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VOLUME LXII
JULY–DECEMBER 1916

WITH TWENTY-ONE PLATES AND ONE HUNDRED AND FIFTY-SEVEN FIGURES

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ERRATA

Vol. LXI

P. 490, last line, for very intruding read very few intruding
P. 495, line 14 from top, for longa read longae; for diaphanis read diaphanae
P. 508, line 13 from top, for plates read plats
P. 511, line 17 from top, for 95 per cent of alcohol read 95 per cent alcohol
P. 514, graph legend, for precipitation from read precipitation, in inches, from

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P. 8, second column of table II, for Arizona read Colorado
P. 20, line 6 from bottom, for Arizona read Colorado
P. 53, lines 12 and 17 from top, for oxidase read oxygenase
P. 144, line 4 from top, for often read open
P. 151, line 10 from bottom, for breviflora read brevifolia
P. 196, last line, for 12° C. read at 12° C.
P. 206, line 7 from top, for poly-hybic read poly-hydric
P. 236, line 10 from top, for 2 M potassium iodide and 2 M acetic acid read 0.2 M potassium iodide and 0.2 M acetic acid

VOLUME LXII NUMBER 1

THE

BOTANICAL GAZETTE

JULY 1916

MEASUREMENT OF THE SURFACE FORCES IN SOILS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 217

CHARLES ALBERT SHULL

(WITH FIVE FIGURES)

I. Introduction

Many investigations of soil moisture have been made, especially during recent years, in attempts to learn something of its mechanics and its relations to plant growth. In a general way the dominating importance of the soil water to plants has long been recognized; but notwithstanding the large amount of work done up to the present time, we still lack some of the most fundamental, elementary facts regarding the physico-chemical relations of the water and soil. This is true generally of that region of soil moisture which lies between what is called the wilting coefficient of the soil and air-dry soil, and more particularly of that critical region immediately below the wilting coefficient.

The discovery of semipermeable coats in seeds in recent years by BROWN (4), SCHRÖDER (30), and others has made it possible to measure approximately the force with which the colloidal gels of the seed attract water. In a former paper (33) it was shown that by means of osmotic solutions whose forces are known the imbibition force of a seed at any given moisture content from saturation to air-dry could be determined approximately.

Because of the rapid establishment of moisture equilibrium relations after disturbance in *Xanthium* seeds, this seed has been chosen for a further investigation of the moisture relations of seeds, with special reference to the moisture held by soil particles. The main purpose of the work was to find some means of measuring the force with which particles of soils of varying fineness retain moisture at different degrees of dryness, and to obtain some more definite knowledge concerning the amount of "back pull" occurring in soils when the total moisture content is so low as to be unavailable to growing plants. Special interest centered in the conditions obtaining in the critical region at and just below the wilting coefficient.

This paper presents the principal results obtained during the last three years. Since the osmotic method of measuring the internal forces of seeds is obviously restricted in practice to such seeds as have a perfectly semipermeable coat, a new method was attempted, based upon a determination of the vapor pressure equilibrium between seeds and osmotic solutions of varying strengths. This method has the advantage of being applicable to all sorts of seeds, regardless of the kind of testa present; but since VON SCHRÖDER (31) and BANCROFT (2) have shown that colloids may not have the same moisture relations to gaseous moisture that they have to water itself, the values obtained by the vapor pressure method have not been used as the basis of comparison with soils in this work. The values for the internal force of seeds as determined by osmotic solutions of various strengths will therefore be used as a basis for comparing the moisture-holding power of fine soil particles.

A number of soils have been used in the investigations, and it is believed that the methods of measurement used here will prove valuable in many kinds of soil moisture studies, since the determinations, while giving excellent data as to the physical relations of the soil moisture, yield at the same time results of considerable physiological significance. The results are more valuable, therefore, than purely physical determinations, because they can be interpreted in terms of plant activity. For, after all, it is the plant in relation to its environment, not merely the environment, that we need to understand.

The work has been carried on in the Plant Physiological Laboratory of the University of Kansas, and in the Hull Botanical

Laboratory of the University of Chicago, where all needed facilities have been generously provided.

II. Historical

The general status of our knowledge of the forces operative in soils was briefly discussed by CAMERON (10) several years ago. It is obvious from this account that up to the present time we have known very little about soil forces within the range of unavailable moisture, that is, between the wilting coefficient and air-dry condition of the soil.

The attempts thus far made at measurement of the surface forces which are known to exist in finely divided matter of all kinds have been made from various angles, but they can be classed under two main heads: (*a*) physical, and (*b*) physiological.

PHYSICAL MEASUREMENTS

A. HEAT OF WETTING METHOD.—The principle of heat of wetting was discovered by POUILLET (26) a good many years ago. He found that all kinds of dry powders, from inorganic substances and porous organic matter, yielded heat on being wet with fluids like water, oil, alcohol, etc. The organic substances yielded the greater amount of heat because, he stated, the organic matter was composed of particles incomparably thinner than the finest inorganic powders.

The literature dealing with the application of this principle to measurements of surface force has been reviewed so recently by PATTEN (25) that it will not be necessary to go into the details of it here. It will be sufficient to point out that through the work of ROSE (28) and JUNGK (19) we gained the conception that water is condensed on the surface of the powdered inorganic or finely divided organic substances, and that the release of heat is due to this compression. The quantitative studies of NÄGELI (23) made it possible for SACHS (29) to calculate the surface forces in starch grains. Since JOULE had shown that 34.3 atmospheres of pressure raises the temperature of water 0.03° C., the amount of heat produced by starch on being wet would indicate much more than 10,000 atmospheres of surface force compression. SACHS assumed, of course, NÄGELI's theory of the structure of organic matter.

The physicists YOUNG, DUPRÉ, and Lord RAYLEIGH have estimated the surface forces of finely divided absolutely dry matter at from 11,000 to 25,000 atmospheres. LAGERGREN'S (20) estimate for charcoal fine enough to have 4 sq. m. of internal surface per gram was 6150 atmospheres.

It is probable that this method would give results too high for soils, for, as MÜNTZ and GAUDECHON (22) have shown, there are other sources of heat release than mere compression when absolutely dry soil and water are mixed. Heat of solution, dilution, and hydration may make considerable errors in estimates of surface forces by this means. The statement frequently made that the force of surface condensation in soils runs from 6,000 to 25,000 atmospheres, as by CAMERON (10), and by BROWN and SMITH (5), is based upon the discussion previously mentioned.

B. COMPRESSION METHOD.—RODEWALD (27) has used a different method in measuring the forces on the surface of starch particles, which has the advantage of being a direct method; that is, the forces of compression are measured by the amount of compression produced instead of by the amount of heat produced. He found that 1 gm. of oven-dry starch absorbed 0.326 gm. of water in becoming saturated. But while the starch swelled, the swelling did not amount to as much as the volume of water absorbed. In other words, there was a volume loss due to compression of the water. The amount of volume loss was 0.0432 cc., and if we refer this to a gram of water, the volume loss is equal to 0.1325 cc. per gm. The compression coefficient of water is calculated by WÜLLNER to be 0.00004659 cc. per gm. for each atmosphere of pressure exerted. This would give a pressure of 2821 atmospheres for the compression actually obtained if we refer the compression solely to the water involved.

By a slightly different method of calculating the force of compression RODEWALD obtained a result of 2523 atmospheres, which is not referred to the water alone, but to the whole system of starch and water. He thinks that the close agreement shows that water alone is involved, or that starch happens to have about the same coefficient of compressibility as water.

The low value obtained by RODEWALD as compared with the values for inorganic bodies, PATTEN thinks is due to the fact that imbibition and absorption are both involved in the starch, and that a much lower value must be obtained than where absorption alone occurs. On the other hand, it will give a higher value than where imbibition alone occurs.

While these determinations of the surface force in absolutely dry matter are interesting, they have no practical value, for such forces as these do not occur in ordinary soils containing capillary moisture, or even in air-dry soils and seeds, for it is evident that the air-dry soil or seed already holds as hygroscopic moisture the water that it would absorb with such remarkable energy if the particles were absolutely dry. However, the figures give us an idea of the power with which these substances retain the last part of their hygroscopic moisture, which must be a force opposite to and equal to that with which wetting occurs.

C. VAPOR PRESSURE AND CENTRIFUGAL FORCE METHODS.—Other physical measurements have been worked out, some of which are very useful, as for instance HILGARD's hygroscopic coefficient (16), a measure based on vapor pressure relations, and the moisture equivalent of BRIGGS and MCLANE (6). The latter is particularly valuable, since BRIGGS and SHANTZ (7) have shown its relation to various physical and physiological amounts of water. But only one of these measurements can be expressed at present in units which permit a comparison of the soil forces with the osmotic forces of the roots of plants.

PHYSIOLOGICAL MEASUREMENTS

The most important attempt at a physiological measurement of the soil forces is that of BRIGGS and SHANTZ (8), who use the wilting coefficient, or percentage of moisture in the soil at the wilting of the plant, in determining unavailable moisture. However, recent work by CALDWELL (9) and by SHIVE and LIVINGSTON (32) shows that within certain ranges the permanent wilting of the plant is a function of the intensity of atmospheric evaporation, and that the wilting coefficient should be rather a measure of the moisture in the plant at the time of wilting than of the moisture in the soil. The constancy of this measure is therefore open to some question, and its value and limitations in physiological studies are to be determined.

Another important physiological study of soil-moisture relations is ALWAY'S (1) investigation of the relation of non-available water to the hygroscopic coefficient. He has shown that some kinds of plants can remain alive for a considerable time after growth ceases from lack of moisture, while others die rather promptly. This is doubtless one of the main differences between xerophytes and mesophytes. In the case of desert perennial legumes, life was maintained even after the soil moisture had fallen slightly below the hygroscopic coefficient. These results emphasize the need of a measure for the surface force of soils which can be expressed, or at least interpreted, in biological rather than physical terms.

There have been few observations on the relation of seeds to soil moisture. BOGDANOFF (3) studied the relation of germinating seeds to soil moisture, and presents many interesting facts. WHITNEY and CAMERON (36) noted the fact that a quantity of cowpeas whose hygroscopic moisture amounted to about 14 per cent, when mixed with an equal quantity of soil which contained 15 per cent of water, took up 12.1 per cent of their own weight, leaving only 1.3 per cent of moisture in the soil. That is, the soil was practically air-dry. In the paper referred to (33) I have shown that the initial internal force of air-dry seeds is little short of 1000 atmospheres; if this condition be general among air-dry seeds, the behavior of the cowpeas can easily be understood. The relation of seeds to soil moisture and vapor pressure will be considered in more detail later.

III. Materials and methods

MATERIAL.—The *Xanthium* seeds used in the experiments discussed in the following section were secured from plants raised on the experimental grounds of the University of Kansas in 1913. Originally all of the seeds planted were from a single plant of *X. pennsylvanicum* Wallr. The 119 plants obtained were very uniform in all their obvious characters, and since it has been shown (34) that the intermingled local types of *Xanthium* are practically isolated by differences in the blooming time of each species, the seeds may be considered as having come from a fairly pure line. This was thought desirable in order that the individual variations of the seeds might be reduced to a minimum, and that consequently more uniform behavior might be obtained under experimental conditions. The other seeds used were obtained from local seedsmen under the names given.

The soils used in the major portion of the work will be characterized briefly. As a representative of heavy clay soil, the subsoil of the Oswego silt loam was chosen. Specimens of this subsoil were obtained from Riley County, Kansas, on an area about 2 miles west of Manhattan. The Oswego silt loam is a residual soil derived by weathering from underlying unbedded shales and sandstone, with the shales predominating. Its subsoil forms a hard, compact, brittle soil, with a gray to dark brown color. The average composition as determined by mechanical analysis is given in table I.

TABLE I

SAND				SILT	CLAY
Coarse	Medium	Fine	Very Fine		
0.4 per cent	0.5 per cent	4.4 per cent	3.2 per cent	61.3 per cent	30.4 per cent

The moisture equivalent is 35.2 per cent, and the wilting coefficient is 19.1 per cent. The general details in regard to the Oswego silt loam and its subsoil may be obtained from the Eighth Report, Field Operations of the Bureau of Soils (11).

As a contrast to the heavy silt clay, a fine quartz sand, the no. 2/0, which is manufactured by the Wausau Quartz Company from quartz rock, was chosen. This grade passes through a 124-mesh screen, and over a 147-mesh screen. The average diameter of the particles is very close to 0.10 mm. The chemical analysis given below shows it to be a very pure quartz sand.

Silicon dioxide	99.07 per cent
Iron oxide	0.17
Aluminum oxide	0.52
Hygroscopic moisture	0.06
Undetermined	0.18
	100.00

The moisture equivalent is 2.41 per cent and the wilting coefficient 1.3 per cent.

For a comparative study of the moisture relations of seeds and soils at the wilting coefficient of the soil, a series of soil samples was obtained from Washington, D.C. The necessary data regarding these soils are given in table II.

TABLE II

Sample number	Locality	Soil type	Moisture equivalent	Wilting coefficient	Hygroscopic H_2O (per cent)*
1.......	Yuma, Arizona	Sand	1.35±0.04	0.73±0.02	0.205
2.......	Highmore, South Dakota	Loam	23.79±0.10	12.93±0.05	3.13
3.......	North Platte, Nebraska	Very fine sandy loam	15.33±0.08	8.33±0.08	1.836
4.......	North Platte, Nebraska	Loam	22.84±0.03	12.41±0.02	2.28
5.......	Amarillo, Texas	Clay loam	29.65±0.02	16.12±0.01	3.82
6.......	North Platte, Neb.	Clay loam	30.08±0.04	16.34±0.02	5.21
7.......	Akron, Colorado	Fine sand	5.90±0.05	3.21±0.03	0.75
8.......	Yuma, Arizona	Sand	1.53±0.01	0.83±0.01	0.218
9.......	Akron, Colorado	Loam	19.91±0.12	10.82±0.06	2.3

* The hygroscopic moisture was determined at the time of use in an ordinary dry oven. The other figures were furnished by Dr. LYMAN J. BRIGGS, of Washington, D.C.

METHODS.—While the internal forces of *Xanthium* seeds have been approximated by osmotic means, many seeds lack semipermeable coats. For such seeds a vapor pressure method has been used which gives results which are in a way comparable to the osmotic measurements. It consists essentially in measuring the vapor pressure equilibrium of the air-dry seeds over sulphuric acid of varying strength, and calculating the internal pressure of the seed from the vapor pressure of the solution over which it was found to be in equilibrium. Owing to our slight knowledge of the concentrated solutions and of the exact relations of colloids to water vapor, the calculations can give only a rough estimate of the internal forces of the seeds, but they are near enough to the osmotic determinations to be of great interest.

The sulphuric acid series was chosen with some reference to the LANDOLT-BÖRNSTEIN tables to facilitate calculation. Beginning with water, the series included 16, 26.5, 35, 39, 50, 54, 57.5, 66, 73, 84.5, and 96–99 per cent H_2SO_4. These fluids were placed in tightly sealed, small, wide-mouthed bottles. The seeds to be tested were suspended in shallow paper baskets a few millimeters above the surface of the acid, the baskets being hung on cotton threads fastened to the corks with carna-uba wax. All metallic condensers were thus avoided. After the seeds were carefully weighed and arranged, the bottles were sunk in a trough of running water to prevent any considerable changes in temperature. Condensation effects due to change of temperature could not occur except over water, for Mitscherlich (21) has shown that even 10 per cent sulphuric acid will prevent deposition of dew in determining hygroscopic coefficients of soils. It may be questioned whether the inclosed space actually reaches the vapor pressure of the solution, for, as Hilgard (17) points out, it is most difficult to secure complete saturation in the case of water vapor. However, the space of air to be brought into equilibrium with the solution vapor pressure in these experiments is very small, and it seems probable that the whole system of liquid, air, and seed comes to an equilibrium pressure in the time of the experiment, except possibly in the case of water. After allowing 15 days for reestablishment of equilibrium by the seeds, the point of no change was determined by weighing.

The osmotic pressure of the sulphuric acid is roughly determined by the use of the vapor pressure formula $P=\frac{f-f^{1}}{f}\cdot\frac{SRT}{M}$, in which f is the vapor pressure of pure water at the temperature of the experiment, f^{1} the vapor pressure of the acid, M the molecular weight of the solvent's vapor, T the absolute temperature, S the density of the sulphuric acid, and R the gas constant. The osmotic pressure (P) is given in grams per square centimeter, and must be reduced to atmospheres. This formula has been developed for dilute solutions and does not hold accurately for high concentrations, but there are at present no data on which to base more accurate estimations. The boiling-point method yields a result close to that given by this formula for sulphuric acid, as will be shown later.

The earliest soil measurements were made with no. 2/0 sand. Seeds of known weight were packed firmly in sand of known water content in paraffined wire baskets, and allowed to come to equilibrium. The tests were confined finally to the region of soil moisture from air-dry to the wilting coefficient, for with a higher moisture content the seeds always became saturated with water. In the case of this sand it was not until the water content was reduced to about 1 per cent that a noticeable "back pull" was developed by the soil.

This method is obviously open to the criticism that friction retards the movement of water in dry soils, and that the seeds therefore do not reach actual equilibrium with the total soil mass, but only with the soil lying very near them. In order to meet this difficulty a rotation method was adopted which brings the seeds constantly into contact with fresh soil particles.

A definite amount of dry soil, usually 60 gm., was taken, and the desired amount of water thoroughly mixed with it. In this condition the soil was divided finely enough to pass through a 2 mm. sieve. The moist soil was then placed with dry seeds in a wide-mouthed 200 cc. bottle, without completely filling it, so that rotation would constantly mix the soils and seeds. The bottles were carefully sealed with heavily shellacked corks to prevent loss of water during the period of rotation.

These bottles were arranged on rotating wheels, driven by a motor and controlled by a speed reducer (fig. 1). The range from air-dry to wilting coefficient was divided into 10 fairly equal divisions, giving 11 tests in each series. The rotation was continued for 15 days, this time having been chosen after making tests as to the effect of differences in duration of the experiment on the amount of intake by the seeds. For instance, no. 2/0 sand with about 0.2 per cent moisture added permitted an intake of 22 per cent of their weight by the seeds in 5 days, and a parallel test showed 21.7 per cent intake in 10 days. Fifteen days, therefore, seems ample time for the establishment of equilibrium. At the end of the time the bottles were opened, and the seeds very rapidly separated from the soil and brushed free of all dust with a camel's-hair brush. The soil and seeds were both placed in weighing bottles as quickly as possible, to prevent serious loss of water by evaporation. The soil was weighed carefully and dried at 104° C. until loss ceased. The seeds also were weighed. The moisture content of the seeds and soils at the time the bottles were opened expressed the equilibrium relation of that soil moisture content.

Since means are at hand for determining the internal force of the seed at practically any moisture content, it is possible to determine from the data the forces of the soils which are in equilibrium with those of the seed.

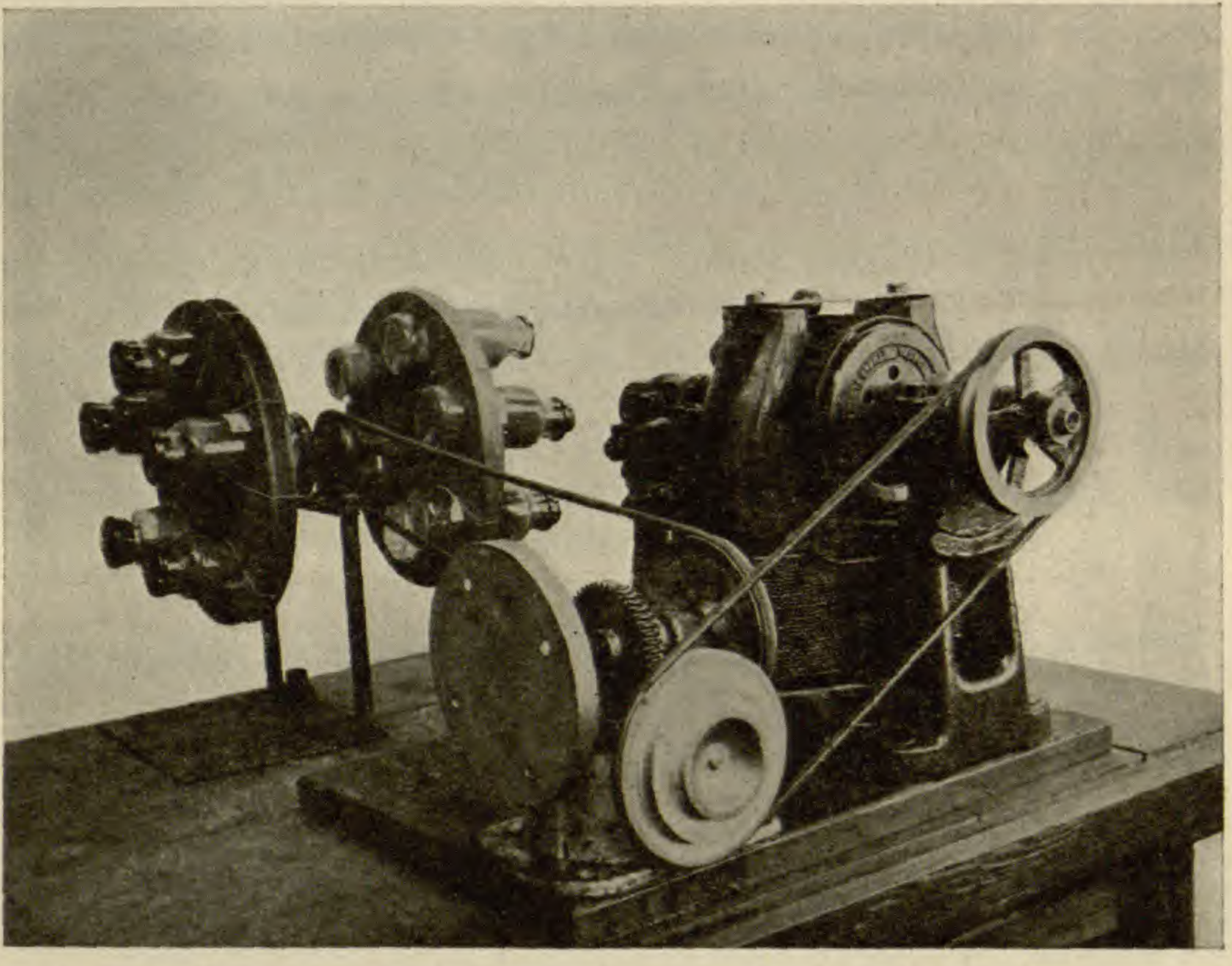

FIG. 1.—Rotator used in these experiments, with motor and speed reducer.

The principal sources of error lie in the fact that moist soils and seeds cannot be handled in ordinary atmospheres without some loss by evaporation during the handling, and in the fact that hot-air ovens for drying are not as accurate as vacuum driers. No claim is made for greater accuracy than these methods will permit. Of course, every precaution was taken to reduce errors to a minimum, and the work was done with the greatest speed and accuracy possible. It is confidently believed that the results to be obtained by more refined methods and more expensive apparatus would in no way change the nature of the conclusions to be drawn from the results.

IV. Experimental results

A. MEASUREMENT OF THE SEEDS.—The measurement of the internal forces of *Xanthium* seeds by means of NaCl and LiCl solutions has been repeated and extended with full confirmation of the previously published results. The data are presented in tabular form for the sake of convenience in table III, and these figures may serve as a basis of comparison in the soil experiments, where the surface forces of the soil particles, instead of osmotic pressure, are pitted against the internal forces of the seed. The data were secured with the lower seeds of *Xanthium pennsylvanicum*.

TABLE III

MOISTURE INTAKE OF *Xanthium* SEEDS IN OSMOTIC SOLUTIONS; TEMPERATURE 23.5° C.; INTAKE IN PERCENTAGE OF AIR-DRY WEIGHT

Solutions volume molecular	1 hour	4 hours	7 hours	10 hours	24 hours	48 hours	Osmotic pressure in atmospheres
H_2O............	16.39	44.38	48.78	50.38	51.18	51.58	0.0
0.1 M–NaCl....	16.79	39.43	45.87	46.48	46.39	46.33	3.8
0.2 M–NaCl....	17.12	38.67	45.00	45.57	45.93	45.52	7.6
0.3 M–NaCl....	16.07	34.05	40.75	41.95	42.24	42.05	11.4
0.4 M–NaCl....	14.36	31.21	38.08	39.97	40.33	40.27	15.2
0.5 M–NaCl....	13.96	30.26	35.87	38.08	38.70	38.98	19.0
0.6 M–NaCl....	13.80	25.57	32.41	33.57	34.77	35.18	22.8
0.7 M–NaCl....	13.32	26.29	30.99	31.73	32.79	32.85	26.6
0.8 M–NaCl....	13.13	25.22	29.21	29.95	31.12	31.12	30.4
0.9 M–NaCl....	12.58	24.34	27.64	28.95	29.14	29.79	34.2
1.0 M–NaCl....	11.90	22.92	25.42	26.48	26.21	26.73	38.0
2.0 M–NaCl....	8.19	14.55	18.25	18.43	18.60	18.55	72.0
4.0 M–NaCl....	4.81	8.37	9.84	10.08	11.00	11.76	130.0
Sat. –NaCl....	3.42	4.94	5.24	5.84	6.21	6.35	375.0
Sat. –LiCl.....	−0.67	−0.77	−0.58	−0.58	−0.58	−0.29	965.0

The results obtained by the vapor pressure method over sulphuric acid are shown in table IV, and some of the curves of moisture intake showing the point of natural equilibrium are shown in figs. 2 and 3.

As the table and curves show, the seeds are initially in moisture equilibrium with sulphuric acid of 46–54 per cent strength. In general the seeds which have carbohydrate reserves in greatest abundance seem to have a somewhat lower equilibrium point than those with high fat and protein content.

The osmotic pressure of the sulphuric acid calculated from the vapor pressure formula given runs from 1000 to 1350 atmospheres. The validity of the vapor pressure formulae will be discussed later. If colloids absorbed as much moisture from a saturated atmosphere as from water, it might be safe to assume that the internal force of the seeds is equal to the osmotic force of the solution. But if VON SCHRÖDER'S work holds for all colloids, this vapor pressure method may give abnormal values. If the colloids always tended to take up more water when in contact with the fluid, above the equilibrium point as well as below, the values given here would be too low, as the equilibrium point would be shifted toward the stronger acids. If, on the other hand, intake is increased below the equilibrium point, and loss is increased correspondingly above the equilibrium point, the shape of the curve would be changed, but the equilibrium point would remain fixed.

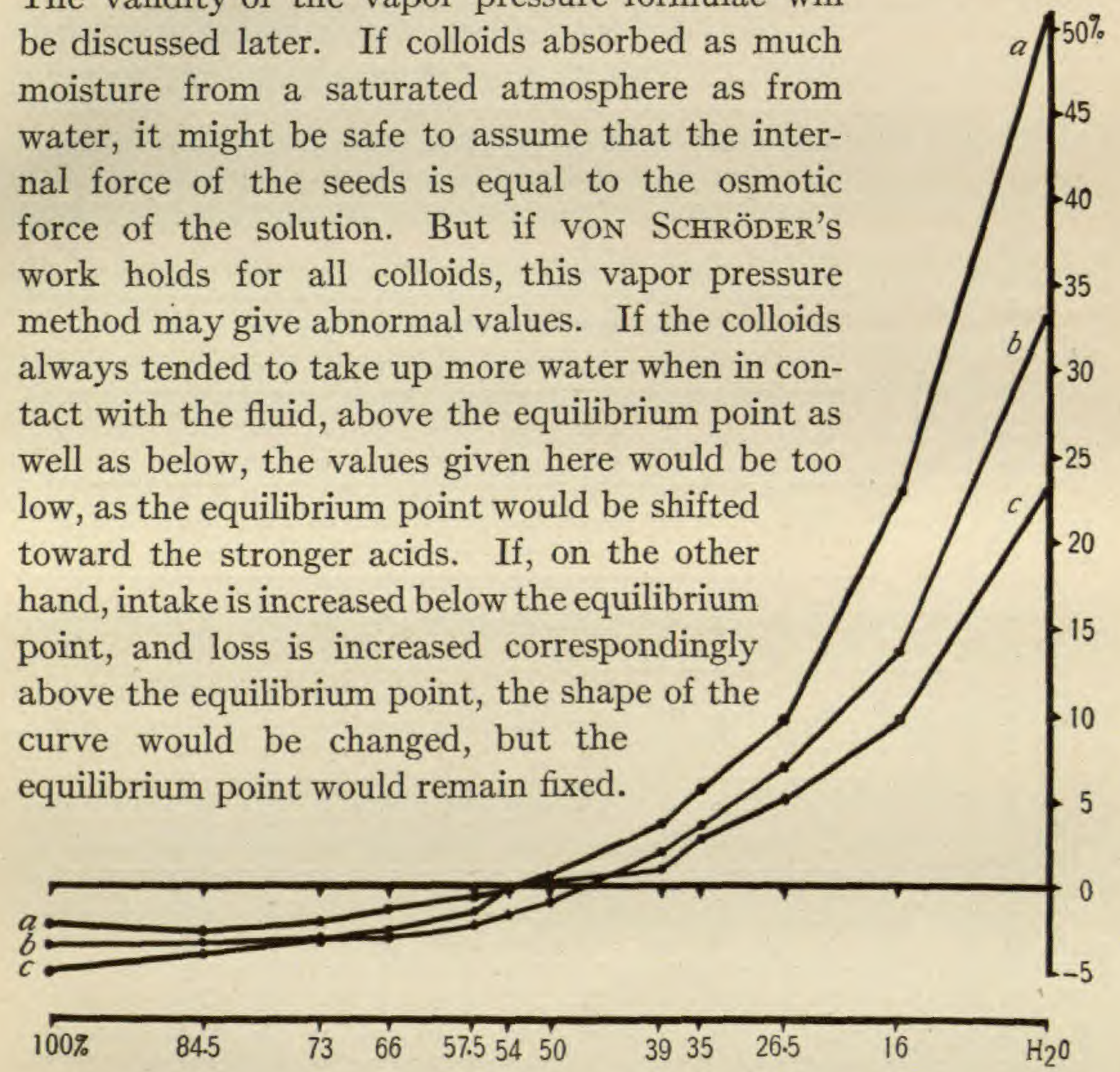

FIG. 2.—Curves of moisture equilibrium of seeds suspended over sulphuric acid: *a*, *Pisum sativum;* *b*, Stowell's evergreen sweet corn; *c*, *Xanthium pennsylvanicum;* abscissae, strength of sulphuric acid; ordinates, moisture intake by seeds in percentage of air-dry weight.

The values run higher with the H_2SO_4 than with the lithium chloride solution, as given in table III. There is one source of possible discrepancy which needs to be mentioned. The vapor pressure tests were all made in Kansas, where the climate averages drier than at Chicago, while the osmotic measurements were all

TABLE IV

VAPOR PRESSURE MEASUREMENT OF SEEDS; GAIN AND LOSS IN WEIGHT OF SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT

SEEDS	GAIN					GAIN AND LOSS	LOSS						EQUILIBRIUM POINT	HYGROSCOPIC MOISTURE	INTERNAL FORCE CALCULATED IN ATMOSPHERES
	Specific gravity H_2SO_4→	1.110	1.190	1.258	1.294	1.398	1.436	1.473	1.566	1.647	1.772	1.835			
	H_2O	Percentage H_2SO_4→ 16	26.5	35.0	39.0	50	54	57.5	66	73	84.5	96.99			
													Per Cent	Per Cent	
Oryza sativa.....	20.09	10.52	5.77	3.11	1.51	−0.81	1.78	2.62	3.92	4.71	4.97	5.29	46.15	10.12	1008.0
Oryza sativa.....	18.1	12.40	7.70	3.50	1.70	−0.80	0.77	1.70	2.80	4.30	5.80	5.90	49.50	9.9	1182.0
Reid's yellow dent corn..........	18.99	10.97	6.89	3.31	2.16	−0.67	1.46	2.39	3.43	3.51	3.46	3.97	47.40	11.96	1060.0
Stowell's evergreen sweet corn	33.42	13.96	6.87	3.36	1.85	−0.89	1.68	2.26	3.07	3.08	3.33	3.42	46.40	11.63	1019.0
Feterita.........	27.00	11.96	7.02	3.62	2.88	+0.74	0.40	1.54	3.23	4.47	5.09	5.43	52.60	6.06	1284.0
Xanthium pennsylvanicum	22.9	9.86	5.09	2.61	0.98	+0.42	0.00	1.68	2.72	3.11	3.88	5.11	54.00	5.28	1354.0
Xanthium pennsylvanicum	36.95	10.11	4.46	2.86	1.36	+0.46	0.08	0.37	1.13	1.73	3.02	3.05	53.40	5.83	1324.0
Xanthium pennsylvanicum	20.63	15.86	9.45	5.85	3.01	+1.63	0.13	0.48	1.33	1.77	2.98	1.99	53.70	5.16	1340.0
Ricinus communis.....	18.1	6.67	2.75	1.47	1.20	+0.15	0.18	0.49	0.93	1.9	2.4	2.5	51.80	3.32	1246.0
Triticum sativum.	40.53	14.20	8.84	5.18	3.23	+0.78	0.00	0.64	2.54	3.24	3.70	3.44	54.00	10.19	1354.0
Pisum sativum (wrinkled seeds)	50.56	23.01	9.76	5.61	3.54	+0.60	0.26	0.56	1.33	2.15	2.57	2.19	52.80	8.14	1294.0
Soy beans.......	40.46	18.59	8.59	3.72	3.22	+0.70	0.00	0.62	1.50	2.90	3.12	3.16	54.00	6.88	1354.0
Lupinus albus....	24.06	5.58	4.13	2.59	2.05	+0.85	0.31	0.31	1.07	2.05	1.87	2.02	52.90	8.02	1300.0

made at Chicago. The hygroscopic moisture of the seeds was uniformly near 5.5 per cent in Kansas,[1] while at Chicago the hygroscopic moisture was rarely less than 7 per cent, usually above. The equilibrium point would naturally run higher, therefore, in a drier climate. However, it is not claimed that the discrepancy would entirely disappear if repeated with seeds of the same moisture content.

There seems to be little doubt as to the high osmotic strength of the sulphuric acid, for the boiling-point method of measuring osmotic pressure gives a value in close agreement with those just given. The boiling point of 53 per cent H_2SO_4 is 128.5° C. If for each rise of 0.52° C. we may assume an osmotic pressure equal to one mole of dissolved substance (HÖBER, Phys. Chem. p. 19), this strength of sulphuric acid should have an osmotic pressure of 1227 atmospheres.

At all events, it is safe to conclude that air-dry seeds possess a very high internal attraction for water which at the initial moment of intake is but little short of 1000 atmospheres. Owing to uncertainty of the figures for sulphuric acid, only those obtained by direct contact with osmotically active solutions will be used as a standard for the following soil experiments:

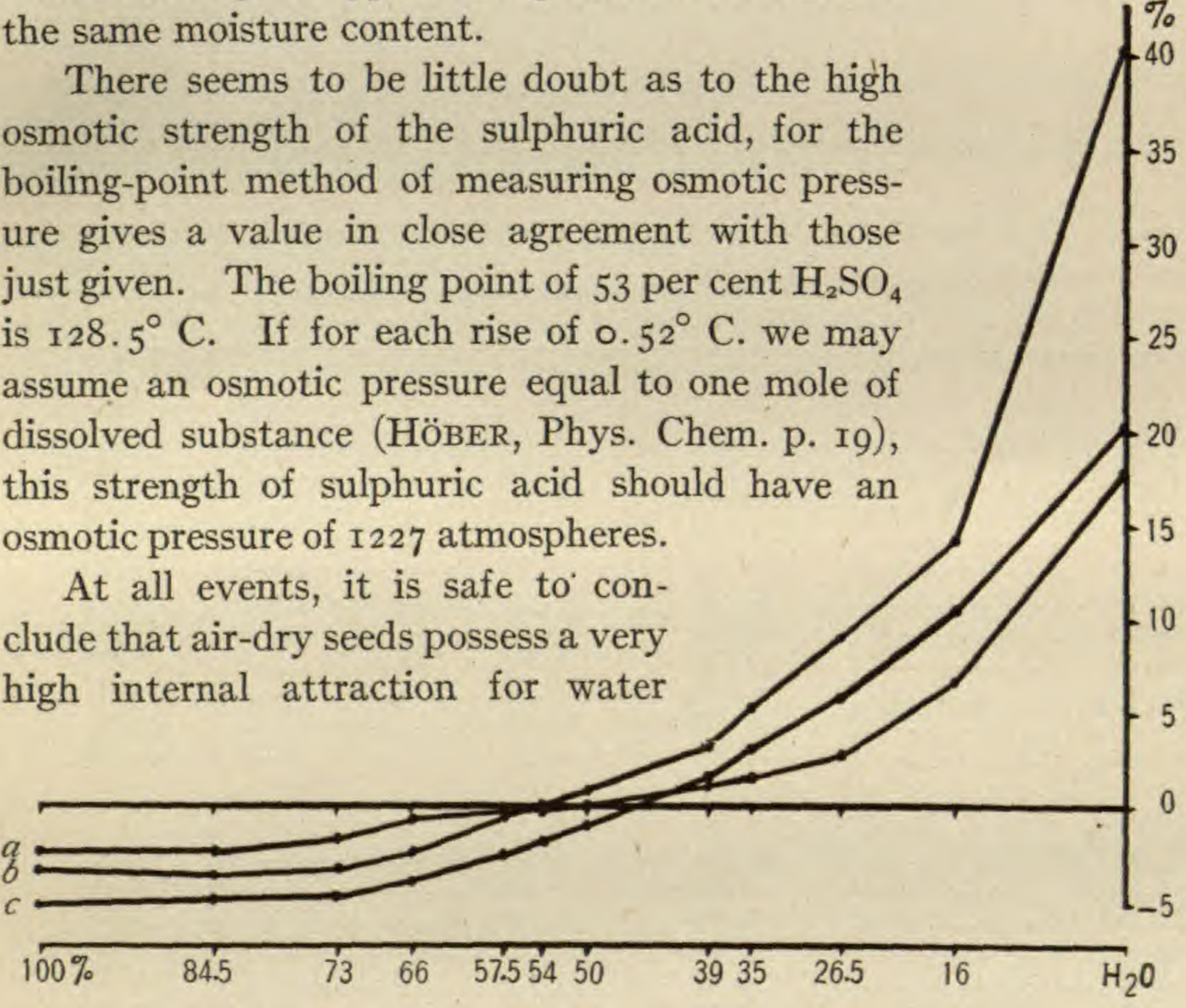

FIG. 3.—Curves of moisture equilibrium of seeds suspended over sulphuric acid: *a*, *Ricinus communis;* *b*, *Triticum sativum;* *c*, *Oryza sativa;* abscissae, strength of sulphuric acid; ordinates, moisture intake by seeds in percentage of air-dry weight.

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[1] Seeds sent to Washington were dried in the vacuum oven by Mr. A. B. CAMPBELL. Upper seeds averaged 5.48 per cent of the absolute dry weight, and lower seeds averaged 5.72 per cent of their absolute dry weight.

B. THE SURFACE FORCES OF SOILS.—1. *The Oswego silt loam.*—A number of tests were made with the subsoil of the Oswego silt loam, the results of 4 of which are shown in table V. The air-dry soil apparently holds its moisture with about the same force as do air-dry seeds, a result one might expect, since both are in moisture equilibrium with the same atmosphere at air-dry.

TABLE V

RELATION OF SOIL MOISTURE IN SUBSOIL OF OSWEGO SILT LOAM TO WATER INTAKE BY SEEDS OF *Xanthium pennsylvanicum*

Soil H_2O in percentage of absolute weight	Intake H_2O in percentage of air-dry weight	Soil H_2O in percentage of absolute weight	Intake H_2O in percentage of air-dry weight
I		III	
6.66 (air-dry)........	0.97	5.95 (air-dry)........	0.38
7.85................	3.35	6.15................	1.58
8.92................	6.25	7.61................	3.73
10.26................	9.58	8.68................	6.16
11.79................	11.94	10.32................	10.76
12.74................	17.46	11.60................	15.79
14.78................	20.62	13.16................	21.36
16.06................	34.00	14.88................	28.61
17.35................	39.77	16.75................	33.86
18.07................	41.79	18.07................	45.15
19.80................	46.54	19.34................	49.31
II		IV	
5.83 (air-dry)........	0.00	4.65 (air-dry)........	−0.53
6.23................	1.91	6.46................	+1.06
8.27................	5.18	7.88................	3.68
9.16................	6.55	9.36................	6.47
10.81................	9.81	11.16................	10.82
13.56................	19.93	12.46................	15.81
14.23................	23.88	13.91................	21.11
15.34................	31.54	15.18................	32.60
17.10................	37.70	17.12................	41.98
17.93................	43.25	18.87................	47.26
19.71................	43.79	20.04................	50.00

As soil moisture is increased, the moisture intake by the seeds increases at a much more rapid rate, until the approaching saturation of the seed begins to cut down the absorption rate. This happens as the soil moisture approaches the wilting coefficient. Reference to fig. 4 will make the relationship of soil moisture content to seed intake clear.

The general situation is strikingly illustrated by table VI, which combines the results of the 4 experiments of table V. While there are a number of discrepancies, as one might expect, it is evident that the method can be used in measuring approximately the forces residing on the surfaces of soil particles at various soil moisture contents. For instance, when this soil contains about 9.36 per cent of moisture (3.5 per cent above air-dry), it is in moisture equilibrium with seeds at 6.47 per cent above their air-dry weight. The seeds attain about the same equilibrium point with saturated NaCl solution, which has an osmotic pressure of 375 atmospheres,

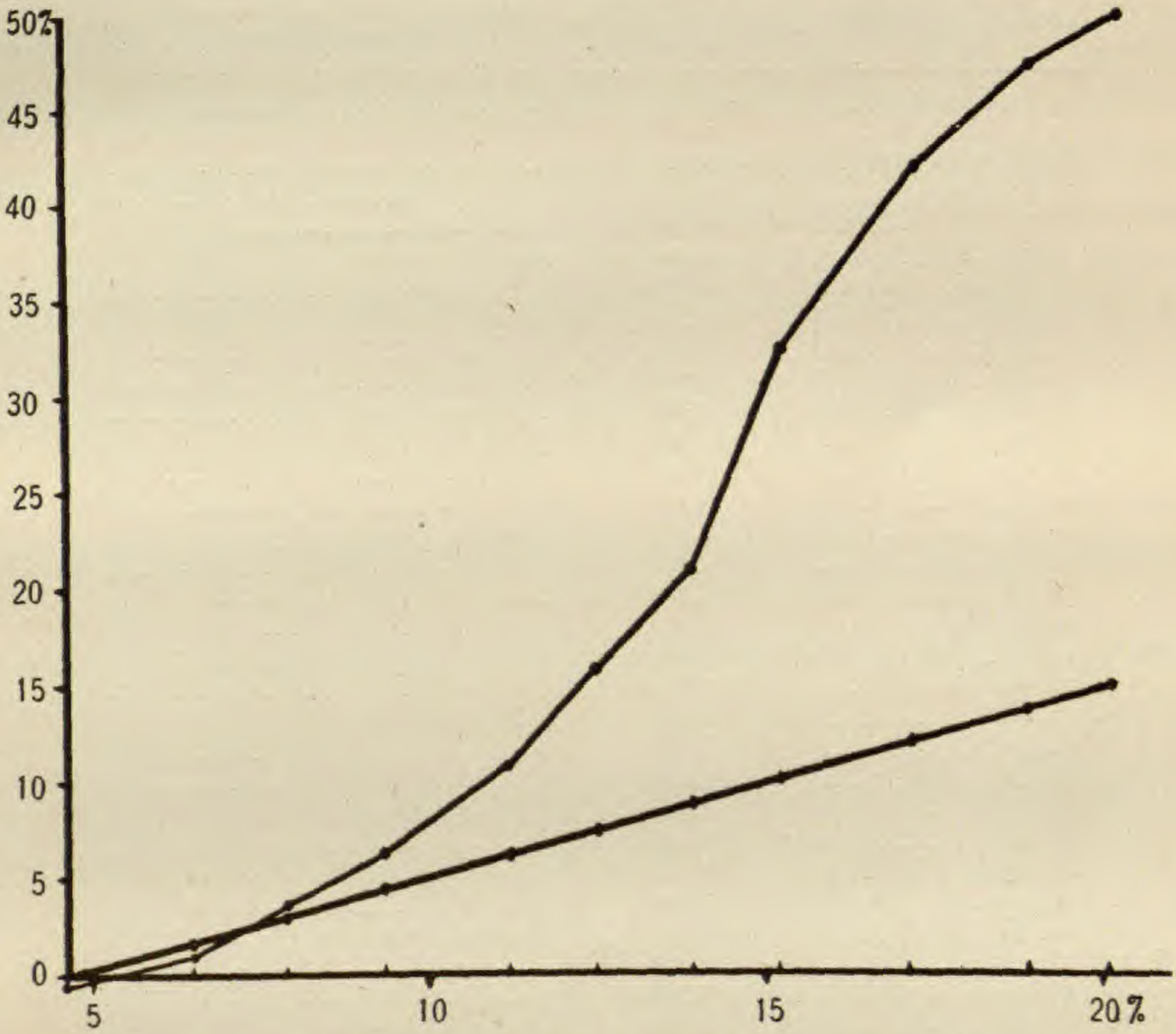

FIG. 4.—Curves showing relation of moisture intake by seeds to increasing soil moisture, plotted from experiment 4, table V; abscissae, percentage of soil moisture in terms of absolute dry weight; ordinates, percentage of increase of soil moisture above air-dry weight for the soil moisture curve (straight), and percentage of intake by seeds in terms of their air-dry weight.

from which it follows that the surface force of the soil particles at the given moisture content is also approximately 375 atmospheres.

TABLE VI

RELATION OF SOIL MOISTURE TO INTAKE BY SEEDS; DATA OF TABLE V COMBINED

Soil moisture in percentage of absolute weight	Intake by seeds in percentage of air-dry weight	Osmotic pressure equal to surface force in atmospheres
4.65 (air-dry)....	−0.53	....
5.83 (air-dry)....	0.00	LiCl saturated = 965 atmospheres
5.95 (air-dry)....	+0.38	....
6.66 (air-dry)....	0.97	....
6.15............	1.58	....
6.23............	1.91	(697)[*]
6.46............	1.06	....
7.61............	3.73	(532)
7.85............	3.35	....
7.88............	3.68	....
8.27............	5.18	....
8.68............	6.16	(418)
8.92............	6.25	....
9.16............	6.55	....
9.36............	6.47	NaCl saturated = 375
10.26............	9.58	....
10.32............	10.76	....
10.81............	9.81	....
11.16............	10.82	....
11.60............	15.79	....
11.79............	11.94	4 M.NaCl = 130
12.46............	15.81	....
12.74............	17.46	....
13.16............	21.36	2 M.NaCl = 72
13.91............	21.11	....
14.23............	23.88	....
14.78............	20.62	....
14.88............	28.61	M.NaCl = 38
15.18............	32.60	....
15.34............	31.54	....
16.06............	34.00	$M.C_6H_{12}O_6 = 22.4$
16.75............	33.86	....
17.10............	37.70	0.5 M.NaCl = 19
17.12............	41.98	0.4 M.NaCl = 15.2
17.35............	39.77	....
17.93............	43.25	0.3 M.NaCl = 11.4
18.07............	41.79	....
18.07............	45.15	0.2 M.NaCl = 7.6
18.87............	47.26	0.1 M.NaCl = 3.8
19.34............	49.31	....
19.71............	43.79	....
19.8............	46.54	....
20.04............	50.00	....
....	51.44	Saturated = 0.00

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[*] Values in parenthesis calculated from the curve of moisture-holding power of the soil as determined by the known values.

When the soil moisture reaches 6 per cent above air-dry, the moisture intake by seeds indicates a force equivalent to 4M. NaCl solution, which is estimated to exceed 130 atmospheres. At 11 per cent above air-dry the holding power of the soil has fallen to 22.5 atmospheres approximately.

In this manner, comparing the percentage intake from the soil with that from the solutions, as given in table III, one may estimate the surface force for any given moisture content of the soil, each soil type, of course, having specific relations. If it were possible to make absolutely accurate determinations for several points in the curve of moisture intake by seeds, as related to the curve of moisture increase in a particular soil, it would be a simple mathematical problem to calculate the exact water-holding power of the soil particles at any soil moisture content whatsoever for that soil.

2. *The no. 2/0 sand.*—By preliminary tests ranging from 17.5 per cent to 1 per cent of moisture it was found that there was no significant water-holding power in this sand until the moisture content fell to less than 2 per cent. At 1 per cent of soil moisture the seeds took in over 45 per cent of their own dry weight.

The results of a series of tests running from air-dry (0.14 per cent) to a little beyond the wilting coefficient (1.3 per cent) are shown in table VII.

TABLE VII

RELATION OF MOISTURE IN NO. 2/0 QUARTZ SAND TO MOISTURE INTAKE OF *Xanthium* SEEDS

Soil H_2O in percentage of absolute weight	Intake H_2O in percentage of air-dry weight
0.14 (air-dry)	−0.306
0.159	+1.407
0.175	5.02
0.203	21.81
0.44	33.98
0.81	42.40
1.03	45.64
1.49	47.46
1.79	52.06
2.14	72.85*

*Four seeds showing incipient germination, hypocotyls averaging 3 mm. long.

In this sand the very rapid decrease in the force with which its particles hold water as moisture content increases stands in sharp contrast to the slower decrease in the heavy clay subsoil. While the air-dry sand gives the same kind of result as the air-dry clay, by the time the sand contains 0.44 per cent of water the seeds secure as much water as they do in a molecular solution of non-ionizable salts. This indicates a force of 22.4 atmospheres.

3. *Various soil types.*—The results obtained with the subsoil of the Oswego silt loam and the no. 2/0 sand suggested that there might be a general relationship between soils and seeds as regards the amount of moisture seeds will absorb at the wilting coefficient of the soil, whatever value the wilting coefficient might have. To clear up this point, the soil types of table II were used. Each soil was brought as nearly to the wilting coefficient as possible by addition of water. The closeness of the experimental conditions to the wilting-coefficient determinations is shown in columns 3 and 4 of table VIII.

TABLE VIII

RELATION OF WILTING COEFFICIENT TO MOISTURE INTAKE BY SEEDS

Soil types	Percentage of hygroscopic moisture	Percentage of wilting coefficient	Percentage of soil H_2O	Percentage of seed intake
1. Sand (coarse)........	0.205	0.73±0.02	0.65	34.44
2. Loam................	3.130	12.93±0.05	12.66	49.02
3. Sandy loam (very fine)	1.836	8.33±0.08	7.86	48.38
4. Loam................	2.280	12.41±0.02	13.30	49.01
5. Clay loam............	3.820	16.12±0.01	16.01	49.49
6. Clay loam............	5.210	16.34±0.02	17.78	47.31
7. Fine sand............	0.750	3.21±0.03	3.19	49.77
8. Sand (coarse)........	0.218	0.83±0.01	0.80	40.98
9. Loam................	2.30	10.82±0.06	10.51	50.42

The percentage of moisture taken up by dry seeds placed in each soil is shown in the last column of the table. With the exception of the two sands from Yuma, Arizona, which are coarse, the results are fairly uniform. In two cases the soils adhered badly to the seeds, making accurate work very difficult; but corrections were made as carefully as possible. In all the other soils the seeds remained clean, or were easily brushed free of adhering particles. The average intake for the 7 types, excluding the coarse sands, is approximately 49 per cent, and this average agrees rather closely with the intake in the Oswego silt loam subsoil and in the no. 2/0 sand at their wilting coefficients. This probably means that the wilting coefficient represents a fairly definite water-holding power for the soil particle, regardless of its size. By comparison of the results given in table VIII with those given in tables III and VI, it is seen that the "back pull" of the soil particles, the force with which they withhold water from seeds and plants at this critical moisture content, is not more than the equivalent of 0.1M. NaCl solution, or 3–4 atmospheres. This value is surprisingly low.

V. Discussion

In this paper the term internal forces of seeds is used to designate the resultant of all forces within the seed tending to cause intake of moisture; and by surface forces of soils is meant the resultant of all the forces of adhesion, cohesion, surface force, etc., as determined by size, chemical composition, and character of the surface of the soil particles, which cause the soil to retain moisture.

In order to make clear the nature of the problems involved in this work we shall first consider rather fully the moisture relations between seeds and their environment, and then the moisture relations of soils to seeds and to the root hairs of living plants. A careful study of the moisture relations existing between organic bodies, like seeds, and the atmosphere, the soil, and osmotic solutions, if the seeds have semipermeable coats, convinces one that the entrance or exit of water from the seed is due to the interplay of such internal forces as capillarity, imbibition force, colloid hydration forces, etc., with external forces such as atmospheric evaporation, the surface forces active on soil particles, osmotic pressure, etc., according to the environment of the seed. Moisture flows into or out of the seed as one or the other of these sets of forces is the greater. Movement of water continues only until the two forces, unequal at the start, become equal. This establishes moisture equilibrium, and further movement of water must be consequent to some disturbance, external or internal, of the balanced condition of forces. Moisture equilibrium may obtain at any moisture content of the seed, if only the two forces are equal.

The attempt to measure the internal forces of seeds with semipermeable coats by means of osmotic solutions was based upon this conception of the moisture relations, and on the assumption that the total osmotic pressure of the solution is transmitted through the membrane as force when pitted against the internal forces of the seed through the agency of the semipermeable coat. The results of this attempt have been highly satisfactory, and I can see no good reason for doubting that the internal forces of air-dry seeds are approximately equal to 900–1000 atmospheres.

The vapor pressure method, using sulphuric acid, involves another assumption whose validity may be a little more questionable. It is generally admitted that the osmotic pressure of a solution can be calculated with some degree of accuracy from its vapor pressure. I have assumed in addition that the vapor pressure of the seed hydrogels measured against the vapor pressure of strong solutions can be used as a measure of the internal forces of the seed. The assumption seems reasonable enough, but it would be difficult to offer definite proof of its validity at present.

The principal difficulty with the vapor pressure method is the uncertainty as to the osmotic pressure of sulphuric acid. It may seem quite unwarranted to some even to think of estimating the osmotic pressure of strong sulphuric acid by means of a formula, knowing as little as we do of all the factors which enter into the problem in this case, and knowing that these formulae have all been developed for the dilute "ideal" solutions. I realize fully the danger of basing any conclusions upon results obtained by such precarious methods, and offer the results merely for their suggestive value. The formula I have used for calculating the osmotic pressure from the vapor pressure is that given by WALKER (35), with S representing the density of the solution rather than that of the solvent. This is necessary in order to make the formula fit a concentrated solution with high density. This same formula has been used by others for the same purpose, as by DRABBLE and DRABBLE (12).

Attention is called to a difference in the formulae given by NERNST (24) and WALKER. In WALKER'S discussion, $\frac{f-f^1}{f}$ represents *relative lowering of the vapor pressure* of the solution as compared with that of water. His formula actually gives the relative lowering. NERNST, however, uses $\frac{p-p^1}{p^1}$ for this factor in the equation, and with concentrated solutions it is quite a different thing. In a concentrated solution, where the relative lowering of the vapor pressure is 0.75, NERNST'S formula will give a value for the osmotic pressure 4 times as great as that given by WALKER'S formula.

In the third English edition of NERNST he gives an equation for this calculation which he claims gives a very exact value for the osmotic pressure from vapor pressure. His equation is as follows:

$$P=\frac{0.0821.T.\quad 1000\,S}{M}.\,ln\frac{p}{p^1}$$

The physical chemists claim that this formula should hold in so far as it includes the factors involved. But even this exact formula does not take care of the change in volume occurring on dilution of the acid, nor for the heat of dilution, which is very considerable in the strong acid solutions over which equilibrium of moisture vapor was obtained in these experiments.

I have chosen to use WALKER'S formula because it actually gives the relative lowering of the vapor pressure, as it is supposed to do. However, it is perfectly clear from this discussion that the osmotic pressure of sulphuric acid of any given concentration, especially of high concentration, cannot be measured accurately by any single vapor pressure formula suggested to date.

The measurements made over sulphuric acid give values, therefore, which are merely suggestive. They indicate that the internal forces of air-dry seeds of all kinds are very high, and confirm the high values obtained by the osmotic solution method. But the latter method only, I believe, can be relied upon for the measuring of the internal forces of seeds until the vapor pressure relations are more perfectly understood.

The main conclusion in regard to these seed measurements may be stated briefly thus: It is possible, with seeds having a perfectly semipermeable coat, to measure the water-attracting internal forces residing in the seed substance at any moisture content between saturation and air-dry. The actual values for this range in *Xanthium* seeds are given in the last column of table III.

The results obtained by measuring the soils with measured seeds are of the greatest interest. While it is important to have means of determining in terms of atmospheric pressure the water-retaining power of soils at any degree of dryness between saturation and air-dry, it is still more important to understand the moisture relations of the plant to the soil at and just below the wilting coefficient. Does wilting occur because capillary soil forces and osmotic root forces reach equilibrium? If so, why should BRIGGS and SHANTZ (8) have found a uniform wilting coefficient for all kinds of plants when we know that root cells vary somewhat in osmotic concentration from species to species? And why should this uniformity fail in the presence of intense evaporation, as shown by CALDWELL (9), and by SHIVE and LIVINGSTON (32)? These questions, and the discrepancies between the excellent work done at Washington and at Tucson can probably be answered intelligently, and explained in the light of these experiments.

The moisture equivalent of a soil is the percentage of water left in the soil after centrifuging for a certain time under a force of 1000 gravities, a force about equal to one atmosphere. We have been accustomed to find the wilting coefficient empirically, or to divide the moisture equivalent by 1.84. But we have not known how much greater are the soil forces at the wilting coefficient than at the moisture equivalent. From the results obtained, the pressure value of the wilting coefficient seems to be about 4 atmospheres.

As the soil becomes drier and drier, the forces become greater and greater, until on the approach of air-dry conditions a very small change in moisture content makes a very large change in the forces involved. The increase of the force with decreasing soil moisture is shown graphically in fig. 5, which shows the curve of increasing force for the Oswego silt loam subsoil, and for no. 2/0 sand. The difference in the curves for clay and sand is striking. In the clay, where the surface films are relatively thick, the force decreases very slowly for a considerable distance. But as the films become very thin, the surface force and forces of adhesion of water to particles become very great. This force would reach its maximum presumably when the particles were surrounded by a film of water just one molecule thick. The curve for the sand is very similar, except that the period of slow increase of the soil forces is very short, and that the whole curve lies much nearer to the absolutely dry condition. These relations, of course, are conditioned mainly by the size of the particles.

Let us see now how the soil forces at the wilting coefficient compare in value with the average osmotic pressure of the root hairs. A few years ago HILL (18) showed that the root hairs of *Salicornia* can in a measure accommodate themselves to changes in the osmotic concentration of the surroundings, through increase or decrease of the cell sap concentration parallel to the changes in the environment. That the cell sap of leaves and other exposed parts increases in concentration with xerophytic habitat has been shown by DRABBLE and LAKE (13), FITTING (14), and others. The general conclusion reached by DRABBLE and DRABBLE (12), that the osmotic strength of cell sap varies with the physiological scarcity of water in any area, seems most reasonable, and it doubtless holds true of root cells under xerophytic soil conditions. As the soil becomes deficient in water supply, and the surface forces are rapidly increasing in magnitude, we might then expect this increased force of the soil to be paralleled by increase in the osmotic forces of the root hairs, owing to progressive concentration of the cell sap. Such increase might amount to many atmospheres.

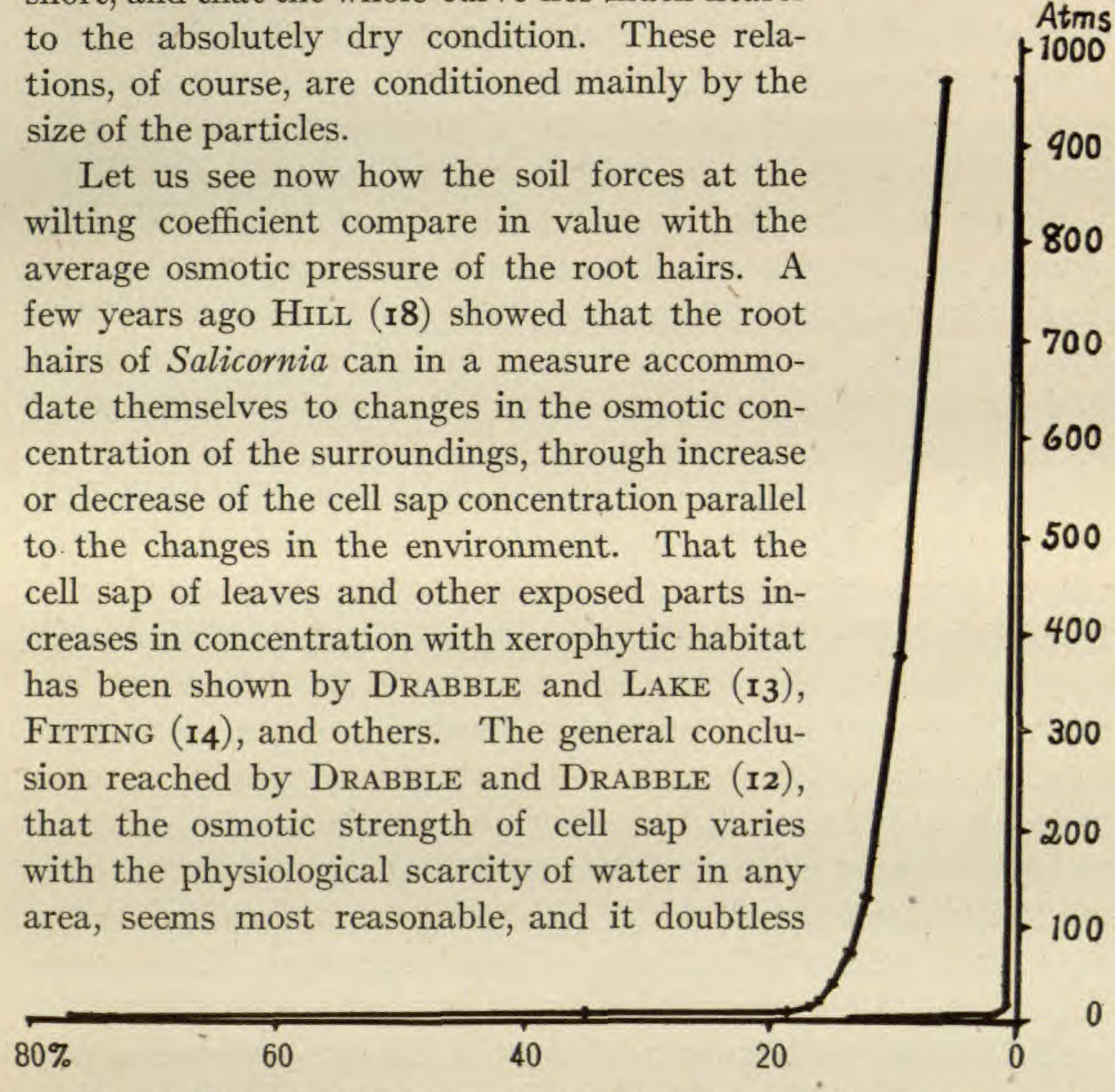

FIG. 5.—Curves showing increase in the surface forces of soils as drying proceeds; to the left, for subsoil of the Oswego silt loam; to the right, for no. 2/0 sand.

The most careful, extensive, and valuable study of the osmotic pressure of the sap of root cells under ordinary conditions of soil moisture which has been made up to the present is that by HANNIG (15). He finds that the average root cell sap pressure for 64 species of plants is 0.21M. KNO_3, or equivalent to 7 or 8 atmospheres. It is seen, therefore, that the water-holding power of the soil at the wilting coefficient is only about half that of the average osmotic pressure of the sap of the root cells. Certainly wilting at the wilting coefficient cannot be due to lack of water, for seeds come within a few per cent of taking up as much moisture at the wilting coefficient as when placed in water itself. Nor can it be due to equalization of forces between root hair and soil water, for there is still a gradient of 4 atmospheres of force in favor of the plant. Moisture and gradient for movement of water toward the plant are both present, and yet the plant wilts!

Even in cases where the soils are drier than the wilting coefficient, the accommodation of the root hairs mentioned above would probably maintain this gradient of a few atmospheres in favor of moisture intake. This idea is strongly supported by unpublished work of Miss EDITH A. ROBERTS, who, working in this laboratory, has shown that seedlings of mustard and radish grown in sugar solutions develop root hairs with osmotic pressures usually about 4 atmospheres in excess of the medium in which they grow, the same amount of excess as this gradient at the wilting coefficient. This relationship of internal to external forces was maintained, in her work, up to volume molecular solutions of cane sugar. It is exceedingly probable, therefore, that as soils dry out beyond the wilting coefficient the root hairs maintain an osmotic pressure a few atmospheres in excess of the soil forces until those forces become relatively very high. Nevertheless, permanent wilting occurs within a narrow range of soil moisture under moderate conditions of evaporation.

There seems to be but one reasonable explanation for this situation. The wilting of plants at the wilting coefficient of the soil must be due to the failure of water movement from soil particle to soil particle, and from these to the root hairs, rather than from lack of moisture or gradient. This does not mean complete cessation of movement of film water toward the plant. It is a question of rates. Evaporation continues from the leaves in accordance with atmospheric conditions somewhat regardless of conditions below the soil surface. At the wilting coefficient the film water becomes so stable, and the friction of movement becomes so great, that the rate of movement of water toward the root is quite inadequate to meet the needs of the plant, and permanent wilting ensues.

It becomes clear at once why BRIGGS and SHANTZ, working under rather uniform conditions of evaporation, found the same wilting coefficient for all kinds of plants in a given soil, regardless of variability of root sap concentration and other variable factors, for these variables do not affect the point at which capillary movement of water over the soil particles ceases to be effective for the plant. This is determined by the physical properties of the soil, the fineness of the particles being the chief factor. We should expect, therefore, this uniform behavior under moderate conditions. On the other hand, when the evaporation rate is very intense, the plant might be caused to wilt permanently before this wilting coefficient is reached, owing to the fact that after all it is a question of rates. The rate of movement in the soil fails to be adequate sooner.

In concluding this discussion, may I suggest that the methods used and the conclusions reached in this work should receive very critical consideration by plant physiologists, soil physicists, and all others interested in these problems. We have lacked even the most elementary facts concerning these important moisture relations of the soil. This is a first attempt to throw light upon an unexplored region of soil physics. It is hoped that other methods may be devised for testing the correctness of the conclusions reached by the methods presented here. The apparatus is quite simple and easily used. If the results obtained can be fairly substantiated by other methods, the method will be exceedingly valuable in physiological and ecological investigations of many kinds.

VI. Summary

1. The force with which the seeds of *Xanthium pennsylvanicum* absorb water has been measured by two methods: (*a*) osmotic

solutions, and (*b*) vapor pressure equilibrium. The osmotic method is at present the more reliable.

2. The air-dry seeds of *Xanthium* show an initial attraction for water of nearly 1000 atmospheres.

3. The attraction which exists at any moisture content of the seed between air-dry and saturation can be approximated. Table III gives the data.

4. The seeds have in turn been used to measure the complex moisture-holding forces of soils, with the following results:

a) The air-dry subsoil of the Oswego silt loam holds its hygroscopic moisture with about the same force as an air-dry seed, that is, about 1000 atmospheres.

b) As the moisture content of the soil increases, the surface force decreases rapidly. When about 3.5 per cent of water has been added to the air-dry soil, the force remaining is about 375 atmospheres. When the soil moisture reaches 6 per cent above air-dry in this soil, the moisture is held with a force of 130 or more atmospheres. At 11 per cent above air-dry the holding power has fallen to 22.4 atmospheres.

c) At the wilting coefficient of the soil (13.3 per cent above air-dry in the Oswego silt loam subsoil) the "back pull" of the soil particles amounts to not more than that of a 0.1M. NaCl solution, that is, not more than about 4 atmospheres. This is shown to hold true for a number of types of soil with widely varying wilting coefficients.

5. This water-holding power of soils at the wilting coefficient is less than the osmotic pressure of the root hairs of many kinds of plants, as shown by HANNIG and others.

6. The wilting of plants at the wilting coefficient of the soil cannot be due to lack of moisture in the soil, nor to lack of a gradient of forces tending to move water toward the plant.

7. The view is held, therefore, that the wilting at this critical soil moisture content must be due to the increasing slowness of water movement from soil particle to soil particle, and from these to the root hairs, the rate of movement falling below that necessary to maintain turgidity of the cells of the aerial parts, even under conditions of low transpiration.

My thanks are due to Professor L. E. CALL, of the Kansas State Agricultural College, Manhattan, for sending me the subsoil of the Oswego silt loam used in these experiments, and to Dr. LYMAN J. BRIGGS and his assistants in the biophysical laboratory of the Bureau of Plant Industry for making determinations of the moisture equivalent of the local soils, hygroscopic determinations of the seeds in the vacuum driers, and for the various soil types which were so kindly sent to me. I desire also to express my appreciation of the generous way in which the Hull Botanical Laboratory has provided all needed facilities for the work, and especially my indebtedness and gratitude to Dr. WILLIAM CROCKER for much helpful advice and encouragement received during the progress of the work.

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A REPORT ON SOME ALLOCTHONOUS PEAT DEPOSITS OF FLORIDA[1]

PART I: TOPOGRAPHICAL

CARL C. FORSAITH

(WITH DIAGRAM)

As an introduction to the following report[2] on the allocthonous peat deposits of Florida, it seems advisable to discuss, very briefly, the geological and climatic conditions which obtain in this region, in so far as they bear a significant relation to the deposition of peat.

The entire state lies wholly within the Atlantic coastal plain, which extends, as an area of varying width, from Long Island to Texas, and represents a region of more or less recent origin, generally flat or rolling in character, with little or no evidence of folding or faulting. A stratum of Vicksburg or later limestone forms a continuous deposit which is now covered with Pliocene clay or Pleistocene sand, the latter being predominant at the surface (4). The gently rolling country forms numerous depressions which are below the level of the water table, and consequently contain a permanent water supply in the form of lakes. In the case of depressions above this water table, or not sufficiently far below it to form lakes by the above-mentioned plan, the limestone may be locally dissolved so as to form an outlet for the continuous underground water system, and in the manner of a natural artesian well a body of water is maintained to which there is no apparent external inlet. This condition is illustrated by the well known Silver Springs near Ocala. There are many other instances, also, where the limestone strata may have been locally dissolved by the action of surface or underground water

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to such an extent that it is not able to support the superincumbent sand or clay, which, falling, leaves a bowl-shaped valley. Depressions thus formed may contain more or less permanent ponds, known as "lime sinks," which are often important as sources of peat. Examples of this type of lake production will be mentioned in the later discussion.

Reference to any good map of the United States will show that there are more lakes in Florida than in the remainder of the coastal plain, the larger number of which, curiously enough, occur in the higher or central regions of the state. These lakes vary in many respects, but may conveniently be divided into 5 classes, as follows: large deep and large shallow lakes of irregular outline; small deep and small shallow lakes of more or less circular outline, and filled lakes which may present an outline characteristic of either of the two previously mentioned types. There is a great variation in size, from Okechobee, which is the largest lake wholly in the United States except Lake Michigan, and Lake Apopka, with its 62 miles of coast line, to those which are only a few rods in diameter.

The semitropical climate of Florida is very favorable for the growth of peat-forming plants. There is an average temperature of 72° C., with a more or less uniform variation from January with a mean of 58° C. to that of 83° C. in July. The average rainfall is about 49.6 inches per annum, the greater part of which is precipitated during the warmer months. This condition compensates for the loss by evaporation during the summer season, and preserves a more constant level for the lakes than would be possible if the greater precipitation occurred within the cooler season of the year. The texture of the soil, also, is very favorable for the formation and stability of lakes, since the porous coastal plain sand at once absorbs the greater portion of the rainfall, which gradually seeks the lower levels to reappear as permanent bodies of water.

The object of the present investigations is to determine the relative amounts of allocthonous peat (that type of peat which has been deposited by a gradual accumulation of floated, drifted, and wind-blown vegetable material in permanent and more or less quiet bodies of open water), and of autochthonous peat (that type of peat which represents the amassing of successive generations of plants in the presence of a more or less constant, but stagnant and concealed, water supply, permitting the growth of peat-forming plants *in situ*). The methods for determining the relative distribution of these two forms of peat are those which were devised by DAVIS (1). Samples were taken with a sounding instrument, the Eberbach probe, invented by DAVIS, at one foot intervals, in sufficiently numerous localities in the deposits to show the average conditions. The samples were forced into cloth sacks, after a superficial study of the gross characters, and stored for subsequent investigation in water rendered antiseptic with carbolic acid or formalin. A study of the gross characters of the material is generally sufficient to determine the method of deposition, and reference to the microscopic results will be made only where such seems necessary for greater accuracy. These investigations have led to the same conclusions that DAVIS (2) reached in regard to allocthonous peat deposits in general in the United States, which he has stated as follows: "At the present time peat deposits of this type (lacustrine) are numerically more important than any other in regions where peat formation is most common."

In addition to the different types of lakes just described, there are other areas where peat may be found, namely, swamps, river estuaries, etc. Examples of these localities will be mentioned later.

Since the types of peat deposition fall so conveniently into two classes, they will be discussed separately, with special reference to the gross characters of the samples. Autochthonous peat (*in situ*) may be very varied, both in respect to composition and gross appearance, depending upon the species of plants which enter into its formation and the amount of oxidation that has taken place. For example, in those deposits which have been formed by the growth of *Cladium*, *Sagittaria*, Cyperaceae, etc., or, to be more general, where the peat is entirely herbaceous in texture, the samples appear as fibrous, light brown, spongy material in which the individual parts of the plants can be distinctly seen and separated. On the other hand, where the quality of the peat is of a woody nature, tests show a deep brown granular substance, in which there is much fine amorphous material intermixed with the more or less decayed woody substance. This granular property may be taken as evidence of the accumulation of *Taxodium*, *Ilex*, *Magnolia*, etc., which plants occur only in those areas which have become somewhat drained, thus permitting decay to act more readily than in the case of the more herbaceous forms. This condition naturally results in a greater reduction, and consequently a more amorphous content. It seems necessary to add that in the apparent *in situ* herbaceous peat there is much evidence of drifted material revealed by the microscope, which naturally places the major part of the saw grass (*Cladium*) and other marshes under the head of allocthonous rather than of autochthonous formations, with the exception of the upper two or three feet of the strata. In speaking of *in situ* herbaceous peat in the later paragraphs, therefore, reference is made only to the uppermost layers, and not to the deposit in general.

Apparently these two characteristics of herbaceous and woody autochthonous peat formations are owing to the natural sequence of their occurrence, for it is a recognized fact that when the water level becomes sufficiently low to permit the growth of the higher plants, those of a herbaceous variety are the first to appear, which naturally accounts for the presence of the grassy peats below the more woody species where both are present in the same region. These two characters also show another distinct difference in respect to the amount of decay that has taken place, resulting entirely from the presence or absence of a permanent water supply. In the herbaceous peats there occurs little evidence of decay because of the presence of a constant water covering, as well as natural plant acids which prohibit the oxidation of the air and the destructive action of fungi, thus preserving its spongy, fibrous nature. When, however, the continual accumulation of these plants has raised the mass to such a height that it is above water for a part of the year at least, the monocotyledonous plants give place to more woody dicotyledonous species, which build up a stratum of a granular quality. This granular property is due to the decayed state of the amassed vegetation, brought about by the action of the air and the enzymes of bacteria, which are permitted to work on account of a lack of permanent protection by water. The less resistant plant organs are consequently reduced to a structureless mass, together with a partial decay of the more resistant elements, thus presenting samples of a rusty brown plastic property, in which, however, portions of decayed wood, cuticular parts, and stigmarioid roots can still be seen.

Although these two types of woody and herbaceous peat appear to be distinct, they have been classed under the same head, owing to a similarity in origin, and the apparent difference is due only to the nature of the included plants and the amount of comminution that has taken place.

In contrast to this formation there is the lake or allocthonous peat, which occurs as a layer of finely divided plant material as well as entirely microscopic organisms on the floors of permanent and deep bodies of fresh water. This type owes its existence to the action of winds, currents, and sedimentation. Although the deep lake formed deposits bear a strong superficial resemblance to the much decayed woody peat, with the exception of a habitual absence of a granular content, they are in fact very dissimilar, since the deep brown, plastic character of uniformly fine texture does not represent the product of comminution of grosser parts of plants, resulting from oxidation and destruction by fungi, but rather the accumulation of originally minute organic material which has not suffered decay, owing to a permanent saturation.

Like *in situ* peat, lake formations may vary much in color. Those samples which are mostly organic in composition present a deep brown, uniformly fine appearance. Those, on the contrary, in which there is a large inorganic content may be red, pink, or gray. These latter colors are due to a more or less abundant silt, shells of infusoria, etc., which produce an admixture with the sedimentary, drift, and wind-blown material, imparting to it, where predominant, their characteristic hue.

As the writer has previously stated, the aim of this paper is to supply a brief account of the regions which were visited and a short description of the samples of peat collected in so far as they bear a significant relation to the methods of peat deposition in a semitropical climate. Reference to later microscopic investigations of the material will be made only where such seems necessary in order to form an adequate opinion as to the plan of accumulation in a special deposit. The general analysis of Florida peat, together with samples from Canada, New Hampshire, and Massachusetts, will be reserved for a later paper on the relation of the present lacustrine deposits to the formation of coal. The regions chosen for investigation were those which presented typical conditions in respect to topography and the flora now found upon the shores; an estimate of the extent of the several deposits is likewise included where such is possible.

The first deposit examined was at Lake Newnan, Alachua County, which represents a large deep lake with an area of about 16 sq. m. This entire body of water is surrounded by a sand plain bearing a scattered upland flora, which extends to the very shore, except at the southern end, where there is more or less of a marshy border. *Taxodium*, *Nyssa*, *Magnolia*, etc., grow abundantly on the banks, and there is a fringe of species of the Nymphaeaceae, *Sagittaria*, *Piaropus* (*Pontederia*) *crassipes*, and other deep water plants which extend several feet into the lake.

The lacustrine peat deposit was tested in 18 localities (diagram, 1–18), and samples were taken at one foot intervals which show the following characters: Those from the bottom are nearly black or deep brown in spite of a large admixture of sand, but there appears no coarse organic material except close to the shores, where the *Piaropus crassipes* has become a part of the mass. Above this foot of more or less gritty material there occurs a rusty brown, uniformly fine substance, becoming less compact and lighter in color near the top, and, as usual in such deposits, there is a characteristic absence of coarse material, except near the shores, where there is an addition of drifted portions of aquatic plants.

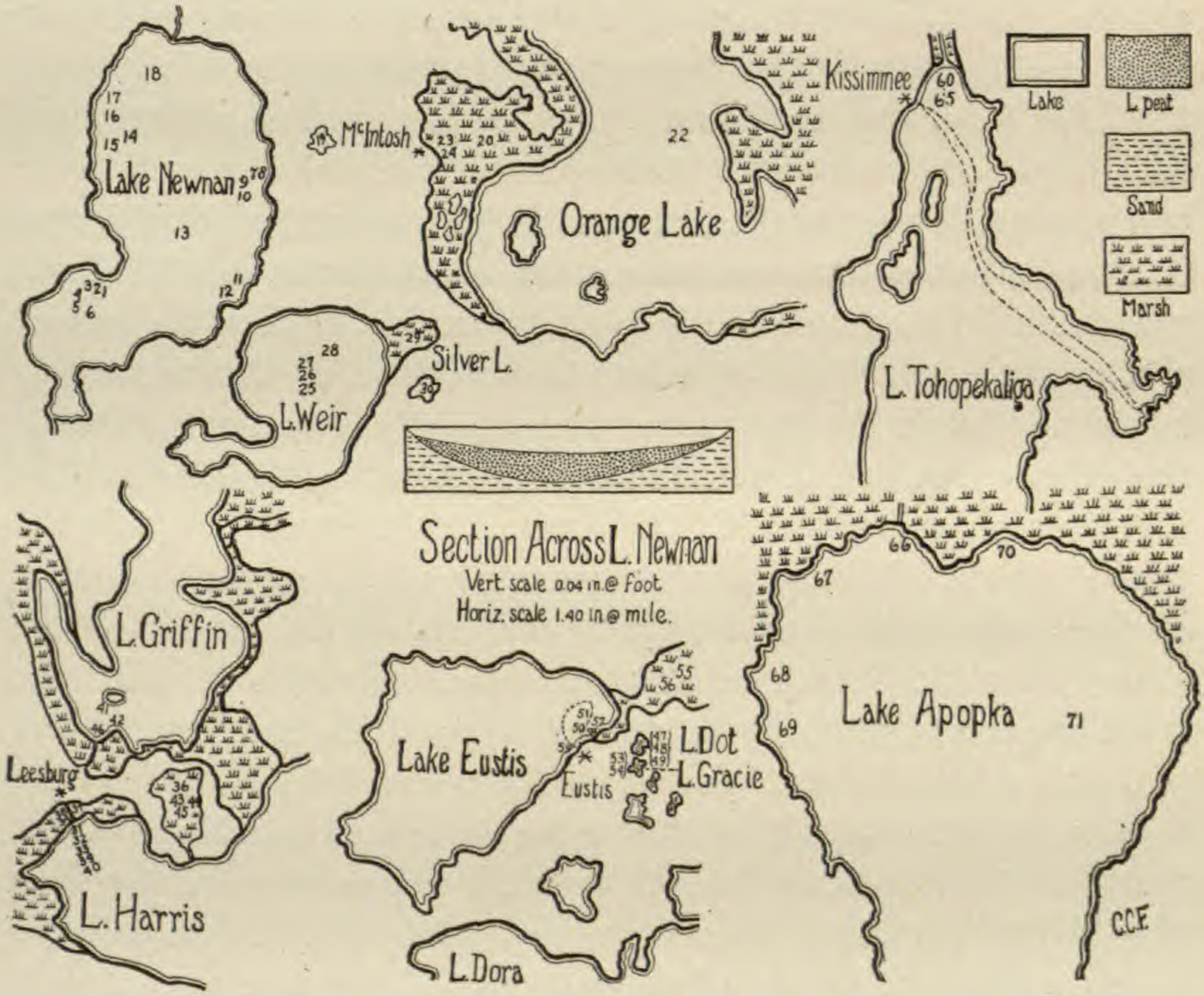
Some idea of the extent of this organic stratum can be obtained from a study of the cross-section (diagram), which was drawn to scale from the results of soundings in the center of the lake and other localities of varying distances from the shore (diagram, 1–18), the results of which can be learned from an application of the accompanying scales. Assuming that these tests are representative for the entire deposit, it is evident that there is a continuous layer of aquatic peat of varying depth extending over the entire floor of the lake, with the exception of the first few yards offshore.

Between Gainesville and Lake Newnan there is a hardwood and cypress swamp, representing the typical condition of fallen tangled logs, intermingled with finer plant débris. Upon investigation there appears only a few inches of peatlike material, which may be better characterized as humus, owing to the almost complete decay of the included vegetation. This type of swamp deposit proves to be very typical, and when compared with more aquatic formations is not of special importance from the numerical and quantitative standpoint.

The next investigations were made on a filled lake 2 miles west of McIntosh, in Marion County (diagram, 19). This represents a small shallow lake which has become entirely filled with herbaceous peat, composed of Cyperaceae, *Solidago*, *Eupatorium*, Gramineae, etc. The 10 samples at one foot intervals show a light brown fibrous material in which the individual plants can be seen distinctly. Upon a later microscopic examination it became evident that the lower two-thirds of the deposit is of allocthonous origin, a condition indicated by the presence of sponge spicules, as well as other evidences of drifted and sedimentary material which must have been laid down in a more or less permanent open pond. The upper stratum, however, has become reduced to a deep brown amorphous mass through the action of the air and fungi, following artificial drainage 25 years ago.

Orange Lake, as can be seen from the accompanying diagram, is bordered by a broad marsh on the western side. According to the reports of the inhabitants, the area of this marsh was used for cultivation 30 years ago, at which time it was inundated, and is now constantly covered with water. Species of the Nymphaeaceae, *Cladium*, *Sagittaria*, etc., grow there in profusion. A sample taken about a mile from shore (diagram, 20) presents a coarse brown fibrous character with the usual evidences of allocthonous material. Since this layer, 6 inches in depth, has been formed during the past 30 years, it may present some evidence as to the rapidity of the accumulation of fibrous peat, which would be at the rate of an inch in 5 years. Obviously, however, more data would be necessary to form a definite opinion concerning the building up of such deposits.

In the center of the lake (diagram, 22) a series of samples indicates a deposit 3 feet in depth, and tests in other parts show this to be a more or less uniform measure. In character the samples are very similar to those found in Lake Newnan. At the bottom there is a mixture of sand with the rusty brown fine material, with the usual absence of coarse fibrous matter, which generally occurs only near the shores. The upper portion differs from this only in a



A diagram to indicate the lakes mentioned in the discussion, each lake represented by a diagram enlarged from the map included in MATSON and SANFORD'S report (4), drawn to scale as accurately as the means at hand would allow; the small figures in the diagram represent the locality of each series of samples named in this paper, the relative position of which can be learned by an application of the scale in miles; the cross-section of Lake Newnan shows the average depth of the allocthonous peat deposit in relation to the breadth and deepness of the lake.

lighter coloration and less firm texture, and naturally enough without an admixture of sand.

On the shores at McIntosh there occurs a deposit, such as is often found on the shores of lakes, very much resembling lacustrine peat, as it presents a fine black structure, but it should not be confused with lake formations proper, for the reason that this condition is owing to the result of the action of the air and fungi on coarse fibrous material during periods of drought.

Lake Weir, Marion County, may be classified as a large deep lake, bordered by hills with their upland flora, which enter into the composition of peat in the form of wind-blown material only, that is, pine and angiospermous pollen, strips of epidermis, and deciduous fragments. The shores are of a sandy nature without the border of *Castalia*, etc., which is very characteristic of the previously mentioned lakes. Owing to the deepness of the water (30 feet in the central portion), it was difficult to determine the depth of the deposit with accuracy with the apparatus at hand. With what tests it was possible to make (diagram, 25–28), however, it became evident that there is no lacustrine material of an organic nature nearer than one-fourth mile from shore, but beyond this limit there appears a gradually increasing layer which is at least 5 feet in depth in the center of the lake.

A series of samples from this region differ very much from those previously observed, as those from the upper portion show a deep gray coloration which gives place to a pink below. There is no admixture of sand in any of the samples; all are fine, firm, and of a uniform texture. This gray and pink hue is apparently owing to the presence of an inorganic silt, shells, and crustacea, sufficient in amount to obscure the characteristic color of the normal allocthonous peat. In order to be more certain that this material is not entirely inorganic in nature, a microscopic test was made which showed, in addition to a large amount of silt and shell formations, sponge spicules, diatoms, plant fragments, pollen, and spores.

Since there was a possibility that a marsh, apparently a former arm of the lake, just north of the town of East Lake (diagram, 29), might show evidences of a transition of lacustrine to *in situ* peat, probings were made, which showed the deposit to be of a uniformly fibrous nature. The samples reveal the fact that the stratum is generally herbaceous and has been formed by the gradual accumulation of the plants which now grow upon the surface, among which Cyperaceae, *Solidago*, *Eupatorium*, etc., are the most abundant. The lower portion, however, showed evidences of an allocthonous origin on later microscopic examination.

Silver Lake, a small circular lake one mile east of the previously mentioned town, presents external characters very similar to those already described for Lake Weir. Many tests proved that the floor is of a uniformly sandy nature, with a general absence of allocthonous formation except a very shallow and gritty layer in a sheltered cove on the eastern side (diagram, 30).

Lake Harris, in Lake County, was visited next. This represents a large deep lake of irregular outline, bordered in many places by a broad saw grass (*Cladium*) marsh, as indicated by the shaded portion of the accompanying diagram. In many places there is a dense cypress swamp between this marsh and the rolling upland country, which gives place to hard woods where the shores are too steep to allow the growth of *Cladium* and other marsh-forming plants. Where the water is too deep to permit the growth of these species there occurs a fringe of *Castalia*, *Nymphaea*, etc., generally in more or less of a floating condition.

One-eighth of a mile from shore (diagram, 31) a series of tests showed some very interesting characters. The bottom layer of the deposit presents samples of a pinkish color, owing to the presence of silt, infusoria, univalvate shells, etc. The substance is of a firm, plastic nature, with the usual absence of coarse material, as was revealed by a microscopic test by means of which much fine plant material was observed. In addition to this fine material there appears a variety of univalvate shells, which are apparently quite characteristic of this region, since they were also found in material from Lake Eustis. There has been no opportunity to have the species of these shells determined, but there is a possibility that they are very similar to those which HARPER (3) mentions as identified by BRYANT WALKER, of Detroit, Michigan. In this report there are 25 species named, among which the genera *Amnicola* and *Planorbis* supply the most species. These shells have a tendency to disappear in the layer above the pink formation, and although this stratum is very similar to the one just described, there occurs a change from the salmon to a decidedly reddish hue, which in turn gives place to the rusty brown plastic peat already described. A striking uniformity in the several layers appears out to about three-fourths of a mile from shore, where there occurs a gray formation below the pink, an exactly opposite sequence from that found in Lake Weir. The several probings (diagram, 31, 32, 33, 39, and 40) showed that this laminated deposit varies in depth from a few inches at the border of the marsh to 9 feet a mile off shore, and when, too, the large area of this lake is taken into consideration, it is evident that, providing conditions similar to those found in Lake Newnan obtain (diagram, cross-section, and description above), there must be an enormous amount of allocthonous peat in Lake Harris.

Lake Griffin, just north of Leesburg, shows external conditions very similar to those already described for Lake Harris. Between the broad saw grass marsh and the upland pine plains there appears a dense cypress swamp which occasionally gives place to a dense growth of hardwood trees, among which *Magnolia glauca* and *Nyssa biflora* are the most abundant. There also appears the usual fringe of floating *Castalia*. Tests were made about a mile from shore (diagram, 41) near a small island, where the floor proved to be of a sandy nature, a condition very habitual for shallow water lakes, owing to a more or less constant agitation by waves. Soundings near the shore of the mainland show a shallow deposit of allocthonous peat, which gradually grows deeper toward the border of the marsh. A series of samples taken just outside the range of the deep water plants presents the following characters (diagram, 42 and 46). The deposit, unlike that found in Lake Weir and Lake Harris, is of a uniform deep brown quality, except for a stratum 2 feet from the bottom, in which the material is deep red, very loose in texture, and showing a tendency to break into a crumbling mass, in contrast to the more plastic claylike structure so commonly found in such deposits. The portion of the allocthonous layer above this red formation presents the usual deep brown plastic samples normally present in the upper strata of lacustrine peat.

Since the saw grass marshes were so abundant in this region, a description of the one bordering Lake Harris (diagram, 34, 37, and 38) may be of interest. The entire surface is now covered by an almost impenetrable growth of *Cladium effusum* (saw grass), *Sagittaria lancifolia*, *Spartina Bakeri*, as well as other marsh plants, which has gradually built up a deposit of light brown fibrous peat, which at the present time has attained a depth of about 10 feet. The samples show a striking uniformity in the lower 8 feet of the stratum, and the presence of sponge spicules, pollen, sporangia, diatoms, etc., show it conclusively to be of allocthonous origin. Owing to permanent saturation as well as natural acidity, the lower portion has not suffered decay, as can be seen by the spongy fibrous character of the peat; but, on the other hand, the upper 2 feet show the result of decomposition, for the reason that there has been a sufficient accumulation of plant material to raise this part above the low water mark, permitting decay to take place. Natural drainage has favored oxidation and comminution to such an extent that the original fibrous mass has been reduced to a deep brown, finely grained substance in which only the more resistant parts of the plants appear, imbedded in an amorphous matrix of the less resistant, and consequently more decayed, portions of the included vegetation.

Localities 36, 43, 44, and 45 (diagram, north of Lake Harris) represent a series of samples from a filled lake. Apparently, as a study of the material and the natural topography of the region will show, this was at one time either a separate lake or an arm of Lake Harris, which has since been filled by a gradual accumulation of plant material representative of both allocthonous and autocthonous forms of peat deposition. The surface, where it has not been disturbed, is now covered by a dense growth of *Magnolia glauca*, *Nyssa biflora*, *Pinus Elliottii*, etc., and in those portions where the growth has been artificially cleared species of Cyperaceae, Gramineae, Compositae, and *Eupatorium capillifolium* grow in profusion. The upper foot of this bed is in the form of a firm, black, consistent mass, owing to an almost complete decay of the included plants, as a result of artificial drainage. This upper layer might be considered as the initial stage in the formation of humus. Below this almost completely broken down stratum there is present a uniformly fine deposit of a decidedly woody nature, which appears as a deep brown granular accumulation of softened coniferous débris, nearly or entirely reduced to a structureless condition. Below this woody deposit there is quite an abrupt transition to a light brown herbaceous peat of the saw grass variety, similar to that described for the marshes on the shores of Lake Harris (diagram, 34, 37, and 38). This coarse undecayed deposit is underlaid by a fine, deep brown lacustrine formation and the absence of any coarse material, together with the unaltered condition of the samples (determined by later microscopic investigations), would indicate that this stratum had been amassed in the bottom of a permanent and deep body of open water by an accumulation of drifted and wind-blown plant material. At the bottom there appears a deposit of bluish gray clay.

From a study of the samples it is apparent that at the time when the lower 6 feet of fine allocthonous peat had been formed the amassed vegetation, as drifted and wind-blown material, had reached a sufficient height to permit the growth of herbaceous water-loving plants (a condition parallel to that represented by the constantly extending marshes on the shores of the adjoining and still existing lakes). When these plants had formed 2 feet of fibrous material, corresponding to the saw grass stage, another change took place, and this type gave way to a more woody growth. This last transition was owing to the fact that the allocthonous variety had so accumulated that the very upper portion of the deposit was above water for a part of the year, thus bringing about a condition favorable to the growth of trees. These gradually drove out the more herbaceous species, and, by the amassing of successive generations of woody plants, slowly built up a layer of autochthonous origin.

The next lake visited was Lake Dot, a small deep lake one-half mile east of Eustis, Lake County (diagram, 47-49). This body of water is situated in a circular depression in the form of a large bowl 70–80 feet deep and 200 yards broad, which naturally leads to the conclusion that it is of "lime sink" origin, formed by the gradual solution of the underground limestone. Although the lake is small in diameter, the water level is permanent and deep, thus affording excellent opportunity for the accumulation of allocthonous peat. The first series of samples were taken at a point about 100 yards from shore, where the deposit proved to be 10 feet in depth and situated under 8 feet of water. The bottom samples present a deep brown plastic appearance, in which there is no coarse material with the exception of a slight admixture of sand, which disappears a few inches from the floor. There occurs an exception to this uniformly fine plastic condition 2 feet from the base in the form of a light brown herbaceous peat 2 feet in depth. Apparently, when the character of the formation of the lake is considered, this sudden variation in the composition of the peat was caused by the opening of an underground outlet, which allowed the water to escape and rendered the lake sufficiently shallow to permit the growth of aquatic marsh plants. This is quite a common occurrence in this region, of which condition the Alachua sink (locally known as Paynes Prairie) near Gainesville furnishes a good example. To quote directly (4, p. 27):

> The Alachua sink is important because it illustrates some of the changes which sink holes undergo. In the early days of the state this sink, which receives the drainage of a large stream crossing Paynes Prairie, appears to have been in about the same condition as it is today; later, owing to the closing of the outlet, perhaps by logs and other rubbish, a large lake was formed. About 1891 the sink reopened and the basin was drained, effectively ending the steamboat traffic there had developed on the lake.

To return to the discussion of Lake Dot, it is apparent that after 2 feet of this herbaceous peat had been formed the outlet was closed (possibly through the amassing of the vegetable material to such an extent that the exit was filled, and the seepage and rainwater were prevented from escaping), and the level of the water was raised to its present position. This second transition killed out the herbaceous species, which are not able to exist in more than 10–12 feet of water (1). This condition is also emphasized by a return of the fine deep brown lake peat above this more fibrous stratum.

Lake Eustis, a large deep lake just west of the town of the same name, furnishes some interesting features. The shores are generally sandy with the exception of an occasional cypress swamp, but the broad saw grass marshes so commonly encountered around the lakes near Leesburg were generally absent. The northern portion was first examined, but no material was found, because the shallow nature of the lake in this region had prevented the accumulation of allocthonous deposits. A series of samples (diagram, 50, 51, 52, 57, 58, and 59) show the general character of the material near the shore. The basal stratum presents black, fine specimens of a decidedly sandy and diatomaceous nature, so commonly encountered in the lower layers of lacustrine formations. This black gritty mass soon gives place to a deep gray lamina of similar texture. The only coarse débris found in this lake was an abundance of univalvate shells of the same species as those mentioned in the discussion of the samples from Lake Harris. In this bed of peat there appears no transition from gray to pink, as is the case in the adjacent lakes, but the gray stratum is overlaid by a deep brown, fine, plastic deposit without the intervention of other formations.

One and one-half miles east of Eustis there is a bog which may be classified as a small filled lake (diagram, 55 and 56). In gross characters this bog furnishes strata similar to those already described for localities 34, 37, and 38, and consequently may be considered as representative of like methods of accumulation and as having passed through parallel cycles of sedimentation. The deposit is now covered by a dense growth of species of *Pinus*, Cyperaceae, Gramineae, Polypodiaceae, etc. Samples taken in the usual way show the following qualities: The upper layer presents a fine, deep brown, plastic character, in which there is much evidence of partially decayed lignified elements intermixed with the less resistant, and consequently more reduced, portions of fallen plants. This type of structure is very uniform to about 9 feet in depth, where it gives place, as usual, to a layer of herbaceous peat. The basal region furnishes specimens of a fine black nature in which there is a large admixture of sand. From results obtained by a study of the soundings it is evident that this bed has pursued a filling process similar to that mentioned in the discussion of the filled lake near Leesburg. The lowest allocthonous peat was formed in deep open water, which condition continued until a sufficient mass of this type had accumulated to render the water shallow enough for the growth of herbaceous water plants. These plants gradually crept in from the shores until the surface was covered, and the accumulation of the remains of successive generations of aquatica, together with the drifted and wind-blown material, eventually raised the amassed substance to such a height that it was above the low-water mark. This condition finally resulted in a change of flora to that of a more woody type, which has since formed the upper stratum of the deposit, the *Verländung* of German authors.

Lake Apopka, in Lake and Orange Counties, was the largest lake visited, and conditions were found to be very similar to those already described for Lake Harris. The entire northern shore is fringed by a broad saw grass marsh, 4 miles in breadth, with a limited region of cypress and hard woods between this and the higher land, and locally, where this woody growth extends to the water's edge. The western shore is bordered by pine hills which extend directly to the shore without the intervening marshy areas. A series of samples from the edge of the *Cladium* marsh (diagram, 66) show material of a herbaceous nature, which is evidently composed of amassed *Cladium effusum, Sagittaria lancifolia, Castalia odorata, Scirpus validus,* etc., light brown and very fibrous in character. The basal portion, however, was of an allocthonous nature, in the form of a deep black, finely grained deposit, gradually becoming coarser in the upper strata. This material is situated above a deep gray marl. On the western shore, just outside the floating *Castalia* and *Utricularia* (diagram, 68 and 69), there appears a layer of fine black lacustrine peat over 20 feet in depth, and, as in other regions where similar formations occur, there is no coarse material present, but all the specimens are uniformly deep brown and fine, with a habitual absence of coarse gray or pink material. The eastern shore presents, instead of the fine allocthonous peat, a partially decayed bed of *Taxodium* and other woods of so firm a nature that it was impossible to force the probing instrument through it, and consequently samples could be taken only from the top, each of which presents a very coarse woody character. The presence of this type of peat formation a mile from shore (diagram, 71) would indicate that at the present time this lake is much larger than it was at some earlier period when this deposit was formed, since the plants which have entered into its composition can only exist in a partially drained locality, while at present there are 10 feet of water above the stratum.

Many small shallow lakes at Zellwood, Eustis, and Orlando were visited, but each one showed a sandy floor and a characteristic absence of allocthonous material. This condition is owing, apparently, to the more or less intermittent nature of the lakes in which periods of drought permit the destructive action of the air and fungi to break down what little material there may have accumulated during periods of greater precipitation. Then, too, there is the action of the waves, which keeps the water in a state of agitation and prevents the gradual amassing of plant débris which may fall or float into the lake.

An estimate of the extent of the peat deposits in Lake and Orange Counties is manifestly impossible when one considers that there are over 2,000 lakes in this vicinity which vary in size from Apopka, with its more than 60 miles of coast line, to those which are only a few rods in diameter. In order to make determinations with accuracy, it would be necessary to resort to a careful survey of each one individually, which is beyond the scope of the present investigations. Consequently, no attempt is made to give even the probable extent further than to state that the allocthonous deposits are of greater numerical and quantitative importance than those of an *in situ* character, a condition true, also, for the other regions visited in Florida. Further, when one considers the probable amount of lacustrine peat, both from the standpoint of the number of lakes and the extent of the deposits as revealed by probings in the chosen regions, one must infer that the allocthonous type of peat in these 2,000 lakes and surrounding marshes must be enormous, and, when compared with those of an autocthonous nature, their relative superiority is very striking.

Lake Tohopikaliga, Osceola County, represents a large shallow lake with an area of about 30 sq. m. In following the dotted line in the plan of this lake there appears no place where the water is over 10 feet in depth (the observations were made in November, 1914, at a time when the water was at a low level), which accounts for the absence of any peat deposits, except in sheltered bayous, where the action of the waves has had no opportunity of prohibiting the accumulation of plant débris. Many of the sheltered portions, also, present no material of an organic nature, since the shallow character of the lake and the large fluctuation of the water level cause them to be dry for a part of the year, at which time the amassed material becomes desiccated and oxidized and floats away at the next inundation.

The only region where peat was collected at Tohopikaliga was in a sheltered bay formed by the broadening of the inflowing creek (diagram, 60–65), where there appears a very shallow layer of sandy, drifted, sedimentary, and wind-blown allocthonous peat. Thus it is very evident that this lake presents conditions very similar to those found in the small shallow lakes previously mentioned, in that the bottom is sandy and there is a general absence of vegetable accumulations.

At Pablo Creek, Duval County, there is a broad marsh with an area of over 4,000 acres, entirely covered by a uniformly bluish black peat to the depth of 10 feet. This deposit evidently owes its existence to the accumulation of river drift and wind-blown material, in addition to the constant amassing of fallen herbaceous plants. The first 8 feet present a series of samples of a bluish color, in which there appears no coarse material. One very noticeable feature in this locality is a strong sulphurous odor, which may be due to the presence of an abundance of *Beggiatoa*. Below this fine plastic mass there is a thin stratum of woody material, probably representative of an ancient inundated forest, which must have grown at some time when the water was at a much lower level than it is at present, and on its subsequent rising this flora gave place to more herbaceous and more water-loving species. The very basal layer consists of a shallow deposit of fine lacustrine peat, which has been formed upon the blue clay floor of this ancient lake. The material from this region is now used for agricultural purposes, after it has been artificially dried and mixed with commercial fertilizers, and the composition thus formed is placed on the market as "prepared humus," which should be very valuable when one considers the paucity of such natural formations in the upland portions of the state.

From a study of the peat deposits of Florida it is evident that lacustrine formations are much more numerous and much more abundant than those that have been laid down *in situ*, that is, in bogs, a situation apparently due to two causes. In the first place, there are many large lakes in the central portion of the state which contain much allocthonous material, and perhaps cover a much more extensive territory than that represented by the low marshy areas. Moreover, in addition to the relative differences in area, climatic conditions favor rapid decay of all the fallen vegetation unless it is protected by a constant water covering, as is the case of the lacustrine formations, in contrast to the less constant, and consequently more exposed, marshy areas.

It seems to be quite universal that the large or rather the deep lakes contain peat deposits, consisting of drifted, sedimentary, and wind-blown material. Moreover, although their deposition is due to the same agents, and has taken place under similar conditions, the individual accumulations of peat may present considerable variation in respect to color and texture. The variation in color is due to the relative amounts of organic and inorganic material present in the strata, for those which are mostly composed of plant débris present a uniformly deep brown coloration; but, on the other hand, where there is a large admixture of an inorganic nature, the samples may show a gray, pink, or red hue, depending upon the characteristic shade of the included silt, shells, etc. The texture is generally fine and plastic, with an absence of coarse material, except near the shore, where there is an addition of sediment resulting from water plants. The consistency may vary, however, depending entirely upon its position in the stratum.

When these lake-formed deposits have been built up to a point about 10 feet below the average water level, species of the Nymphaeaceae, *Cladium*, *Sagittaria*, etc., appear and gradually lay down a coarse, light brown fibrous peat of an allocthonous nature. In this type there is little evidence of decay until the deposit has reached such a level that the upper portion is above water for a part of the year, when the destructive action of the air and fungi has an opportunity to partially break down the successive generations of marsh-forming plants to a more or less amorphous mass of an autochthonous nature. When this stage is reached finally, the herbaceous plants give place to more woody species which, by a constant accumulation of deciduous matter, bring about the formation of a deep brown layer of granular texture, resulting from the only partial comminution of the resistant lignified elements.

All the previously mentioned stages in the formation of filled lakes were seen, and Lake Weir may be chosen as representative of the initial phase, since the floor and shores are uniformly sandy, with the exception of a deep gray allocthonous deposit in its central or deeper portion. The eastern side of Lake Eustis represents an example of the second stage in the process, since there is a more organic stratum above the gray formation. Lake Dot shows a still further transition in the form of a border of grass along the shore and the presence of a few of the deeper water plants growing on the surface of the brown lacustrine material. A more advanced degree of invasion may be seen in Lake Griffin, where there occurs a quantity of allocthonous peat covering the deeper part of the lake, and already the extending *Cladium*, *Sagittaria*, etc., have formed a broad surrounding marsh, the outer border of which is in turn bounded by a dense encroaching growth of more woody species. The final stage in the filling process is indicated by the bog near Leesburg, where the grassy plants have long since covered the entire area, and are now replaced by a dense growth of lowland trees. Conditions similar to these may be seen in many of the smaller deep lakes in this region.

Shallow lakes, whether large or small, show a general absence of peat formations, owing to their intermittent character, which, during periods of low water, permits the oxidation and comminution of what plant débris may have gathered; and the agitation by the waves prevents the accumulation of wind-blown and drifted material, as well as the sedimentation of water plants where such occur on the shores.

Conclusions

1. There are two main types of peat formation, namely, those deposits which are represented by a gradual amassing of drifted,

wind-blown, and sedimentary vegetable material in permanent, open, and quiet bodies of water (allocthonous), and those which have resulted from a gradual accumulation of successive generations of plants *in situ*, in the presence of more or less permanent but concealed water (autochthonous).

2. Allocthonous peat is characteristic of deep permanent lakes and the lower portions of marshes; autochthonous peat is characteristic of the upper portions of marshes, the upper strata of filled lakes, and swamps.

3. From what studies it was possible to make in regard to the average extent of these two types of peat formation it is evident that those of a lacustrine character (allocthonous) are of vastly greater numerical and quantitative importance than those of an autochthonous nature.

In conclusion, the writer wishes to express his most sincere thanks to Dr. C. A. DAVIS, of the Bureau of Mines, Washington, D.C., for very valuable advice in regard to the collection of peat, for a report on the uses of peat (1), and for letters of introduction; to Mr. O. F. BURGER, of the Bussey Institute of Harvard University, for letters of introduction to many of his friends in Florida; to Mrs. N. M. G. PRANGE, of Jacksonville, Florida, for valuable information in regard to the work; to Professor P. H. ROLFS, Director of the State Experiment Station at Gainesville, for information in regard to the locations of peat deposits. To Dr. E. C. JEFFREY, of Harvard University, I wish to express sincere thanks for advice.

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ON THE MECHANISM OF OXIDASE ACTION

G. B. REED

(WITH FOUR FIGURES)

The suggestion was made some years ago by BACH,[1] and later by KASTLE and LOEVENHART,[2] that the action of the oxidases depends on the fact that these bodies have a special aptitude for forming peroxides; that molecular oxygen in contact with complex autoxidizable substances combines with them to form unstable peroxides which in turn can give up their oxygen, in part or completely, to any oxidizable substance present in the cell. These changes may be represented diagrammatically as follows: (1) $A+O_2=AO_2$ (oxygenase); (2) $AO_2+2B=A+2BO$; or (3) $AO_2+B=AO+BO$; and (4) $AO_2+A=2AO$; where A is the autoxidizable substance (or substances) contained in the cell, which, by uniting with oxygen, forms the oxidase AO_2. The oxidase, in contact with an oxidizable substance B, may give up its oxygen in a variety of ways, depending upon conditions. All of the oxygen may be absorbed by B as in equation (2), in which case A is liberated and may begin the cycle again by combining with atmospheric oxygen; but if only a part of the oxygen of the oxidase is absorbed, as in equation (3), the resulting AO is assumed to be inert and incapable of regenerating new oxygenase. Finally, if no combustible substances are present, a part of the oxygen of the oxygenase may combine with a second A, which thus loses its regenerative power.

This conception of biological oxidation, originally based upon TRAUBE'S peroxide theory of oxidation, finds abundant support in many discoveries made during the last 10 years. TRAUBE was of the opinion that hydrogen peroxide is formed as a primary product in many, if not in all, oxidation processes, and that through its agency oxygen is transferred to the bodies finally undergoing combustion. ENGLER and his collaborators have been able to show

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[1] Compt. Rend. Acad. Sci. 124:951–954. 1897.

[2] Amer. Chem. Jour. 26:539–566. 1901.

that a peroxide of the substance undergoing oxidation is the usual intermediate product. Thus, when rubidium burns in air it is converted quantitatively into rubidium peroxide: $Rb+O_2=RbO_2$; and as BAEYER and VILLIGER[3] have shown, when benzaldehyde is exposed to oxygen, or to the air, it first absorbs oxygen to form benzoyl-hydrogen peroxide:

$$C_6H_5CHO+O_2=C_6H_5CO-O-OH.$$

benzaldehyde benzoyl-hydrogen peroxide

If allowed to remain in contact with water, the benzoyl-hydrogen peroxide is hydrolyzed with the production of benzoic acid and hydrogen peroxide:

$$C_6H_5CO-O-OH+HOH=C_6H_5COOH+H_2O_2.$$

benzoyl-hydrogen peroxide benzoic acid

If a second molecule of benzaldehyde comes in contact with the benzoyl-hydrogen peroxide, however, the former is oxidized and two molecules of the acid are produced:

$$C_6H_5CO-O-OH+C_6H_5CHO=2C_6H_5COOH.$$

benzoyl-hydrogen peroxide benzaldehyde benzoic acid

Finally, if an oxidizable substance, such as indigo, be present, it is oxidized by the benzoyl-hydrogen peroxide with the production of isatin and benzoic acid, thus:

$$C_{16}H_{10}N_2O_2+C_6H_5CO-O-H=2C_8H_5NO_2+2C_6H_5COOH.$$

indigo benzoyl-hydrogen peroxide isatin benzoic acid

Hence through the intermediary action of the benzoyl-hydrogen peroxide benzaldehyde is oxidized to benzoic acid; or a second oxidizable substance, which is not acted upon by atmospheric oxygen, may also be oxidized by the peroxide.

More direct evidence that the oxidations within the organism follow this peroxidase procedure has been given by BACH and CHODAT,[4] who were able to isolate a peroxide from the fresh juice of *Lathraea squamaria* at a time when it exhibited active oxidase action. After long standing, however, when the juice had lost its oxidase activity, it contained no peroxide. These investigators accordingly concluded that the oxidase itself is of a peroxide nature or contains a peroxide as one of its constituents. This conclusion

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[3] Ber. Deutsch. Chem. Gesells. 33:1569–1585. 1900.

[4] Ber. Deutsch. Chem. Gesells. 35:2466–2470. 1902.

was later[5] confirmed and extended by separating an oxidase into two constituents, a peroxide-like substance which they called oxygenase, and a body capable of activating this peroxide, namely, a peroxidase. By treating the fresh juice of *Lactarius vellereus* with 40 per cent alcohol, a precipitate was obtained which (in watery solutions) exhibited the properties of a very weak oxidase. The filtrate, however, when taken alone showed no oxidative activity, but was capable of imparting great activity to either hydrogen peroxide or to the precipitate which contained the oxygenase.

A considerable number of data indicate that all the so-called "direct oxidases" (that is, those which blue gum guaiac without the addition of hydrogen peroxide) consist of two such substances. BACH[6] has shown that the peroxidase separated from tyrosinase will activate either its own oxygenase or hydrogen peroxide. The writer has also obtained similar results with an oxidase of the laccase type from potatoes by the following method.

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Equal volumes of finely grated potato peelings and 55 per cent alcohol were mixed in a mortar, ground together until the potato was completely macerated, and then filtered. The filtrate was found to contain a peroxidase; while the residue, after washing with 55 per cent alcohol, extracting for several hours with an equal volume of water and finally filtering, gave an oxygenase solution, as the following experiments indicate.

The oxidizing activity of each of these fractions on pyrogallol, acting separately or in conjunction, was determined by mixing them in the proportions indicated in table I. After 4 hours the

TABLE I

cc. 5 per cent pyrogallol	cc. potato oxygenase	cc. potato peroxidase	cc. horse-radish peroxidase	cc. water	cc. 0.05M $KMnO_4$ required for titration
25	10			10	1.4
25		10		10	0.3
25			10	10	0.8
25	10	10			8.2
25	10		10		6.4

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[5] Ber. Deutsch. Chem. Gesells. **36**:600–605. 1903.

[6] Ber. Deutsch. Chem. Gesells. **39**:2126–2129, 3329–3331. 1906.

purpurogallin resulting from the oxidation of the pyrogallol was filtered off on asbestos under pressure, washed free of unoxidized pyrogallol, dissolved in 10 cc. of concentrated sulphuric acid, and the amount determined by titrating with 0.05M potassium permanganate. The results are indicated in the last column of table I.

The amount of pyrogallol oxidized in 4 hours by different combinations of potato and horse-radish peroxidase with potato oxygenase is indicated by the amount of $KMnO_4$ required for titration (the greater the amount of $KMnO_4$ required the greater the oxidation). It is evident that, like the *Lactarius* oxygenase, the potato oxygenase has some power to oxidize pyrogallol, but that this action is greatly accelerated by the peroxidase from the same source and to a less extent by that obtained from other sources (for example, from the horse-radish).

These results furnish additional evidence in support of the conclusion that all the *direct* oxidases consist of oxygenases plus peroxidases. Some tissues or extracts possess the *indirect* oxidase action (that is, they react only after the addition of a peroxide) and are capable of activating the oxygenases, hydrogen peroxide, or various organic peroxides, and it appears that they differ from those exhibiting *direct* action only in the absence of oxygenase or in lacking the ability to regenerate oxygenase.

BACH and CHODAT[7] consider that the oxygenases are formed as a result of an enzyme reaction or that they are themselves enzymes. MOORE and WHITLEY,[8] while agreeing with the former investigators as to the occurrence of oxygenases, consider them to be merely unstable peroxides resulting directly from contact of atmospheric oxygen with various substances in the cell, such as BAEYER and VILLIGER (*l.c.*) have shown that benzoyl-hydrogen peroxide will form. The solution of this problem awaits further investigation.

Whatever the origin of the oxygenase may be, it is clear that there is a substance, peroxidase, possessing enzyme properties, and capable of transferring oxygen from oxygenases (or from peroxides of known constitution) to oxidizable substances. The

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[7] Ber. Deutsch. Chem. Gesells. **36**:600–605. 1903.

[8] Biochem. Jour. **4**:136–167. 1909.

mechanism of this reaction, however, has been quite unknown. A suggestion in regard to it was made by BACH and CHODAT,[9] who found that an excess of either peroxidase or hydrogen peroxide had no effect on the rate at which pyrogallol was oxidized by hydrogen peroxides and horse-radish peroxidase. They concluded that the peroxide and peroxidase always take part in the reaction in constant proportions. BACH and CHODAT thus arrived at the conclusion which had previously been advanced by KASTLE and LOEVENHART (*l.c.*) from theoretical considerations, namely, that the peroxidase forms a definite compound with hydrogen peroxide, exhibiting more energetic oxidizing properties than the peroxide alone.

The writer has obtained results of a much more definite and conclusive character by experimenting with platinum black and by applying the suggestions gained in this way to the study of enzyme reactions found in living tissue. The present paper contains an account of the experiments with platinum black.

In connection with certain experiments it was observed that different samples of colloidal platinum and colloidal silver (prepared by passing a direct current between two electrodes of the metal under water) behaved differently toward solutions of gum guaiac. In some cases the guaiac was oxidized directly, in others only after the addition of a peroxide. This condition suggested to the writer that samples of colloidal metal might contain different proportions of oxygen, and, moreover, that the amount might be varied by suitable treatment. Subsequent experimentation has proved this supposition to be correct.

Instead of a solution of colloidal platinum, a platinum surface covered with the colloidal metal has been employed. A large platinum crucible with a surface of about 150 sq. cm. was platinized in the ordinary manner, by making it a cathode in a solution containing 2 gm. platinum chloride and 0.16 gm. lead acetate in 60 cc. of water, until its surface was uniformly coated with a black deposit of the colloidal metal. It was then subjected to active hydrogen by making it a cathode in a dilute solution of hydrochloric acid through which was passed a current of one ampere at 110 volt. To prevent the oxygen generated at the opposite pole from reaching

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[9] Ber. Deutsch. Chem. Gesells. **37**:1342–1348. 1904.

the colloidal platinum the electrolysis was carried out in two beakers connected by a siphon.

After this treatment with active hydrogen, the platinum crucible, when placed in a solution of gum guaiac[10] free from peroxide, or in a solution of potassium iodide, produced no oxidation. But after subjecting the colloidal metal to active oxygen (by making it the anode in the same electrolysis apparatus as used before) it produced rapid oxidation when placed in a solution of either gum guaiac or potassium iodide. This was shown by the appearance of a blue color in the former and after the addition of starch paste in the latter. Although this reaction took place rapidly, it was observed that only a very small amount of material was oxidized. This suggested that the amount of oxygen taken up by the platinum was sufficient for only a limited oxidation.

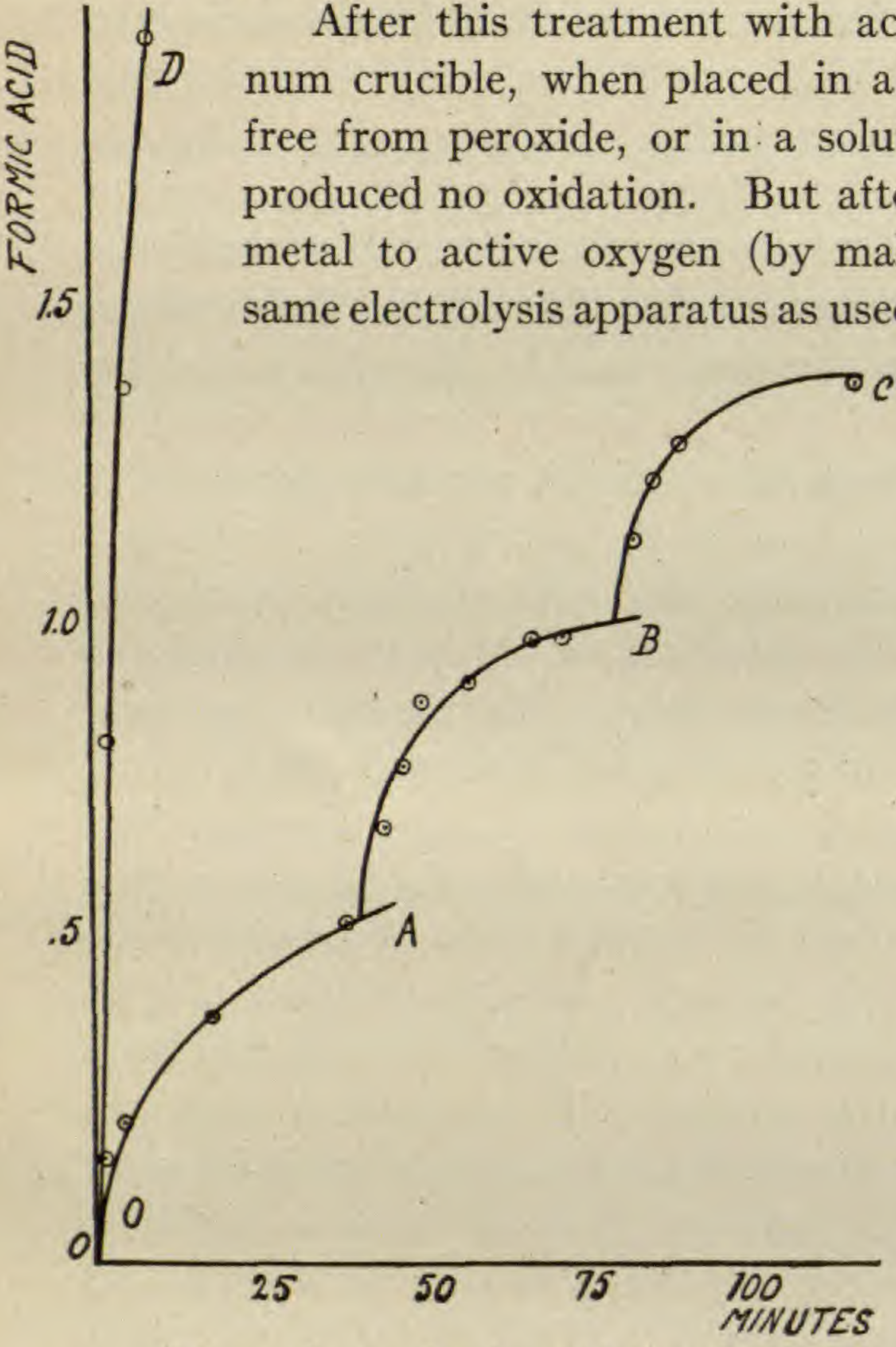

FIG. 1.—Curves showing oxidation of formaldehyde; lower curves *OA*, *AB*, *BC* represent successive oxidations by platinum black which had been charged with oxygen by making it an anode in dilute acid at the beginning of each operation; upper curve *OD* represents the oxidation by hydrogen peroxide in the presence of platinum black; ordinates represent amount of formic acid produced (expressed as the difference in the number of cc. 0.05M HCl required to neutralize the NaOH in 5 cc. of the mixture at the beginning of the experiment and after a part of it had been neutralized by the formic acid produced); abscissae represent time in minutes.

To determine whether this was true, quantitative experiments were undertaken. For this purpose the oxidation of formic aldehyde to formic acid suggested itself as a reaction, which is

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[10] It was shown by MOORE and WHITLEY (Biochem. Jour. 4:169) that ordinary alcoholic solutions of gum guaiac frequently contain traces of peroxide which may be removed by boiling the tincture with animal charcoal.

catalyzed by platinum black and which can be accurately and conveniently measured.

About 80 cc. of a solution containing 0.05M NaOH and approximately 0.3M formaldehyde was placed in an open beaker in a water bath maintained at a constant temperature of 30° C. The platinum crucible was freshly platinized and exposed to active oxygen (as previously described) for 5 minutes. It was then washed by rapidly dipping it into three changes of distilled water,[11] after which it was introduced into the solution of formaldehyde. The mixture was kept thoroughly stirred throughout the experiment, and at frequent intervals 2 cc. were removed and titrated with 0.05M HCl. This showed the amount of NaOH neutralized by the formic acid generated in the reaction. The results are expressed in the curve *OA*, fig. 1.

The amount of formic acid formed in this reaction is very small when compared with the amount of formic aldehyde present at the start. When equilibrium is reached, therefore, it must be the oxygen (presumably the oxygen that was combined with the platinum in the charging process), and not the aldehyde, that is used up. If this be true, by furnishing more oxygen combined with the platinum the reaction should proceed much farther. This condition was realized experimentally by recharging the platinum with oxygen.

After the first reaction between oxidized platinum and aldehyde had almost reached an equilibrium, the platinum electrode was removed from the solution and subjected to active oxygen as in the former case; it was then washed and returned to the same formaldehyde solution. Renewing the supply of oxygen on the platinum in this manner caused the formation of more formic acid. The results plotted in the curve *AB*, fig. 1, indicate that the reaction proceeded at approximately the same rate as with the first charge of oxygen. Repeating the operation a third time gave the similar results plotted in the curve *BC*, fig. 1.

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[11] As a check experiment, the platinum after this amount of washing was placed in 0.05M NaOH without formaldehyde; titration showed no decrease in alkalinity, hence the washing was sufficient to remove the very dilute acid of the solution in which it was oxidized.

It is evident that the oxygen concerned in this reaction is derived from the platinum, and that the more frequently the platinum is recharged with oxygen the higher will be the rate of formation of formic acid. It is of theoretical interest to determine whether the rate can thus be made to approximate that of the production of formic acid in the presence of hydrogen peroxide. The latter reaction was accordingly investigated.

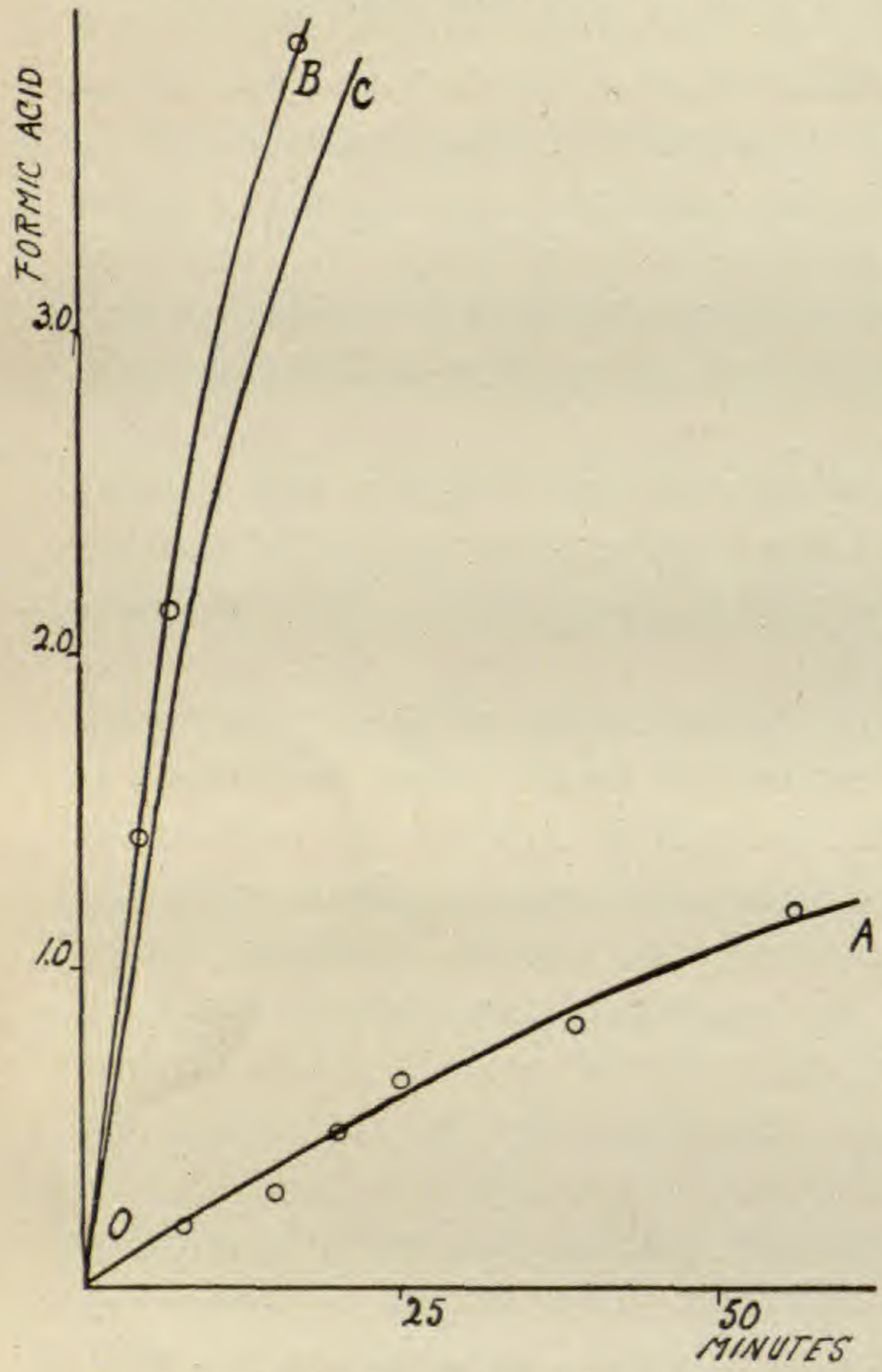

FIG. 2.—Curves of the rate of oxidation of formaldehyde by hydrogen peroxide: *OA* represents oxidation in absence of a catalyzer; *OB* represents oxidation in presence of colloidal platinum; *OC* represents activity of the catalyzer; ordinates represent amount of formic acid produced (stated as the difference in number of cc. 0.05M HCl required to neutralize the NaOH in 5 cc. of the mixture at the beginning of the experiment and after a part of it was neutralized by the formic acid produced); abscissae represent time in minutes.

Since hydrogen peroxide has considerable oxidizing action on the aldehyde in the absence of a catalyzer, it was necessary first of all to determine the effect of the colloidal platinum on the reaction. For this purpose the following method was adopted. To a solution containing the same concentration of formic aldehyde and sodium hydroxide as used in the previous experiment sufficient hydrogen peroxide was added to make the concentration 0.5M. The rate of oxidation of the formic aldehyde in the mixture (which was kept well stirred and at a constant temperature of 30° C.) was determined, as in the former case, by titrating samples at frequent intervals with HCl. The results are indicated by the curve *OA*, fig. 2. The freshly platinized platinum crucible was then placed in a similar mixture of formic aldehyde, alkali, and hydrogen peroxide, and the rate of oxidation under the new conditions determined as before. The results are shown in the curve *OB*, fig. 2. By subtracting the ordinates of the curve *OA* from the ordinates of *OB* we obtain the curve *OC*, which expresses the effect of the catalyzer. For subsequent comparison the curve *OC* is also plotted as curve *OD*, fig. 1.

As has been shown, the speed of the reaction between aldehyde and oxidized platinum, in the absence of any other source of oxygen, is a function of the amount of oxygen which can be furnished by the charged platinum; and it is evident from an inspection of the reaction curve *OA* of fig. 1 that by starting with a sufficiently high concentration of oxygen on the platinum the initial velocity could be made equal to the velocity of the catalyzed peroxide reaction represented by *OD*. As the curves indicate, the former reaction in the absence of a continual supply of oxygen slows down much more rapidly than the peroxide reaction; but it is clearly possible to prevent this by recharging the platinum with oxygen at sufficiently short intervals. It seems evident, therefore, that the hydrogen peroxide acts by recharging the platinum with oxygen as soon as a portion of the oxygen has been removed by the formaldehyde. This is of great theoretical interest as an explanation of the means by which the catalyzer produces its effect, that is, by combining with one or both of the reacting substances.

Measurement of the oxidation potential of this platinized surface when connected as a cathode through a formic aldehyde mixture similar to that previously used led to the same conclusions.[12] When freshly charged with oxygen the platinum exhibited a *high* potential, but on contact with the aldehyde solution this rapidly dropped to equilibrium at very nearly zero potential. In the presence of hydrogen peroxide and the aldehyde mixture,

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[12] Reed, G. B., Measurement of oxidation potential and its significance in the study of oxidases. Bot. Gaz. 61:523–527. *figs. 2.* 1916.

however, the oxidation potential did not fall so low, and it remained approximately constant until the peroxide was exhausted.[13]

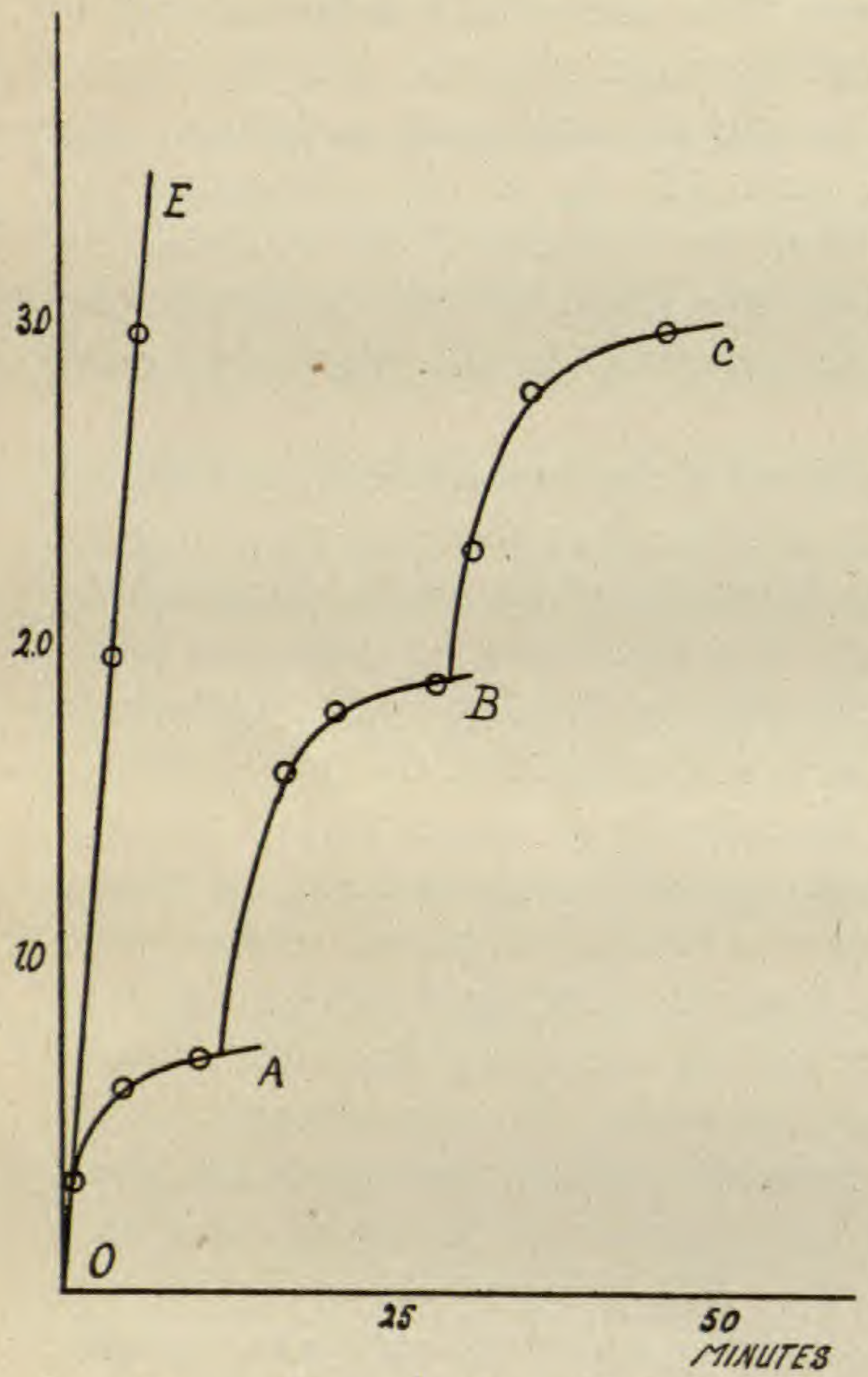

Fig. 3.—Curves showing oxidation of potassium iodide; lower curves *OA*, *AB*, *BC*, represent successive oxidations by platinum black which had been charged with oxygen at the beginning of each oxidation; curve *OE* represents oxidation by hydrogen peroxide in presence of colloidal platinum; ordinates represent drops of 0.01M $Na_2\ S_2\ O_3$ required to combine with the iodine in 5 cc. of the reaction mixture; abscissae represent time in minutes.

From these results it may be concluded that in the oxidation of formic aldehyde by hydrogen peroxide in the presence of platinum black two reactions are concerned: the platinum combines with oxygen from the hydrogen peroxide, as it combined with oxygen when subjected to anodic oxidation; this compound of platinum then gives up its oxygen to the formaldehyde, producing formic acid.

In a similar manner it was possible to show that the catalytic action of platinum black on the oxidation of potassium iodide by hydrogen peroxide was due to an intermediate platinum-oxygen compound. A freshly platinized platinum crucible, which had just been subjected to active oxygen in the manner described in the previous experiment, was

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[13] The oxidation potential in hydrogen peroxide is much lower than would be expected from its activity as an oxidizing agent (Lewis, Jour. Amer. Chem. Soc. 36: 1696), which is probably due to the fact that it may act as both an oxidizing and as a reducing agent.

placed in 100 cc. of a 0.2M solution of potassium iodide, which was maintained at a constant temperature of 20° C. Portions of 5 cc. were removed at frequent intervals and titrated with 0.01M sodium thiosulfate (with starch indicator) for the amount of iodine liberated in the oxidation. The curves *OA*, *AB*, *BC*, fig. 3, represent the velocity of oxidation of potassium iodide after successive chargings of the platinum with oxygen.

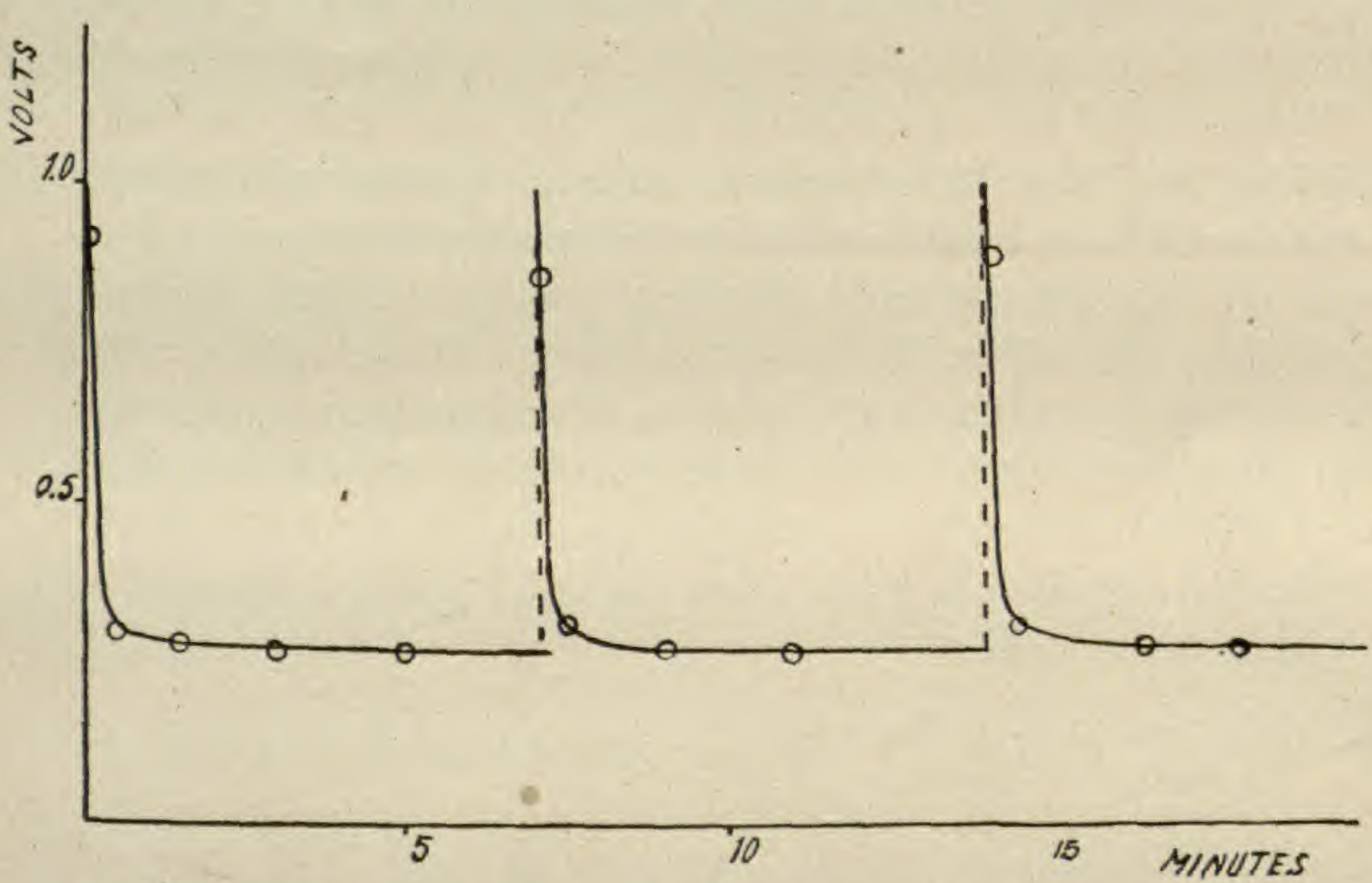

FIG. 4.—Curves showing fall of oxidation potential of colloidal platinum in 3 successive oxidations of a potassium iodide solution after the platinum had been charged with oxygen at an anode at the beginning of each oxidation; ordinates represent volts; abscissae represent time in minutes.

By connecting the platinum, as a cathode, to an apparatus for the measurement of the oxidation potential it was found that the rate at which the oxygen potential dropped, that is, the rate at which oxygen was given up by the platinum electrode in the potassium iodide solution, followed very nearly the velocity of the oxidation reaction. This may be seen by comparing the curves in fig. 4, showing the changes in the potential of the platinum after three successive chargings with oxygen, with the rates of oxidation produced by the platinum as plotted in fig. 3. It is evident that the drop in potential is somewhat more rapid than the oxidation as measured, but this is doubtless owing to the fact that the products of the reaction must diffuse from the spongy platinum into the solution before they can be detected by titration.

It seems evident, therefore, that in this reaction, as in the oxidation of formaldehyde, the platinum is capable of carrying oxygen into the solution, and it appears from fig. 3 that if the oxygen were supplied at sufficiently frequent intervals the reaction could be made to follow the curve *OE*, which represents the action of colloidal platinum in the oxidation of potassium iodide by hydrogen peroxide.

From these two reactions it may be concluded that when colloidal platinum is introduced into a mixture of hydrogen peroxide and an oxidizable substance the platinum takes up oxygen from the peroxide, thereby forming a more efficient oxidizing agent than the original hydrogen peroxide. The catalytic action of the platinum in this case, that is, its peroxidase action, therefore, depends upon its aptitude for forming unstable oxygen compounds when it is in contact with hydrogen peroxide.

Similar experiments with plant material will be reported on in a subsequent paper.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

THE TEMPERATURE COEFFICIENT OF THE DURATION OF LIFE OF CERAMIUM TENUISSIMUM

ARTHUR H. AYRES

Reaction velocities at various temperatures have become of much interest to biologists, on account of the possibility they present for the identification of certain physiological processes as chemical or physical, since it has been found that certain common physical and chemical reactions have rather characteristic temperature coefficients. Thus, if we suspect that a certain reaction is due to a chemical process, we can determine whether or not the velocity of the reaction at various temperatures is of the magnitude demanded by a chemical reaction. Since the temperature coefficients for all chemical reactions do not always have the same order of magnitude as demanded by the van't Hoff rule on the dependence of the rate of reaction of chemical processes upon temperature (9), the test is not wholly conclusive, but indicates rather a probable explanation of the phenomenon in question.

Animal physiologists in recent years have given much attention to temperature coefficients, but very little work has been done upon this subject by plant physiologists. CLAUSEN (2) was the first to show that the rate of evolution of carbon dioxide from seedlings and buds was about doubled with an increase of 10° C. Miss MATTHAEI (5) found that van't Hoff's rule applied to the fixation of carbon dioxide by leaves in sunlight and to the evolution of this gas by leaves in the dark. PRICE (7) determined the temperature coefficient for the opening of flower buds, and found it to have the magnitude demanded for a chemical reaction.

It will be seen that these experiments deal with growth phenomena. LOEB (4) was the first to call attention to the fact that the temperature coefficient of the duration of life of sea urchin eggs differs widely from that of their development, the temperature coefficient of development being 2.86 for a rise in temperature of 10° C., while that of duration of life is nearly 2 for a rise in temperature of 1° C. MOORE (6) found that the temperature coefficient of the duration of life of *Tubularia crocea* stems was of the same order of magnitude as that found by LOEB for sea urchin eggs, being about 2 for each degree centigrade rise in temperature. The results of these investigations on animals led GOODSPEED (**3**) to determine the temperature coefficient of the duration of life in barley, which was found to be 1.27 for an interval of 1° C., or about 11 for 10° C. This result is much lower than that found by LOEB for sea urchin eggs, and by MOORE for hydroid stems, and is much higher than the temperature coefficients determined for growth phenomena in plants.

The results obtained with barley involved the use of relatively high temperatures, and it was suggested to me that similar investigations upon the marine algae, involving a lower range of temperature, might yield data which could more properly be compared with the results obtained with simple animal organisms by LOEB, MOORE, and others. Another reason for using an alga is that we then deal with a much simpler structure than was used by GOODSPEED (*loc. cit.*), thus eliminating to a certain extent the possibility of unknown factors entering into the experiment.

Physiologists have recognized that the degree to which van't Hoff's rule applies to physiological processes is determined by the degree of uniformity in which experimental material is affected by the environmental changes. Thus, whenever it is possible, isolated cells are chosen for experimental purposes, a small energy change being the most influential cause of uniformity in physiological reactions (cf. BARRY **1**). In this connection it is to be noticed that the tissues used for observation in the present experiment were those in the apical region, and were thus embryonic in character; hence, the following observations are more nearly comparable with those of other investigators who have used embryonic animal cells or tissue in their investigations.

Of the material available, certain members of the Rhodophyceae were chosen, especially those species which are known to show a more or less definite death point by a rapid and distinct change of color. In a number of preliminary experiments the plant which passes commonly as the Pacific Coast form of *Ceramium tenuissimum* gave the sharpest death point color reaction, and for this reason was used exclusively.

The material was collected from floats in a tidal estuary of San Francisco Bay. The temperature of the water in which *Ceramium tenuissimum* occurs undoubtedly varies somewhat with the change of seasons, but during the winter the temperature is practically constant at 9–10° C. The plants to be brought into the laboratory were placed in jars of sea water at the place of collection. In the laboratory they were kept at a temperature of 12–14° C. by immersing the jars in a stream of running tap water. By replacing the sea water occasionally the material was kept growing normally in the laboratory for several days.

Two methods were employed for obtaining and maintaining the desired temperatures. In the first place, the temperatures were kept constant within an asbestos-lined hood by means of a double water bath heated by a Bunsen burner, which was automatically regulated by a mercury thermo-regulator. By placing covered finger bowls containing sea water on shelves in the hood above or around the water bath, a considerable number of constant temperatures was obtained. The second method consisted in placing a double bell jar filled with water over a heavy glass dish containing the material in sea water. This apparatus was then placed on top of a glass case which inclosed a 16-candle-power incandescent light. A free circulation of air maintained the temperatures constant to ±0.5°. The various temperatures desired were obtained by shifting the position of the bell jar and the dish with reference to the light. The results secured by these two methods of obtaining constant temperatures agreed so closely that both were employed.

A finger bowl, containing 100 cc. of sea water, covered to prevent evaporation, was brought to the desired temperature and maintained constant there for about 30 minutes; a number of plants were then transferred directly from the aquarium to the finger bowl. At intervals some of the material was removed from the finger bowl and transferred to a small quantity of sea water at the temperature of the laboratory. "Duration of life," as used in table I, was taken as the time for which a given temperature must act to cause a change in color of the apical cells of the branches from maroon, the normal color, to brick red color, which is characteristic of dead material. This change in color is due probably to a change in the permeability of the chromatophores, thus allowing the phycoerythrin to diffuse out into the cell contents. The larger cells of the plant show this diffusion of the pigment before smaller cells at the tip show any such change. The change in color is most evident after the plant has been left standing at room temperature for 6–10 hours after removal from the constant temperature chamber. Branches which remained the normal color after heating were found to proliferate and grow to some length when allowed to stand, while those which showed diffusion of the coloring matter from the chromatophores did not grow further. This fact justifies the use of the color reaction as an indicator of the duration of life. Table I expresses the average results of a number of experiments.

TABLE I

Temperature (centigrade)	Duration of life (minutes)	Mean temperature coefficient for 1° C.
28..............	300–340	320
		......1.21
29..............	240–285	262.5
		......1.12
30..............	210–255	232.5
		......1.16
31..............	185–215	200
		......1.15
32..............	160–185	172.5
		......2.15
33..............	75– 85	80
		......1.18
34..............	60– 75	67.5
		......1.42
35..............	40– 55	47.5
		......1.58
36..............	25– 35	30
		......2.14
37..............	12– 16	14
		......1.64
38..............	7– 10	8.5

Average temperature coefficient for 1° C., 1.47.

The temperature coefficient of the duration of life of *Ceramium tenuissimum* has been determined thus for the temperatures 28–38° C. inclusive, and found to average 1.47 for each degree centigrade rise in temperature. This average temperature coefficient corresponds closely with that found by GOODSPEED (1.27), whose experiment involved the use of much higher temperatures for barley grains, but is less than that found by LOEB for sea urchin eggs and by MOORE for hydroid stems. This result is of far too great a magnitude for any of the well known physical processes, whose temperature coefficients seldom reach a magnitude as great as 2 for a 10° C. interval (cf. SNYDER **8**).

Although there is some question as to the value of temperature coefficients as indicating the exact nature of physiological processes, it seems fitting to make this contribution to our knowledge of a subject which may sometime give us a better understanding of fundamental biological phenomena.

I wish to thank Professor SETCHELL and Dr. GOODSPEED of the botany department of the University of California for their continued interest and helpful criticism as this work has progressed.

UNIVERSITY OF CALIFORNIA

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BRIEFER ARTICLES

QUADRAT STUDIES IN A MOUNTAIN GRASSLAND

In connection with an extended study of dry grassland areas in the mountains of Colorado, the writer has examined a large number of meter square quadrats. A report[1] already published deals with 16 quadrats located at different stations in Boulder Park, Tolland, Colorado (altitude 8889 feet). In the present paper the vegetation is described for a series of 158 quadrats in the same park. These extend 138 m. in an east-west line, with an additional row of 20 running south from quadrat 45. The general slope is toward the west, with consequent exposure to prevailing winds.

Each quadrat was studied and recorded separately, according to the plan described in an earlier paper.[2] The records for all quadrats of each society were then added and averaged. Apparently this kind of work does not appeal to most ecologists, as I have found no published figures on xerophytic grasslands that could be used for comparison.

Six minor assemblages of plants are included in the quadrats studied. The list, together with the quadrat numbers, follows:

Xerophytic ridge (*Erigeron multifidus* society)	1–16
Xerophytic slope (*Muhlenbergia-Comandra* society)	37–76, 139–158
Xerophytic slope (*Muhlenbergia-Antennaria anaphaloides* society)	117–3
Xerophytic slope (*Muhlenbergia-Aragallus* society)	92–110
Hypoxerophytic slope (*Muhlenbergia-Danthonia* society)	32–36, 77–91, 127–138
Glacial sink	111–126

The systematic list which forms the chief part of this article is prepared from collections made in the midsummer of 1914; censuses were made at that time and verified in 1915. It will be seen that grasses form a large part of the vegetation, 18 species out of a total of 79. Sedges are not so important as is often the case in mountain grasslands or indeed as in other parts of the same park. There is an entire absence

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[1] Ramaley, F., The relative importance of different species in a mountain grassland. Bot. Gaz. **60**:154–157. 1915.

[2] ———, The amount of bare ground in some mountain grasslands. Bot. Gaz. **57**:526–528. 1914.

SYSTEMATIC LIST

	1 Erigeron multifidus (16 quadrats)	2 Muhlenbergia -Comandra (60 quadrats)	3 Muhlenbergia -Antennaria (15 quadrats)	4 Muhlenbergia -Aragallus (19 quadrats)	5 Muhlenbergia -Danthonia (32 quadrats)	6 Glacial sink (16 quadrats)	Average of 158 quadrats
Bare ground and rocks*	42.0	31.1	29.9	25.5	24.6	25.6	29.6
Parmelia molliuscula (lichen)*	1.0	1.0	0.6	1.0	0.5		0.8
Selaginella densa*	11.3	2.9	2.0	3.4	2.3	1.5	3.5
Agropyron violaceum*	0.8	0.8	1.3	0.5	1.0	2.2	1.0
Agrostis hiemalis					0.3	1.5	0.1
Avena americana*	0.4	0.9	1.0	0.6	0.2		0.6
Bromus Pumpellianus		1.5			0.5	1.2	0.8
Danthonia intermedia		0.1	0.2		0.2	9.2	0.1
Danthonia Parryi*	0.9	1.7	3.1	4.8	6.4	0.2	2.9
Festuca ingrata	1.0	2.3	0.7	1.6			1.2
Festuca saximontana*		5.4	11.1	1.6	11.9	7.8	5.9
Koeleria cristata*	5.4	3.8	5.0	6.7	4.6	0.5	4.3
Muhlenbergia gracilis*	3.4	11.4	0.6	14.8	8.6	11.8	9.8
Phleum alpinum					0.1	1.2	0.1
Poa pratensis and compressa						1.0	0.1
Poa crocata	0.4	0.9	1.2		0.4		0.6
Poa interior*	0.4	0.7	0.1	1.4	2.0		0.9
Poa rupicola	0.1	0.2	0.8	0.6			0.3
Poa subpurpurea					0.1		0.1
Stipa comata	0.7	2.3		0.1	0.1		1.0
Stipa viridula						3.8	0.4
Carex filifolia*	1.0	0.2	2.2	1.0	1.0		0.5
Carex obtusata				1.2	0.6		0.3
Carex siccata					0.1	0.4	0.1
Carex stenophylla*	4.6	1.0	0.3	3.9	1.6	0.1	1.5
Juncus balticus montanus*	0.7	0.7	0.6	0.6	0.8	1.8	1.0
Allium recurvatum	1.0						0.1
Comandra pallida	0.3	2.3	2.2				1.1
Eriogonum subalpinum		0.1				0.1	0.1
Eriogonum umbellatum		0.6		0.1			0.1

SYSTEMATIC LIST—*Continued*

	1 Erigeron Multifidus (16 quadrats)	2 Muhlenbergia -Comandra (60 quadrats)	3 Muhlenbergia -Antennaria (15 quadrats)	4 Muhlenbergia -Aragallus (19 quadrats)	5 Muhlenbergia -Danthonia (32 quadrats)	6 Glacial sink (16 quadrats)	Average of 158 quadrats
Polygonum Engelmanni	....	0.1	....	....	0.1	0.6	0.1
Chenopodium oblongifolium	....	0.1	....	....	....	....	0.1
Arenaria Fendleri*	1.3	2.0	1.5	1.4	1.0	0.1	1.5
Cerastium occidentale*	0.7	1.5	3.0	1.4	3.0	0.6	1.7
Silene Hallii*	....	0.2	0.3	0.2	0.8	0.1	0.1
Ranunculus cardiophyllus	....	....	....	0.1	....	0.1	0.1
Arabis divaricarpa	....	0.1	....	....	....	0.2	0.1
Erysimum Wheeleri	....	0.1	....	....	0.1	....	0.1
Thlaspi purpurascens	....	0.2	0.1	....	0.2	....	0.1
Sedum stenopetalum*	1.1	1.0	1.0	0.7	1.3	0.2	1.0
Dasiphora fruticosa*	0.2	1.3	3.1	0.1	0.2	0.6	0.9
Potentilla concinna*	1.3	0.1	0.8	0.1	0.1	....	0.2
Potentilla Hippiana*	0.1	0.1	0.3	0.7	0.4	0.6	0.3
Potentilla pennsylvanica strigosa	1.2	0.3	0.2	0.1	0.4	0.1	0.3
Potentilla pulcherrima	....	....	0.1	....	0.1	0.2	0.1
Sieversia ciliata	....	....	0.4	....	0.3	0.1	0.1
Aragallus deflexus	....	0.3	0.2	1.8	0.8	....	0.6
Aragallus Lambertii*	5.6	4.0	3.4	5.4	2.7	0.1	3.6
Aragallus Richardsonii	....	0.1	....	1.6	0.7	0.1	0.4
Thermopsis divaricarpa	0.1	....	....	....	....	....	0.1
Pseudocymopterus tenuifolius*	....	0.5	1.3	0.6	1.0	1.2	0.7
Vaccinium caespitosum	....	....	....	....	0.1	7.3	0.7
Androsace puberulenta*	....	0.2	0.3	0.2	0.2	0.6	0.2
Amarella plebeja	....	....	0.1	....	....	0.1	0.1
Dasystephana Parryi*	....	0.1	0.3	0.4	0.3	0.1	0.2
Mertensia Bakeri*	1.0	2.8	4.6	2.1	2.7	1.1	2.4
Orthocarpus luteus*	....	0.3	0.1	2.1	0.6	0.1	0.5
Pentstemon procerus*	....	0.1	0.2	0.2	0.8	6.7	0.9
Galium boreale	....	0.2	0.1	....	0.3	....	0.1
Campanula petiolata*	....	0.4	0.7	0.8	0.7	1.3	0.6

Achillaea lanulosa*	....	0.7	2.0	0.9	0.2	3.0	1.2
Antennaria anaphaloides	0.2	0.7	3.0	....	0.3	....	0.6
Antennaria aprica and arida	....	0.1	....	1.2	0.6	0.3	0.3
Antennaria microphylla*	....	0.5	0.5	0.5	2.0	0.3	0.6
Artemisia Forwoodii and aromatica*	2.0	1.1	1.3	1.6	1.2	0.2	1.2
Artemisia frigida*	3.0	4.7	3.8	4.1	3.0	0.5	3.7
Aster lonchophyllus	....	....	....	....	....	0.2	0.1
Chrysopsis villosa*	2.5	3.3	3.3	2.1	2.0	0.5	2.5
Erigeron eximius	....	....	....	0.1	0.1	0.4	0.1
Erigeron macranthus	....	....	0.2	....	0.1	....	0.1
Erigeron multifidus	5.0	....	0.2	....	....	....	0.5
Gaillardia aristata	....	0.3	....	....	....	....	0.1
Solidago concinna	....	....	0.1	0.2	0.6	0.5	0.2
Agoseris glauca*	....	0.4	0.3	0.5	0.9	0.4	0.3
Taraxacum Taraxacum	....	....	....	....	....	0.2	0.1
Total	99.7	99.9	99.7	100.4	97.9	98.5	98.4

of such plains grasses as *Bulbilis* and *Bouteloua.* No cacti of any kind are found, although they are present in mountain parks at slightly lower altitudes. The large proportion of Papilionaceae and Carduaceae here noted is to be expected in any area of the Rocky Mountain region.

Many of the plants are recorded from all of the minor communities in the list; some from only a part. The species here noted include most of the common ones of xerophytic areas of higher parks, but only about one-half of the entire number of species in the dry grassland of Boulder Park.

The figures in the list are for percentages of ground covered. Amounts less than one-tenth of 1 per cent are given as 0.1 per cent. An asterisk (*) is placed after the names of species found in all or all but one of the different communities.

The water requirements of the several species will be understood best if the reader will keep in mind that the most xerophytic plant assemblage is represented in column 1, and that the other columns represent in order less and less arid conditions. Hence a plant shown only at the left of the table is hyperxerophytic, as *Poa rupicola* and *Allium recurvatum.* One recorded chiefly at the right is hypoxerophytic, as *Agrostis hiemalis* and *Stipa viridula.* It should be mentioned, however, that an occasional stray may get in anywhere, as *Thermopsis divaricarpa,* a meadow plant here recorded from the driest situation.—FRANCIS RAMALEY, *University of Colorado, Boulder, Colo.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Ecology of the Santa Catalina Mountains.—The Santa Catalina Mountains of southeastern Arizona afford unusual opportunities for ecological studies by displaying a rich vegetation in the midst of a semi-arid region. They rise from a basal elevation of 3000 feet to a height of 9150 feet, and their proximity to Tucson has lead to their study by members of the staff of the Desert Laboratory and of the University of Arizona. As a result of such an investigation, SHREVE[1] has made an analysis of the vegetation in relation to climatic factors. In general these mountains are characterized by rugged ridges of gneissic rock, and steep, rather narrow, drainage canyons without mountain parks or meadows. Only above 7500 feet is there any modification of the soil due to the accumulation of humus, while below that altitude the pockets and crevices are filled with a loam soil mixed and often with its surface covered with coarse angular fragments. About the base of the mountain are alluvial slopes of straight profile accumulated by violent and intermittent stream flow, and designated by their popular Spanish name "bajada." In this study the vegetation of the upper bajadas is included, giving a picture of the plant life by which the higher elevations are surrounded and from which they derive many of their characteristic species.

Three vegetational regions are distinguished: the desert, with an altitudinal range from 3000 feet on the upper bajadas to 4000–4500 feet upon the mountain slopes; the scrub or semi-desert, here designated "encinal," with an upward limit of 6000–7000 feet; and forest reaching to and most highly developed upon the mountain tops. The desert vegetation of the upper bajadas and mountain slopes differs from that of the lower plains principally in the greater number both of species and of individuals there present, but agrees in general openness of stand, lowness of stature, and diversity of types, comprising principally such forms as stem succulents, microphyllous and sclerophyllous shrubs, macrophyllous deciduous shrubs, perennial grasses, root perennials, and ephemeral herbaceous plants. Conspicuous examples of such types are seen in *Cereus giganteus, Echinocactus* spp., *Opuntia* spp., *Prosopis velutina, Acacia Greggii, Parkinsonia microphylla, Covillea tridentata, Fouquiera splendens, Jatropha cardiophylla,* and *Franseria deltoidea;* while at the upper

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[1] SHREVE, FORREST, The vegetation of a desert mountain range as conditioned by climatic factors. Carnegie Inst. Wash. Publ. no. 217. pp. 112. *pls. 36. figs. 17.* 1915.

transition portion *Prosopis* and other shrubs, together with several grasses, become more abundant, and such forms as *Agave Palmeri* and *Dasylirion Wheeleri* appear. In the encinal the grass cover largely persists, although small evergreen trees and shrubs are the distinctive feature of the vegetation. Among the most abundant of these are several species of *Quercus*, *Juniperus pachyphloea*, *Pinus cembroides*, *Arctostaphylos pungens*, *Mimosa biuncifera*, *Rhus trilobata*, *Garrya Wrightii*, and *Vauquelinia californica*. Herbaceous perennials are also abundant.

The forest begins at about 6000 feet, with an open stand of *Pinus arizonica*, in which the evergreen oaks of the encinal become gradually more scattered. At slightly higher elevations *Arbutus arizonica*, *Pseudotsuga mucronata*, and *Pinus strobiformis* begin to be found, while the shrubby and herbaceous flora gradually changes by the appearance of less xerophytic forms. This forest reaches its highest expression by the appearance of *Abies concolor* above 7000 feet, where the individual trees are larger and the stand becomes close and heavy. Such a mesophytic conifer forest in the midst of a desert calls for a close analysis of the climatic factors involved. The location of the Santa Catalina Mountains in a continental desert gives their lower slopes the climate characteristic of this desert, its main features being a low rainfall unequally distributed, a short winter with severe frost, and a long summer with high temperatures and low humidity. The major portion of the rainfall is confined to a short but well marked rainy season in July and August, within which a little over half of the annual precipitation usually occurs. At Tucson this has averaged 6.6 inches during the years 1907–1912, while in the mountains, although there have been very few observations, the average for the years 1907–1914 inclusive, at stations at 7600 feet, for the humid midsummer, was 17.45 inches, or a probable annual average of 35 inches. The frostless season decreases from a range extending from March to December at Tucson, to one from the middle of May to the beginning of October at 8000 feet, but perhaps the change at higher altitudes most important in its effect upon vegetation is the prolongation of spring shortening the arid fore-summer from 15 weeks on the desert, to 11 weeks at 6000 feet, and 6 weeks at 8000 feet. The influence of this modification will become apparent when it is recalled that this is the most trying season of the year, and the one in which moisture conditions are critical for the survival of individuals or species. The humid midsummer, on the contrary, is the season when conditions upon the desert and in the mountain are most alike, and during which there is the greatest vegetative activity in both habitats. A close analysis of these effects of the variations in the amount and distribution of the rainfall is given, together with a few data regarding the resulting conditions of soil moisture.

The evaporative power of the air was determined by the use of standard atmometers at six stations, situated at regular intervals of 1000 feet from 3000 to 8000 feet. At each station a pair of instruments, one with a north exposure and the other with a south exposure, were used, and some data were secured during 1908 and 1910, and a more complete set of observations was obtained during the summer of 1911. These results are expressed in an interesting series of graphs, with evaporation rates ranging from approximately 100 cc. per day from the standard atmometer at 3000 feet during April and May, and half that amount during the more humid July and August, to about 15 cc. per day during the latter months at 8000 feet. The need of data from a larger number of stations for a series of years is recognized, but from those obtained the following conclusions are drawn: (1) the rate of evaporation through the arid and humid summer seasons is about 3.5 times as great on the desert as it is at 8000 feet; (2) the rates of evaporation are approximately half as great in humid midsummer as they are in arid fore-summer; (3) at the middle and higher altitudes the evaporation on north slopes is less than on south slopes; and (4) the difference between the amounts of evaporation on north and south slopes becomes greater with increase of altitude in proportion to the amounts of each. Corresponding with the high rates of evaporation are low values of relative humidity. A considerable amount of temperature data has been secured, its most important bearings having to do with the altitudinal shortening of the frostless season, the altitudinal fall in temperature, the daily maxima and minima at various altitudes, and the absolute minima of winter. These data show that the lowest temperatures of winter (−2° in 1913 and 15°.5 F. in 1914 at 7600 feet) are less severe on the Santa Catalinas than on the plateau of north central Arizona. The departure from the normal altitudinal gradient, due to the operation of cold air drainage, discussed in an earlier paper[2] and reviewed in this journal,[3] is supported by further data, and its importance in influencing vegetation is noted, and some soil temperature data seem to indicate that its winter minimum is decidedly above that of the air. With such remarkable variety in vegetation and such extremes in physical factors, the correlation of vegetation and its controlling factors become of the utmost importance, and in no part of this report is the excellence of the work better shown than in SHREVE's discussion of the complex problems involved. With such a large gradient of climatic change, it might be expected that vertical distribution of species would be decidedly limited, and such is the case. No plant extends its range from the desert to the upper forest, and very few extend through half that gradient of conditions, while in general it may be said that most are limited to vertical ranges of less than 1500 feet in habitats of the same topographic character. A vertical range of 4700 feet for *Vitis arizonica* in canyons and ravines, 4200 feet for *Agave Palmeri* from the dry slopes at 3200 feet to high open ridges, and *Juniperus pachyphloea* from northern slopes at 4200 feet to ridges at 7900 feet are among the more extreme instances given. Moisture is recognized as the most critical factor controlling the vertical distribution of this vegetation, and the ratio of evaporation to soil moisture is

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[2] SHREVE, FORREST, Cold air drainage. Plant World **15**:110–115. 1912.

[3] BOT. GAZ. **55**:263. 1913.

accepted as the best expression of the conditions which affect the water relations of plants. Unfortunately, the soil moisture data are too scanty to afford a good basis for comparison, and are not expressed in terms indicating what proportion of this moisture is available for plant production. The temperature control has been experimentally studied for a few species, and the results have been previously reported.[4] These are given some further consideration, while slope exposure and topographic relief are carefully discussed. Perhaps nothing shows the unusual character of the factors controlling vegetation more than the fact that succession due to physiographic development and to the reaction of the plant upon its habitat is almost completely absent.

In its efforts to determine in a quantitative manner the climatic and other physical factors involved, and in its careful attempts to correlate these factors with vegetation, this report may be regarded as an excellent example of modern ecological investigation. The illustrations are numerous, well chosen, and reproduced in the excellent manner that has usually characterized the publications of the Carnegie Institution, while the organization of the material presented is decidedly better than that of many similar publications that have come to the attention of the reviewer.—GEO. D. FULLER.

Vegetation and tide levels.—The excellent opportunities for investigating the problems of seashore vegetation at Cold Spring Harbor, New York, afforded by the location at that place of the Biological Laboratory of the Brooklyn Institute of Arts and Sciences and the Carnegie Station for Experimental Evolution has been appreciated by many botanists, and the factors determining the composition and distribution of the various plant associations in the vicinity are becoming better known. In 1912 JOHNSON and YORK[5] made a preliminary announcement of the results of a survey of the inner harbor, and indicated the relations of the various plant associations to tide levels. This was followed by a more general paper by TRANSEAU[6] on the littoral successions of the vicinity, devoted principally to a consideration of the lines of succession followed by the seed plant communities from the salt marsh to the pine barrens. More recently there has come the full report of the careful survey of JOHNSON and YORK,[7] who have confined their attention to the vegetation of the inner harbor.

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[4] SHREVE, FORREST, Influence of low temperatures on the distribution of the giant cactus. Plant World **14**:136–146. 1911.

———, The rôle of winter temperatures in determining the distribution of plants. Amer. Jour. Bot. **1**:193–202. 1914; see review in BOT. GAZ. **59**:502–503. 1915.

[5] JOHNSON, D. S., and YORK, H. H., The relation of plants to tide levels. Johns Hopkins Univ. Circular no. 2. pp. 6. 1912.

[6] TRANSEAU, E. N., The vegetation of Cold Spring Harbor, Long Island. I. The littoral succession. Plant World **16**:189–210. *figs. 1–8.* 1913.

[7] JOHNSON, D. S. and YORK, H. H., The relation of plants to tide levels. Carnegie Inst. Wash. Publ. no. 206. pp. 162. *pls. 24. figs. 5.* 1915.

This body of water is separated from the larger outer harbor by a spit of sand and gravel. The shallowness of its water may be noted from the fact that its area of 110 acres at mean high tide is reduced to 45.5 acres with the 8-foot recession of the waters at mean low tide, while a further drop of 1 foot effects a further reduction to only 17 acres. The various levels were carefully marked by a large series of stakes set along the contour lines. In this way, using mean low water as zero, four vegetational belts were delimited: (1) the region of bottom vegetation from −3 to 1.5 feet, occupied by an algal association composed of *Ulva* and *Enteromorpha* together with an association of seed plants consisting of *Zostera marina* and *Ruppia maritima*, rooted in the muddy bottom; (2) the mid-littoral belt, from 1.5 to 6.5 feet, with the *Spartina glabra* marsh and the algal rockweed associations, the latter being dominated by *Fucus* and *Ascophyllum;* (3) the upper littoral belt, from 6.5 to 8 feet, with associations dominated by species of *Spartina, Juncus*, and *Scirpus;* and (4) the supra-littoral belt, from 8 to 12 feet, containing many species combined in intermingling associations.

In the careful analysis of many of the factors influencing the distribution of littoral plants, the substratum is found to consist of the lower stretches of a valley in glacial gravel and sand. The upper part of the valley is occupied by a small stream of fresh water flowing into the harbor, while in the harbor itself there is superimposed upon the gravel a black mud of varying depth, passing upward into a muddy peat formed by the remains of the salt marsh vegetation. The deposition of this peat is of such a character as to indicate comparatively recent coastal subsidence estimated at a minimum of 6 feet. Boulders of considerable size and the walls of wharves largely determine the horizontal limits of the rockweed association, while the larger plants act as the substratum for a varied epiphytic vegetation comprising most of the red algae; the abundant mussels are seen to be important in anchoring *Ulva* and *Enteromorpha clathrata*. Details of the interaction of the plant life and these substrata are rather carefully worked out.

Water currents due to tidal movements through the narrow channel at the end of the spit and to the inflowing stream of fresh water seem to be important in effecting distribution of the species, both through the transportation of the plants themselves, either broken or entire, and by the dispersal of seeds and spores. A secondary effect of currents is shown to be in changing the concentration of solutes about the plants themselves. Aeration is suggested as one of the important results, and further data are promised as to the distribution and effects of salinity. Tidal movements as effecting changes in water are the factor, or rather the complex of factors, given most attention, and an analysis is made of the character of the tides as related to (1) submergence and exposure, (2) evaporation, (3) aeration, (4) salinity of soil water, (5) effect on exposure to rain, and (6) effect on light supply. Many data are tabulated regarding the duration of submergence and exposure, and the ratio between the duration of the two is determined. The distribution of the various plant associations agrees very closely in every instance with variations in this ratio, the vertical range of littoral species being strictly and sometimes very narrowly limited. Strangely enough, practically no species is found to be distributed, as so often reported, "between tide marks." The data upon the evaporative power of the air are scanty, only going to show that it is high, for as the atmometer readings were for a few hours of daylight only, no significant comparisons could be made with the data of other observers. Aeration due to tidal movements seems to be of some importance, while upon variation of salinity, the direct action of rain upon exposed vegetation, and the variation in light and their effects few exact data have been obtained as yet, but the careful study of the question of relative times of submergence and exposure has clearly defined many dependent problems for future investigation. This is perhaps the largest contribution from the careful and extended work of the authors.

Little less important than the careful analysis of the factors controlling the vegetation is the detailed study of the distribution both of the plant associations and of the species composing these associations. The results are expressed in several excellent vegetational maps of the entire harbor, of the sand spit, and of the estuarial marsh, together with belt transects on a large scale of the spit and the marsh. These provide a basis for future studies, two or more decades hence, that will certainly do much to give a better understanding of the causes and progress of plant succession in this and similar areas. The abundance of the data, the care with which they have been collected and arranged, the number of the maps, diagrams, and tables, and the organization of the discussion, are all matters to be highly commended. The deficiencies and omissions are such as are dependent upon the size of the problem and the multiplicity of the factors involved, rather than upon the neglect or oversight of the investigators, who are to be congratulated upon their patience and care in making so important a contribution to our knowledge of littoral vegetation.—GEO. D. FULLER.

Taxonomic notes.—BARTLETT[8] has described a new Mexican guayule, naming it *Parthenium Lloydii*, collected by LLOYD in Zacatecas.

DIXON[9] has described 10 new species of African mosses, one of which (*F. Wageri*) is made the basis of a new section of *Fabronia*. He also notes and discusses 21 additional rare species.

EVANS[10] has described a new species of *Metzgeria* (*M. grandiflora*) from the Galapagos Islands, included in a collection of liverworts made by ALBAN

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[8] BARTLETT, H. H., *Parthenium Lloydii*, a new Mexican guayule. Torreya **16**:45–46. 1916.

[9] DIXON, H. N., New and rare African mosses, from MITTEN's herbarium and other sources. Bull. Torr. Bot. Club **43**:63–81. *pl. 1*. 1916.

[10] EVANS, A. W., A new species of *Metzgeria* from the Galapagos Islands. Torreya **16**:67–70. *figs. 5*. 1916.

STEWART while acting as botanist to an expedition of the California Academy of Sciences. It is said to be the first representative of the Metzgeriaceae from this group of islands. The specific name refers to the unusually large female branches.

GOLDMAN[11] has described 3 new species of *Quercus* from Lower California, included in a collection made by himself and E. W. Nelson during a general exploration for the biological survey of the United States Department of Agriculture. The period covered by the expedition was from April 1905 to February 1906, during which the entire length of Lower California was traversed. In addition to the 3 new oaks, 19 other new species have already been published from material collected by this expedition.

GRIFFITHS[12] has described 10 new species of *Opuntia* from material collected mostly in the Mexican border region, and chiefly in Texas. It is a noteworthy fact that all of these species have been propagated vegetatively in the *Opuntia* collection growing at Chico, California, have been studied in the field, except in one instance, and the majority of them have been grown to maturity from the seed.

HALLIER,[13] in his study of the flora of Borneo, has described 9 new species.

NAKAI,[14] in this sixth contribution dealing with the woody plants of Korea, has presented the Pomaceae, describing 4 new species of *Pyrus*.

OKAMURA[15] has published a list of marine algae collected in the Caroline Islands in 1915. The list includes 28 species of Chlorophyceae, 11 species of Phaeophyceae (2 new species), and 22 species of Florideae (1 new species).

OSTERHOUT[16] has described a new species of *Phacelia* (*P. denticulata*) from Colorado. It is related to *P. glandulosa* and *P. neo-mexicana*.

PENNELL[17] has begun a series of notes on the plants of the southern states. The first paper consists chiefly of a critical revision of the genus *Commelina* in the United States. The full key to the species, serving the purpose of specific descriptions, is followed by a list of the 9 recognized species with full citation of exsiccatae.

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[11] GOLDMAN, E. A., Plant records of an expedition to Lower California. Contr. U.S. Nat. Herb. **16**:309–371. *pls. 104–133.* 1916.

[12] GRIFFITHS, DAVID, New species of *Opuntia*. Bull. Torr. Bot. Club **43**:83–92. *pls. 2, 3.* 1916.

[13] HALLIER, HANS, Beiträge zur Flora von Borneo. Beih. Bot. Centralbl. **34**:19–53. 1916.

[14] NAKAI, T., Praecursores ad floram sylvaticam Koreanam. VI (Pomaceae). Bot. Mag. Tokyo **30**:15–33. 1916.

[15] OKAMURA, K., List of marine algae collected in Caroline Islands, 1915. Bot. Mag. Tokyo **30**:1–14. *figs. 9.* 1916.

[16] OSTERHOUT, G. E., A new *Phacelia* from Colorado. Torreya **16**:70–71. 1916.

[17] PENNELL, F. W., Notes on plants of the southern United States. I. Bull. Torr. Bot. Club **43**:93–111. 1916.

PITTIER[18] has published his fifth contribution dealing with new or noteworthy plants from Colombia and Central America. Under various subtitles 15 new species are described, representing 12 genera, *Bombacopsis* (Bombacaceae) being a new genus. There is also a revision of *Brownea* and *Browneopsis* (Caesalpiniaceae), as represented in Panama, Colombia, and Venezuela.

TAKEDA[19] has described a new genus (*Dysmorphococcus*) of algae, which resembles externally *Trachelomonas*. The material was found in a small stagnant pond in Richmond Park, Surrey, England.

VAN ALDERWERELT VAN ROSENBURGH,[20] in his seventh paper on new or interesting Malayan ferns, has published as new species or with new names 42 species of ferns, representing 21 genera, and also 3 new club mosses.—J. M. C.

Temperature and growth rate.—LEITCH,[21] working in the plant physiology laboratory of the University of Copenhagen, has done an excellent piece of work upon the effect of temperature on the rate of growth of the main root of *Pisum sativum*. Short experimental periods were used to avoid errors due to changing rate in the course of the grand period of growth. The period of maximum growth rate (root 5–10 mm. long) was also chosen. It was important in determining methods of experimentation that diffuse light or change of temperature, as such, does not modify the rate of growth of this organ. The temperature coefficient, Q_{10}, is as follows for temperatures between 0° C. and 28° C.

Range of temperature	Temperature coefficient	Range of temperature	Temperature coefficient
0–10° C. . . .	8.25	12–22° C. . . .	2.67
2–12°.	6.28	14–24°.	2.44
4–14°	4.58	. . . .	. . . .
6–16°.	3.72	16–26°.	2.31
8–18°.	3.24	18–28°.	2.22
10–20°.	2.88		

The temperature coefficient is typically VAN'T HOFF's only between 10° and 29° C., while below 10° C. the coefficient exceeds the VAN'T HOFF value of 2 to 3. This means little regarding the nature of the growth process, for the coefficient often greatly exceeds 3 in monomolecular reactions *in vitro*. In

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[18] PITTIER, HENRY, New or noteworthy plants from Colombia and Central America. V. Contr. U.S. Nat. Herb. **18**:143–171. *pls. 57–80. figs. 10.* 1916.

[19] TAKEDA, H., *Dysmorphococcus variabilis*, gen. et sp. nov. Ann. Botany **30**:151–156. *figs. 15.* 1916.

[20] VAN ALDERWERELT VAN ROSENBURGH, C. R. W. K., New or interesting Malayan ferns. VII. Bull. Jard. Bot. Buitenzorg **20**:1–28. *pl. 4.* 1915.

[21] LEITCH, I., Some experiments on the influence of temperature on the rate of growth in *Pisum sativum*. Ann. Botany **30**:25–46. *figs. 10. pl. 1.* 1916.

growth, however, as is frequently true in chemical and physical processes, the coefficient falls as the temperature rises above 0° C.

At temperatures above 29° C. the initial rate is not maintained, but fluctuates or falls as time elapses, the well known *time factor* of BLACKMAN. At 30° and 35° C. the initial fall in rate is followed by a rise, which in turn is followed by a continuous fall, while at 40° C. the fall in growth rate is continuous with the elapse of time. This is comparable to the respiratory intensities at higher temperatures as determined by KUIJPER.[22]

The growth minimum for the organ studied is $-2°$ C. and the maximum 44°5 C. In lieu of the errors involved in the old conception of optima, as shown by BLACKMAN and confirmed by many others, LEITCH proposes a new definition for *optimum temperature* in relation to any process in the organism, namely, *the highest temperature at which no time factor enters.* For the organ studied the point is between 28° and 30° C. A fourth cardinal point is defined, namely, *maximum-rate temperature*, as *the temperature at which the process attains its highest intensity*, which is 30°3 C. in this organ.

It is to be regretted that the author did not have an opportunity to examine the excellent work of LEHENBAUER (thesis, Illinois, 1914), who had, in large part, obtained similar results and arrived at similar conclusions.—WM. CROCKER.

Subalpine flora.—In continuing a series of phytogeographical papers, the first of which was recently reviewed in this journal,[23] RYDBERG[24] has discussed the forests and grasslands of the zones immediately below the alpine. He distinguishes two principal areas in the Rockies separated by a break in the range occurring in Wyoming about where the Union Pacific Railroad crosses. This break divides the portion of the region under discussion into the northern and the southern Rockies. The northern Rockies extend from the Yukon southward, and are made to include the Sawtooth Mountains of Idaho, the Tetons and the Big Horns of Wyoming, and the Cypress Hills of Alberta. They are further extended to include the Black Hills and smaller chains in their neighborhood. Over this area RYDBERG says the flora is practically homogeneous, and includes among other trees not found in the southern part *Larix occidentalis, Abies grandis, Tsuga heterophylla, T. mertensiana, Thuja plicata, Taxus brevifolia*, and several species of *Betula, Salix*, and *Populus.* In a further analysis of the flora, species exclusively southern and those common throughout the range are noted. Notes are also made of habits of growth and peculiarities of distribution of the more important trees and of the variation of altitudinal range of the zones.

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[22] BOT. GAZ. **50**:233–234. 1910.

[23] BOT. GAZ. **59**:64–65. 1915.

[24] RYDBERG, P. A., Phytogeographical notes on the Rocky Mountain region. IV. Forests of the subalpine and montane zones; V. Grasslands of the subalpine and montane zones. Bull. Torr. Bot. Club **42**:11–25, 629–642. 1915.

The second of these papers contains an analysis of the flora of the various mountain grasslands, those of the montane zone receiving most attention. Many species are common to the montane and subalpine zones, rather more to the montane and plains, while a smaller number are limited to either the northern or the southern montane zones only. Little attempt is made to analyze the composition and dynamics of the various grassland associations.—GEO. D. FULLER.

Fungus lore.—In his presidential address before the British Mycological Society in 1914, BULLER[25] has given a very interesting account of what he calls the fungus lore of the Greeks and Romans. It is an admirable résumé of the ancient literature of fungi, and gives to the general botanist much information which he will prize. Such topics as the following suggest the kind of information presented: edible and poisonous fungi, the dawn of mycology, the first known illustration of a fungus, the rust disease, the origin of fungi, the medicinal properties of fungi, the origin of poisons in fungi, the cultivation of fungi, the misuse of classical names by LINNAEUS, etc. It is shown that the Greeks and Romans were familiar with many kinds of edible and poisonous fungi. As to the origin and reproduction of fungi, they were in complete ignorance, in general being contented to accept a theory of spontaneous generation.

In a presidential address before the Royal Society of Canada in 1915, BULLER[26] continues the publication of his historical researches by presenting an account of "MICHELI and the discovery of reproduction of fungi."—J. M. C.

Blooming period of Dendrobium.—RUTGERS and WENT[27] have investigated the factors concerned in the blooming of *Dendrobium crumenatum*, whose flowers open simultaneously on different individuals. They find that this coincident blooming of different plants depends upon the prevailing external conditions; and since these may vary in different places, the blooming period shows local variations. They conclude that light cannot exert any definite influence on the time of blooming, although it influences the number of flowers; and therefore they consider temperature or atmospheric moisture, or both factors together, to be responsible. The development of the flowers proceeds slowly up to a definite stage, at which they remain until some inhibitory influence is resolved by external factors. When this has occurred, the last stage in the opening of the flowers is accomplished in a few days. The external

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[25] BULLER, A. H. R., The fungus lore of the Greeks and Romans. Trans. British Mycol. Soc. pp. 66. 1914.

[26] BULLER, A. H. R., MICHELI and the discovery of reproduction in fungi. Trans. Roy. Soc. Canada **9**:1–25. *pl. 4.* 1915.

[27] RUTGERS, A. A. L., and WENT, F. A. F. C., Periodische Erscheinungen bei den Blüten des *Dendrobium crumenatum* Lindl. Ann. Jard. Bot. Buitenzorg **14**:129–160. *pl. 22. figs. 5.* 1915.

conditions result in so vigorous a development of the flowers that the enveloping scales are burst.—J. M. C.

Morphology of Gnetum.—PEARSON[28] has contributed much to our knowledge of *Gnetum*, a genus so suggestive of a relationship to angiosperms as to deserve the most critical study. The present paper is a well organized summary of the known facts in reference to the genus, and while the argument for angiosperm affinity is not convincing, it shows that the case is still open. PEARSON sees in the behavior of the fusing nuclei of *Welwitschia* and *Gnetum* too much suggestion of the behavior of the polar nuclei in angiosperms to be passed over lightly. As the author remarks, "while the whole question is involved in much obscurity, it is surely not desirable at the present stage of inquiry that the search for a primitive type of endosperm, from which that of the angiosperm may be derived, should cease." Aside from the phylogenetic discussion, the paper analyzes the observed facts and theories in reference to the strobilus or "spike" of *Gnetum* in a most suggestive way.—J. M. C.

Temperature of leaves in winter.—Using careful methods of determining differences of temperature by an electrical apparatus, EHLERS,[29] working at Ann Arbor, Michigan, upon *Pinus Laricio*, found that the leaves through the absorption of radiant energy maintain during the winter temperatures of 2–10° C. higher than the surrounding air. For the month of February, 650 readings taken between the hours of 8:00 A.M. and 3:00 P.M., under all kinds of weather conditions, including both cloudy and bright days, gave an average differential temperature of 3°06 C. Increased photosynthetic activity resulting from such increases in temperature would seem to be of considerable importance, and would tend to explain the presence and accumulation of the reserve food material found in evergreen leaves in winter by various workers. EHLERS was unable, however, to obtain any conclusive evidence of carbohydrate formation during the months of January and February.—GEO. D. FULLER.

Structure of Alaria.—In a recent publication of the Puget Sound Marine Station, Miss KIBBE[30] describes the structure of *Alaria fistulosa*. In many features she finds a close resemblance to other Laminariaceae. The chief purpose of the paper is to trace the development of the fistulose or chambered character of the midrib. Severe strain during growth, resulting from continued transverse and radial division of cortical cells after the cells of the medulla

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[28] PEARSON, H. H. W., Notes on the morphology of certain structures concerned in reproduction in the genus *Gnetum*. Trans. Linn. Soc. London 8:311–332. *pl. 31, 32*. 1915.

[29] EHLERS, J. H., The temperature of leaves of *Pinus* in winter. Amer. Jour. Bot. 2:32–70. 1915.

[30] KIBBE, ALICE L., Some points in the structure of *Alaria fistulosa*. Puget Sound Marine Sta. Publ. 1:43–57. *pls. 8, 9*. 1915.

have ceased to divide, produces rifts in the pith tissue; and from these rifts chambers are gradually developed. Meanwhile, hyphal chains and sieve tubes, broken down in the process of chamber formation, form a mucilaginous substance. Septa between the chambers are made up of hyphal chains left unbroken by deep lobing of a ridge sent out along the angled side of the rachis and inclosing a portion of the pith web.—MABEL L. ROE.

A new luminous fungus.—KAWAMURA[31] has investigated a luminous and very poisonous fungus that grows on the decaying trunks of the beech (*Fagus sylvatica*) in the uplands of Japan, and appears in the autumn. It is known by a Japanese name meaning "moon-night mushroom," and proves to be a new species of *Pleurotus* (*P. japonicus*). The light is emitted by the gills only, which are uniformly luminous all over. The range of temperature for luminosity is 3–40° C., the optimum being 10–15° C. Experiments were made by exposing the fungus to nitrogen, hydrogen, ether, and vapor of chloroform, in all of which the luminosity disappeared after a variable interval; while in oxygen there was no change. It is stated that about 100 sq. cm. of luminous area gives enough light for reading, and that the luminosity is very evident at a distance of 30 m. or more.—J. M. C.

Alaskan liverworts.—EVANS,[32] studying the collection of Alaskan liverworts made by Dr. T. C. FRYE, finds that of 70 species in a condition to be identified with certainty, 20 are new to Alaska, 7 new to America, and 3 new to science. The Harriman Expedition yielded 63 species, of which 39 were new to Alaska, 6 new to America, and one species new to science. The total number of species now known in Alaska is 105, and comparatively little intensive exploration has been done. An admirable feature of the paper, and one which should be followed by future explorers, is that the latitude and longitude of each station are given to one minute. This will enable competent collectors to find at any future time almost the exact spot where a collection has been made.—W. J. G. LAND.

Growth and concentration of nutrient solution.—BRENCHLY[33] concludes that barley and wheat do not give complete or maximum growth in a solution containing the amount of potash and phosphoric acid (K_2O 28 ppm. P_2O_5 7 ppm.) stated by CAMERON to exist in soil solutions. The reviewer would suggest that the surface of contact between the root hair or root epidermis and the soil particle, and not the general soil solution, is the medium from which plants

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[31] KAWAMURA, SEIICHI, Studies on the luminous fungus *Pleurotus japonicus*, sp. nov. Jour. Coll. Sci. Tokyo **35**:1–29. *pl. 3*. 1915.

[32] EVANS, ALEXANDER W., Report on the Hepaticae of Alaska. Bull. Torr. Bot. Club **41**:577–616. *pl. 21*. 1915.

[33] BRENCHLY, W. E., The effect of the concentration of the nutrient solution on the growth of barley and wheat in water cultures. Ann. Botany **30**:77–90. 1916.

absorb salts. The former bears salt in much higher concentration than in the latter, owing to excretion of carbon dioxide and probably owing to the presence of mineral acids freed because of the absorption of cations of salts[34] by the gels of the walls of the root hairs and epidermal cells.—WM. CROCKER.

Vegetation of an atoll.—KOIDZUMI[35] has visited and described the vegetation of an oceanic coral island lying in longitude 169°5 E. and latitude 6° N., contrasting the luxuriance of its vegetation with the poorness of its flora. The greater part of this atoll is covered with a luxuriant strand forest in which the cocoanut is prominent. This is a response to a mean temperature of 27° C., and an annual rainfall of 450 cm. Of its 59 species, 40 seem to have reached the island by natural means. The largest families are the Gramineae (6 spp.), Euphorbiaceae (5 spp.), and the Leguminosae (4 spp.), their small representation also pointing to the conclusion that the flora is altogether derivative and of comparatively recent origin.—GEO. D. FULLER.

Parasitism of Comandra umbellata.—Investigating the conditions of growth of *Comandra umbellata*, because of its importance as one of the hosts of the heteroecious rust *Peridermium pyriforme*, so injurious to various pines, HEDGCOCK[36] found that in nature the plant is always a partial parasite, being united to its host by its roots and apparently most dependent in regard to its water supply. Fifty different hosts, scattered through various plant families from the Gramineae to the Compositae, are listed. Proof is cited that *Comandra* can live without parasitism, and that its seeds may germinate without the presence of the roots of host plants, although it is doubtful if it ever does either in nature.—GEO. D. FULLER.

Anatomy of Nephrolepis volubilis.—SAHNI[37] has investigated the anatomy of this climbing Malayan fern. It is remarkable for its extremely long stolons, which scale forest trees up to 16 m., and enable the "lateral" plants borne on them at intervals to reach far above the mother plant, which is rooted in the soil. These lateral plants have no roots, and put out coiled tendril-like stolons that show contact irritability. The vascular cylinder of the stolons is an exarch protostele, and when a stolon branches, the two steles run parallel to each other for some distance, inclosed in the cortical envelope, before they become free. It is a case in which a soil-rooted plant gives rise through stolons to an epiphytic progeny.—J. M. C.

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[34] CZAPEK, F., Jahrb. Wiss. Bot. **56**:97–98. 1915.

[35] KOIDZUMI, GENICHI, The vegetation of Jaluit Island. Bot. Mag. Tokyo **29**:242–257. *figs. 3.* 1915.

[36] HEDGCOCK, G. G., Parasitism of *Comandra umbellata*. Jour. Agric. Research **5**:133–135. 1915.

[37] SAHNI, BIRBAL, The anatomy of *Nephrolepis volubilis* J. Sm., with remarks on the biology and morphology of the genus. New Phytol. **14**:251–274. *pl. 4. figs. 7.* 1915.

A cretaceous genus of Abietineae.—Dr. STOPES[38] has investigated a petrified tree trunk from the Cretaceous of New Zealand, which shows "a striking mixture of abietinean and araucarian characters." She regards it as a new genus, which is named *Planoxylon*, and which is made to contain two species, the one investigated (*P. Hectori*) and another (*P. Lindleii*) that had been referred to *Araucarioxylon* by SEWARD. *P. Hectori* is of special interest because "it comes from Australasia, where no Abietineae or Juniperineae or other forms with abietinean ray structure are endemic at present."—J. M.C.

Morphology of Treubia.—GRÜN,[39] studying material of *Treubia insignis* Goebel from Tjibodas, finds that the thallus develops from a dolabrate apical cell like most of the anacrogynous Jungermanniales; that the archegonia occur in clusters of 8–10 and may have as many as 16 neck canal cells each; that only one sporophyte develops on each thallus; that vegetative reproduction is by means of 3 or 4-celled brood bodies; that mycorhiza are present in the thallus; and that the chromosome numbers are 8 and 16.—W. J. G. LAND.

Plagiochasma.—In a monograph of the genus *Plagiochasma*, EVANS[40] includes as much of the morphology of the group as will be useful for purposes of identification of species, gives a key to the North American species, and describes in detail the 6 species found in North America, one of which is new to science. The reviewer believes that several new species will be added to the group from Mexico when botanical exploration again becomes possible in that country.—W. J. G. LAND.

Endemism in the flora of New York.—TAYLOR[41] has found in the flora of the region near New York 22 endemic species, of which he considers 14 to be accounted for by generic or specific instability; 2 are "habitat endemics," or habitat forms of more widely spread species; while 5 are evidently relic endemics. He is convinced, therefore, that many forces are operative in making endemics, and he gives some discussion of such possible forces.—GEO. D. FULLER.

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[38] STOPES, MARIE C., An early type of the Abietineae(?) from the Cretaceous of New Zealand. Ann. Botany **30**:111–125. *pl. 4. figs. 7.* 1916.

[39] GRÜN, C., Monographische Studien an *Treubia insignis* Goebel. Flora **106**: 331–392. *pls. 3–5. figs. 14.* 1914.

[40] EVANS, ALEXANDER W., The genus *Plagiochasma* and its North American species. Bull. Torr. Bot. Club **42**:259–308. *figs. 8.* 1915.

[41] TAYLOR, NORMAN, Endemism in the flora of the vicinity of New York. Torreya **16**:18–27. 1916.

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AXIAL SUSCEPTIBILITY GRADIENTS IN ALGAE

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Introduction

Before presenting the data which constitute the subject of this paper, it is necessary to make clear the reasons which determined this venture into the botanical field and the presentation of these data to botanists for consideration and criticism. My experimental studies on the lower animals, extending over a period of years, have led me to certain conclusions concerning the nature of the physiological axes of organisms and of physiological individuality, which suggest that the physiological individual as an expression of a dynamic unity and order in protoplasm is fundamentally similar in its origin and nature in both animals and plants. The observations recorded in this paper constitute the first step in an attempt to determine whether and to what extent this is true. In order to make clear the point of view from which the investigation was undertaken, a brief statement of certain results of my experiments with animals and of the general conception toward which they point is essential.

I have shown that various lines of evidence point to the existence of a gradient in the rate of metabolism or of certain fundamental metabolic reactions as a characteristic feature of at least the chief physiological axes, and probably of all such axes in animals. Such a gradient is the earliest demonstrable indication of the axis, and in many forms persists without essential change throughout the life of the individual, while in others it undergoes modification during the course of development. In the axial metabolic gradient of the chief axis the region of highest rate of reaction becomes the apical region or head of the individual, and in other axes the development of organs shows a definite relation to the direction of the gradient.

In the final analysis such a gradient is not self-determined by some sort of organization, but arises as the result of the differential action of factors external to the protoplasm, cell, or cell mass acted upon. If, for example, an undifferentiated cell or cell mass is stimulated at some point by the action of a factor external to it, the resulting increase in metabolic activity is not limited to the region immediately affected, but a wave of change spreads or is transmitted over or through the protoplasm with decreasing energy, intensity, or physiological effectiveness, until, if the mass be large enough, it becomes inappreciable at a greater or less distance from the point of origin. It may be likened to a wave in water or in air spreading from the point of disturbance.

This is the simplest form of metabolic gradient, but while it produces temporary changes in the protoplasm, they are usually evanescent or the lasting effect is inappreciable. If, however, the stimulation be sufficiently often repeated or sufficiently long continued at the same point, more or less persistent changes in the protoplasm may occur, which appear as differences in its capacity to react. In such a case we say that the irritability of the protoplasm is altered. Since such changes are in general proportional to the energy or intensity of the transmitted change which produces them, they must differ at different distances from the point of action of the external factor, and the result is what we may call an irritability gradient, a gradient in the rapidity, capacity, or intensity of reaction of the protoplasm. Such a gradient is, I believe, the simplest form of persistent organic or physiological axis. In the chief gradient or axis, which is that first established, the region of highest metabolic rate or highest irritability becomes the apical region, and in the more highly differentiated animals the head, or more specifically the cephalic part of the central nervous system, develops from it.

In such a metabolic or irritability gradient it is evident that the region of highest rate of reaction must control or dominate other regions within a certain range, simply because the changes transmitted from it possess greater energy or physiological effectiveness than those from other regions, and therefore constitute the chief factor in determining the general metabolic rate in these other regions within the range of its action. In plants the dominance of the apical region over other levels of the axis is a familiar fact, and I have shown that in at least the simpler animals similar relations exist along the main axis, the region of highest metabolic rate being the dominant region.

This relation of dominance and subordination dependent upon a gradient in metabolic rate or in the irritability of the protoplasm is, as I believe, the simplest kind of physiological[1] unity and order in living protoplasm, these transmitted changes are the fundamental form of physiological correlation, and the physiological individual as an expression of dynamic unity and order consists fundamentally of one or more such metabolic gradients.

According to this conception, the individual originates as one or more quantitative gradients in the dynamic processes characteristic of the specific protoplasm concerned. Morphogenesis and differentiation result from the quantitative differences existing at different levels of the gradient because the substances which remain as constituents of or inclosures in the protoplasm differ not only in amount but in kind according to the rate or intensity of metabolic activity. In this way qualitative differences arise from quantitative, and what we call differentiation results. This conception of differentiation is supported by the fact that it is possible experimentally to alter widely the character of morphogenesis by means of conditions which act primarily in a quantitative way.

If this view be correct, the physiological axiate individual is fundamentally a unity resulting from transmitted dynamic changes,

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[1] The word "physiological" is used here by way of distinction. Other kinds of individuality, of unity and order in aggregates, exist in the world and some of them undoubtedly exist in organisms, but by physiological unity and order is meant that kind which seems to be peculiarly characteristic of living things, and which determines orderly space and time relations with respect to an axis; in short, an orderly form and an orderly course of development.

rather than, as often assumed, from the transportation of specific chemical substances. In other words, physiological correlation is fundamentally transmissive rather than transportative in character, and transportative correlation arises only secondarily as the result of the quantitative and qualitative differences associated with the transmitted changes. It is evident that the existence of orderly transportative correlation demands the existence of differences of some sort arranged in an orderly way, and it is this fundamental order from which the other orderly phenomena in the life of the organism result, that this theory of the individual attempts to account for.

It is not necessary to assume, however, that every individual arises in the manner described above. When an axial gradient is once established in a cell or cell mass, it may persist through division or other forms of reproduction, so that each axis of the offspring is simply the axis of the parent or a fraction of it. In such cases the processes of rejuvenescence associated with reproduction bring the different fractions to essentially the same physiological condition, that is, the fraction which represents the lower levels of the parent gradient is brought up to the same metabolic rate or rates as that representing the higher levels. Nevertheless, we must, I believe, go back to a differential action of factors external to the protoplasm concerned for the origin of the metabolic gradient and so of the axis or polarity of which it is the simplest form.

Since the energy or effectiveness of a transmitted change in protoplasm decreases with increasing distance from the point of origin, the length of a metabolic gradient, and therefore the range of dominance of the region of highest rate, is limited, the limit varying with rate or intensity of metabolic activity, irritability and conductivity of the protoplasm, etc. This limit represents the maximum size which the physiological individual can attain and remain entirely an individual under the conditions which determine the limit. If, in consequence of continued growth, of decrease in the metabolic activity of the dominant apical region, of decrease in the conductivity of the protoplasm, any part previously within the range of dominance, that is, within the limit of length of the metabolic gradient, comes to lie beyond this limit, it becomes physiologically isolated, and if the irritability gradient in its protoplasm resulting from its former relations to the gradient persists, or if a new gradient arises in it, the reproduction of a new individual occurs. The space relations in the formation, inhibition, or development of buds in plants and the dominance of the chief growing tip seem to be fundamentally of this character. Growth in size of the plant or removal or inhibition of the chief growing tip results in physiological isolation of other parts, and new buds arise or buds previously inhibited develop.

This in brief is the conception of physiological individuality which has developed from my experimental work on the lower animals.[2] Since I have found metabolic gradients as characteristic features of the axes in animals and have been able to control and modify to a high degree size, development, and reproduction by controlling and modifying these gradients, it seemed desirable to determine whether the existence of similar gradients could be demonstrated in the simpler plants. The behavior in growth and development of the axis of the higher plant is a practical demonstration of the presence of a metabolic gradient with the apical region as the region of highest rate, but I thought it of interest to apply to some of the simple plants methods which I had used for the simple animals.

Methods and material

In the course of experimentation on the lower animals, I have found that certain relations exist between metabolic condition and susceptibility to at least many agents and conditions which interfere with, depress, or inhibit metabolism in some way. These relations are briefly as follows: To agents and conditions which affect the organism in sufficient degree to bring about death in a short time without permitting adaptation or acclimation, susceptibility varies directly with metabolic rate or with the rate of certain fundamental metabolic reactions, probably primarily the oxidations. The higher the rate of reaction the sooner death occurs, and vice versa. To agents and conditions which affect the organism to so slight a degree that more or less adaptation or acclimation is

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[2] For more extended consideration of the subject see: CHILD, C. M., 1–4, 6, 7, 9–11 (chapter ix), 12.

possible, the rapidity and degree of acclimation varies under most conditions directly with the rate of metabolism; consequently in the long run the susceptibility varies inversely as the metabolic rate. Within certain limits under these conditions, the higher the metabolic rate the longer life continues, because the higher the rate, the more rapid and complete the acclimation. Thus with the same differences in metabolic rate, the relative susceptibilities to high concentrations of a given agent are the inverse of those to very low concentrations of the same agent.

These relations have been demonstrated for the cyanides, ethyl alcohol, ether and various other narcotics, acids and in marine forms for alkalies also, as well as for certain other substances and conditions, such as lack of oxygen, presence of carbon dioxide or other metabolic products in the medium, etc. It cannot be supposed that all these agents and conditions act on living protoplasm in the same way, but it is true that they all interfere with the activities of living protoplasm in some way. The general relation between susceptibility and metabolic rate is undoubtedly independent, at least to a large extent, of the particular method of action of a particular agent or condition. With a certain rate of chemical reaction in protoplasm, certain conditions as regards enzymes, aggregation of colloids, permeability, etc., are in general correlated. The chemical reactions may be retarded or inhibited by means of changes in these various protoplasmic conditions, as well as by a direct chemical action; and since all these different factors in the activity of the protoplasm are correlated, its susceptibility to at least many, if not all inhibiting agents and conditions the effect of which is not instantaneous but progressive, must vary in general with its metabolic activity. If the inhibiting effect is so great that the protoplasmic machine cannot adjust itself to the new conditions, the higher its rate of activity, the sooner it is brought to a standstill. But if the inhibiting effect is slight, the higher the rate, the more rapidly and completely adjustment occurs, because adjustment depends, at least in large part, on the activity of the protoplasmic machine, that is, upon metabolism.

These relations between susceptibility and metabolic rate have been demonstrated in many different ways, and within certain limits and with certain precautions can be used very widely as a means of comparing the general metabolic condition of different individuals, and of different regions of the body of a single individual. The method is so simple as to appear crude, but its delicacy and value have been shown very clearly. Practically it consists simply in finding the survival times of the different individuals or body regions to be compared in a concentration, determined by preliminary experiment, of the agents employed. I have found the cyanides particularly satisfactory for animals, because they are effective in very low concentrations and for various other reasons, but many other substances and conditions can be used in the same way.

In the two modifications of the method we have two ways of testing susceptibility and so of comparing general metabolic activity or fundamental features of it in different individuals and body regions. The first, in which susceptibility is directly determined by means of lethal concentrations, I have called the direct susceptibility method; the second, in which it is determined indirectly through the capacity for acclimation, I have called the indirect or acclimation method. The direct method is simpler and more satisfactory for most purposes, but can be controlled in many cases by the indirect.[3]

By means of these methods, checked and controlled by others, I have been able to demonstrate the existence of axial metabolic gradients as a characteristic feature of the animal organism, and various other lines of experiment indicate very clearly that the physiological axes are fundamentally metabolic gradients.

In my attempt to determine whether such axial gradients are present in plants the direct method was used, with the following procedure. The living plants, freshly collected, were first stained with the vital stain, neutral red, and then were placed in a solution of potassium cyanide in sea water. The neutral red was used as an indicator to make possible the determination of the time of death with some degree of exactness. In the plants thus stained and then killed in cyanide or various other agents of the proper

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[3] These methods and some of their results have been considered in the following publications: CHILD, C. M., 4–10, 11 (particularly chapter iii), 12.

concentration, the first indication of approaching death is an increase in the redness of the stain, indicating an increase in acidity in the cell. After this the red color rapidly disappears, often within a few seconds, or changes to yellow and then disappears. In the more transparent cells the disintegration and coagulation and disintegration of the protoplast, sometimes preceded by a rather sudden plasmolysis, can be observed directly. In these cases the protoplasm, which up to this time has appeared under low magnification to be uniformly stained, suddenly becomes turbid and contracts into a small irregular mass in the cell or a number of such masses scattered over the cell wall. These masses contain all the neutral red as well as the phycoerythrin in cases where it is present, and in consequence of the increased acidity and the aggregation of the protoplasm, these masses usually appear blackish or purplish by transmitted light. Beyond question the moment of this change is approximately the moment of death. The appearance of the cell is completely altered in many cases. Instead of being uniformly red, it now appears nearly transparent, except in cases where the cell wall is also colored, and contains one or more blackish masses or granules. Following these changes the color rapidly disappears from the blackish masses of coagulated protoplasm, leaving the whole cell almost transparent and without color except in the wall. These changes are most readily followed in the transparent hairs or hairlike branches which occur on many of the Rhodophyceae, where they can often be observed with great clearness. It seems probable that at least in some cases the neutral red is decomposed with loss of color in the course of the death changes, but in other cases it becomes yellow before disappearing because the alkali of the KCN solution penetrates as the protoplast dies. These various changes, the deepening red color of the stain, followed by its disappearance with change to yellow in some cases, the visible disintegration and coagulation of the protoplast, and finally in the red algae the loss of the natural color by the extraction of the phycoerythrin are very definite and striking, and afford a means of determining with a considerable degree of exactness the time of death of different cells of the plant and so their relative susceptibility.

After testing various concentrations of KCN, I found that although different species showed considerable differences in susceptibility, a concentration of *m*/100 served the purpose for the forms examined, the survival time in this concentration ranging from a few minutes to several hours.

Neutral red itself, particularly in high concentrations, is somewhat toxic to living cells, and in the course of my experiments I found that a strong solution of neutral red in sea water could be used in the same way as cyanide to determine differential susceptibility, the only difference observed being that death occurs much less rapidly in the neutral red alone than in cyanide.

The work was done at the Marine Biological Laboratory at Woods Hole during August and September 1915. From among the forms available at that time, species with definitely axiate, and in most cases with more or less highly branched thallus and with a more or less definite growing tip (at least during the earlier stages of growth), were selected, because it was desired first of all to determine whether characteristic differences in susceptibility were associated with the visible axes of stems and branches. Most of the forms examined were Rhodophyceae, and it might be supposed that the natural color of these forms would interfere with the use of neutral red as an indicator. As a matter of fact, however, the color of the protoplasm stained with neutral red is distinctly different from the natural color, and there was no serious difficulty in observing the change and disappearance of color in any case, and the protoplasmic changes afforded additional means of determining the time of death in many cases.

For the identification of a number of the species I am indebted to Mr. GUSTAVSEN of the M.B.L. Supply Department. Others were identified with the aid of FARLOW'S *Marine Algae of New England*, supplemented by RABENHORST'S *Die Meeresalgen*. Thus far the growing thalli or parts of them have been examined in 14 species.

The susceptibility gradients

Enteromorpha sp.—Many plants of this species were examined, ranging from stems and branches in very early stages of development to large old thalli which had taken on the "intestinalis" characteristics. Young axes in good physiological condition always show a very definite susceptibility gradient from the apical end basally, that is, death with the disappearance of the neutral red begins at the tip of the stem or branch and proceeds basipetally with a high degree of regularity. The progress of the decoloration and death from cell to cell along the axis can be followed under the microscope. Occasionally a cell here and there or a small group of cells may die earlier or later than the other cells of their level, but in general the progress of death in the basal direction from the apical end is very regular in the younger axes.

In the large tubular axes, however, such a uniform death gradient does not appear. The first cells to die are scattered more or less over the whole length of the axis, or in many cases the death gradient is more or less distinctly acropetal in the more basal portions instead of basipetal as in the young axes.

These differences between the young and the large tubular axes are undoubtedly connected with the differences in the regions of growth and cell division in these different stages. In the newly formed developing axis where there is a more or less definite growing apical region, this is the region of greatest metabolic activity, and therefore a basipetal susceptibility gradient appears. Later, however, the activity of the apical region decreases and is apparently replaced by cell division and growth in the basal region or more or less along the whole axis. This change is associated perhaps with a greater or less degree of physiological isolation resulting from the decrease in activity of the apical region, which no longer dominates other parts to the same extent as earlier, and the earlier orderly behavior disappears. The original basipetal susceptibility gradient disappears, therefore, and is replaced by an acropetal gradient, at least in the basal region, or by a susceptibility mosaic. This method then makes possible an optical demonstration of the different localization of the highest metabolic activity at different stages. With a little care axes in all stages of this change can be found. In some the apical region is still active to some extent and a basipetal gradient extends for a short distance from it, while farther down the axis a susceptibility mosaic may appear, or the basal region may show a high susceptibility.

When the plant is killed by a high concentration of neutral red alone, the same axial differences in susceptibility appear as in death from KCN, although of course death begins later and progresses much less rapidly. The simple experiment of gradually killing the plants by overcrowding in standing water was also performed, and the changes in susceptibility and the progress of death were followed. After two days under these conditions, staining with neutral red showed that longer or shorter portions of the apical regions of the small short branches were dead and would no longer take the stain, while other parts still stained as living cells. In the large tubular thalli practically all cells were still alive at this time. After 5 days in standing water most of the short young branches were wholly dead, but in some a short basal region was still alive. The large thalli at this time showed a mosaic or patches of dead cells, often more marked in the basal region. These crude experiments are sufficient to show that differences in the susceptibility of different regions of the plant to overcrowding in standing water are essentially the same as to KCN. Death progresses in the same way in both cases.

One other point of interest in relation to the physiological age of parts may be mentioned. I have found it possible to use the susceptibility method very widely among animals to distinguish differences in physiological age, the young animals being more susceptible than the old because of their higher metabolic rate (11). In *Enteromorpha* the short branches, evidently in early stages of development, are more susceptible than the larger older branches. Where such short branches arise laterally on a larger stem, death proceeds basipetally in the short branch to the point where it joins the stem, but here it stops for the time being and the adjoining cells of the stem may remain alive for several hours after the branch is dead. The branch is evidently much younger than the stem at its level, and consists dynamically of a metabolic gradient of much higher rate than that of the stem, and this gradient ends rather sharply at its base. The gradient in the branch itself is also in a sense an age gradient, the cells of the apical region being youngest physiologically because of their continued reproduction, while basipetally the physiological age of the cells increases. In the large thalli where growth is no longer apical but more or less generally distributed, cells here and there have undergone more or less dedifferentiation, and so have become younger and resumed reproduction.

Where a thallus has been broken or injured, an area of high susceptibility extends for a greater or less distance, often 0.5 mm. or more from the region of injury. In the younger portions of the thallus where the basipetal gradient is still present, this area of high susceptibility arising from an injury extends farther in the basal than in the apical direction. Evidently this area represents the extent of the stimulation resulting from the injury, or at least the more persistent component of it.

The visible changes in the single cells as death approaches are essentially those described in the preceding section. First the color of the stain deepens, beginning in the young branches at the apical end and proceeding basipetally. Then as the protoplast undergoes disintegration and coagulation at death, the cell contents appear as an irregular blackish mass, but from this the color soon disappears and the dead decolorized cell is yellowish green instead of green under low powers, while under high powers the coagulated masses of protoplasm are visible.

The susceptibility method can undoubtedly be used for analyzing the metabolic conditions in *Enteromorpha* and related forms to a much greater extent than the few experiments described here indicate. As regards the question of metabolic gradients, however, these experiments give a definite answer. They show that the vegetative individual is dynamically in its earlier stages such a gradient, that each new branch is a new gradient and so a new individual, and that with advancing age and under certain environmental conditions the gradient may disappear more or less completely and give place to a mosaic of more active regions scattered over the thallus or more distinct basally.

Ectocarpus confervoides.—In this form the branches which are younger as regards development and the more apical regions of most others show a distinct basipetal gradient, while in the more basal regions of the more advanced parts of the thallus considerable irregularity appears, some cells dying earlier or later than in a regular basipetal gradient. These irregularities undoubtedly indicate the disappearance of the regular gradient in the later vegetative stages, with intercalary growth and cell division in the more basal regions. Here, as in *Enteromorpha*, elongation of the axis beyond a certain length, or decreasing activity of the apical region in consequence of advancing physiological age or for other reasons, may lead to a greater or less degree of physiological isolation of the more basal cells, which then again become active and therefore show a higher susceptibility.

Castagnea tuberculosa.—The complex structure and opacity of the thallus of this form interfered with the use of the susceptibility method, but it was found that the apical regions were most susceptible, and here in many cases the cells actually disintegrated in KCN as they do soon after death in many of the lower animals. Over most of the length of the thallus, however, no definite and constant gradient was observed.

The delicate transparent hairs which arise on the surface of the thallus afford interesting objects for susceptibility experiments. They consist of a single series of elongated cells and are unbranched. These hairs are very highly susceptible to KCN, and in *m*/100 they begin to die almost at once. Most hairs show in general a basipetal susceptibility gradient, but there are frequent irregularities, one or a few cells dying out of the order proper to such a gradient. Many if not all of these irregularities are undoubtedly due to injuries, for the hairs are so delicate that even the most careful handling of the plant is likely to break or bend them, but it may be that other factors are also concerned. The usual susceptibility gradient in these hairs indicates that their growth is primarily apical like that of most plant axes, but it is not improbable that in later stages secondary growth and division may occur in other cells as the activity of the apical region decreases.

Callithamnion (*roseum?*).—This monosiphonous form is very favorable for demonstration of axial differences in susceptibility. Both young plants consisting of a single stem with bipinnately arranged branches and more advanced stages in which branching had proceeded much farther were examined with similar results in all cases. In each branch death begins at the apex and proceeds basipetally from cell to cell. In each bipinnate system of stem and branches death begins at the apex of the stem and the most apical branches, and proceeds basipetally in the whole system, that is, the branches nearest the apex of the stem are most susceptible, those lower down are less susceptible, and so on to the base of the bipinnate system. The apex of the main stem of such a bipinnate system is highly susceptible like the apical branches, but the susceptibility decreases rapidly down the stem, so that at any level the stem is less susceptible than even the basal regions of branches at that level. These susceptibility gradients are remarkably regular and uniform with one characteristic exception. In the older more basal branches and in the stem of each bipinnate system of the thallus it was observed that the basal cell or the two most nearly basal cells were usually somewhat more susceptible and died earlier than the next two or three cells apical to them. In other words, the gradient in such branches is basipetal except for one or two cells at the base. The greater susceptibility of these basal cells suggests the possibility that they are the seat of secondary growth activity in consequence of physiological isolation, as in *Ectocarpus*, but whether this is actually the case or not I do not know. It may be merely that these cells are subjected to greater mechanical stimulation than more apical cells as the branches bend with water movements.

The greater susceptibility of more apical as compared with more basal branches in the same bipinnate system is undoubtedly due to the fact that the more basal branches are physiologically older and their rate of metabolism is lower than that of the more apical branches.

Pleonosporium Borreri.—Only very young plants of this species, consisting of a single unbranched axis of a stem with a few lateral branches, were examined. In every case a distinct basipetal susceptibility gradient was found, both in the main axis and in the lateral branches when they were present.

Ceramium rubrum and *C. tenuissimum.*—The susceptibility gradients in these two species are distinctly basipetal. The small cells of the extreme apical region are distinctly more susceptible than the larger differentiated cells below, but in the differentiated region the gradient is very regular for a distance of several millimeters from the apical ends when the plants are in good condition. In the more basal regions of the older, highly branched thalli, however, more or less irregularity usually appears, death occurring at the same time over lengths of 2 or 3 mm., or certain levels of the stem showing a higher or lower susceptibility than adjoining regions. Many very young unbranched individuals of *C. rubrum*, ranging from only 6–8 to 30–40 cells in length, which were found growing on other algae, were examined *in situ* and therefore in wholly intact condition. In these the basipetal susceptibility gradient was found to be very regular, and in the shorter individuals it extended to the base of the plant, while in some of the longer, some slight irregularity appeared in the basal region. As might be expected from the dichotomous branching in *Ceramium*, the susceptibility of all apical regions from the same general portion of a highly branched thallus was found to be about the same, except that in various cases where one member of a pair of young branches had been retarded in its development, the susceptibility of the retarded member was slightly less than that of the other. Different regions of the thallus, however, may show somewhat different susceptibilities even in the apical regions. For example, the lower peripheral portions of a large, highly branched thallus are often less susceptible than the upper parts, probably in consequence of different environmental conditions. *C. rubrum* is rather resistant to laboratory conditions as compared with other forms; the apical regions retain their susceptibility and the gradients persist with little or no change, even after a day or two in standing water.

The differences in susceptibility along the axis in *C. rubrum* are such that it requires several hours in KCN *m*/100 for death to progress from the growing tip to a level 3–4 mm. below it. In young unbranched plants about 20 cells in length, the progress of death over the whole length of the plant required about an hour in cases observed. In a few cases the progress of death from cell to cell was followed and timed for some distance. In the younger plants and the more apical regions of older axes the length of time between the death of one large cell of the axis and the next basal to it is only 2–3 minutes, while in more basal regions, where the metabolic rate is lower, it may be 15–20 minutes. The collapse and disintegration of the protoplast and the disappearance of color take place in a few seconds. In neutral red alone the axial differences in susceptibility are similar to those in KCN, but death begins much later and proceeds more slowly.

A few observations were made on the transparent hairs which arise from the cortical cells. These hairs contain no transverse cell walls, being merely cell outgrowths. In the long fullgrown hairs the susceptibility gradient is distinctly acropetal, while in those which are apparently still growing, it is basipetal. These differences suggest that while the hair is growing its apical region possesses the highest metabolic rate and the axial gradient is basipetal, but that after growth is completed and the apical activity decreases, the gradient becomes acropetal, that is, from the cell with which the hair is connected. The possible significance of this change will be considered later.

Spyridia filamentosa.—Observations on this form are fragmentary, since only a single thallus, detached and apparently not in perfect condition, was available for examination. Each main branch of the thallus, as well as each of the simple lateral branches which arise from it, shows in general a basipetal gradient; and each such system as a whole shows more or less clearly a basipetal gradient from branch to branch, the most apical branches being most susceptible, the next lower in general less so, and so on.

In the plant examined, however, irregularities in these gradients were frequent, both in single branches and in the whole system. Here and there cells were already dead, while others were more or less susceptible than their neighbors, and the same differences appeared between different branches in some cases. These irregularities probably indicate that the plant was not in good physiological condition; as previously noted, it was detached when collected and had been washed about by the waves, so that some of the cells had undoubtedly been injured or killed. The susceptibility of this species to KCN and to neutral red is in general very high, death beginning almost at once in KCN *m*/100, and within half an hour to an hour in neutral red alone. Since I have found that high susceptibility to the cyanides in animals indicates in general high susceptibility to at least many other depressing conditions, I am inclined to regard this species as rather sensitive and readily injured physiologically, and such a high susceptibility would readily account for the irregularities observed in the death gradient.

Champia (*Chylocladia*) *parvula*.—Various stages of development, ranging from unbranched to large much branched thalli, were examined. In general, death begins at the apical end of each axis. A short terminal portion, evidently representing a growing tip, dies first, and then, after a more or less distinct interval, death proceeds slowly basipetally. In the younger, shorter axes the gradient is usually very regular over most of the length, and in the older axes over the apical 2–3 mm. Farther basally, however, irregularities appear in cells or cell groups. Whether these irregularities indicate physiological isolation and secondary growth in these basal regions, or whether they are the result of injuries or other external factors, I do not know. They are certainly more frequent in the longer, older axes than in the shorter, younger ones. In general also the younger axes, or at least their more basal regions, show a higher susceptibility than the older axes, so that while the death of the growing tip may occur at about the same time in both, the basal region of the younger branch may die considerably earlier than that of the older branch.

The transparent, unicellular hairs which develop on the thallus usually show an acropetal susceptibility gradient in the cases examined, like the hairs of *Ceramium rubrum*, although in some hairs the gradient is basipetal and in some death begins at both ends and proceeds toward the middle. Further observations are necessary to determine with certainty the meaning of these differences, but it seems probable that, as in *Ceramium*, the growing hair may have a basipetal gradient, the full grown hair an acropetal gradient, and that those in which death begins at both ends are stages in the change from one condition to the other. It may be, however, that stimulation or injury is responsible for these differences in some cases at least.

Chondriopsis (*Chondria*) *dasyphylla* var. *sedifolia*.—In young unbranched or slightly branched plants in good condition and usually in the more apical regions of the branches in older much branched thalli, the susceptibility gradient is basipetal. Irregularities in the course of death and acropetal gradients observed in some cases probably result from injury or bad environmental conditions which affect the more active regions to a greater extent than the less active regions, or they may indicate perhaps the completion of apical growth in an axis. In general the physiological ages of different branches on a single axis are indicated by their susceptibility, the smaller less advanced branches being the more susceptible.

In some of the plants examined cystocarps were present. In the sterile wall of the cystocarp the gradient is basipetal, as in the vegetative portions of the plant. Differences in susceptibility in the cystocarp contents, indicating differences in physiological age, were also observed. No attempt was made to distinguish the various stages in the development of the carpogonium and carpospores, but it was merely noted that the susceptibility of the fully developed carpospores was much less than that of the cystocarp contents in the earlier stages. This difference indicates that the carpospore has the low metabolic rate characteristic of physiologically old cells.

The transparent hairs which arise in tufts at the apical ends of the thallus branches are beautiful objects for the observation of the susceptibility gradient. Each hair is monosiphonous and dichotomously branched, and the susceptibility gradient in each hair as a whole is very distinctly and uniformly basipetal, the apical cell of each branch dying first and death progressing basipetally from cell to cell in regular order.

In each single cell of the hair, however, the susceptibility gradient is almost invariably acropetal. The disintegration of the protoplast and the appearance of the dark masses resulting from coagulation begin at the basal end and proceed very uniformly to the apical end of the cell, the progress of death over the whole length of the cell often requiring 2–3 minutes. With rare exceptions the death of each cell is completed before that of the next cell basal to it begins.

My observations were limited to hairs which were apparently full grown or nearly so, and only further work can determine whether young actively growing hairs will show the two opposed gradients or not. Discussion of the facts is postponed to the general section of the paper.

Polysiphonia variegata and *P. fibrillosa*.—In *P. variegata* the general axial gradient is clearly basipetal in each axis, with a few irregularities in freshly collected plants. The transparent, dichotomously branching hairs also show a basipetal gradient, both in the hair as a whole and in the single cells, so far as observed. After a day or two in the laboratory irregularities in the susceptibility are much more frequent.

Cystocarps in various stages of development were present on some of the plants examined, as in *Chondriopsis*. The cystocarp wall shows a basipetal gradient. In general the susceptibility of the cystocarp, except in the earliest stages of its development, is lower than that of the cells of the vegetative axis from which it arises, and it was further noted that the region of the branch to which the cystocarp is attached also shows a lower susceptibility than adjoining regions of the axis. This low susceptibility may extend basipetally over 4 or 5 segments of the thallus, and acropetally over 2 or 3, and is more marked in connection with more advanced than with earlier cystocarps. Apparently the cystocarp in its later stages, together with a portion of the vegetative thallus in the region of its attachment, represents a region of lower metabolic activity than the adjoining vegetative regions.

As in *Chondriopsis*, the cystocarp contents in the earlier stages are much more susceptible than the fully developed carpospores which are the least susceptible portions of the whole plant on which they occur. Apparently metabolic activity in the carpospores is relatively slight.

P. fibrillosa is in general much more susceptible then *P. variegata*, and after a few hours in the laboratory the course of death along the axes is very irregular. In freshly collected plants, however, a basipetal gradient usually appears clearly in the more apical regions, but even here the irregularities are very frequent a few millimeters from the tips, and sometimes death occurs first at some level below the tip. I think it probable that these irregularities result from stimulation or injury of the apparently very sensitive cells in collecting and handling. The great difference in susceptibility between the two species is evident from the fact that in *P. fibrillosa* death begins almost at once and is usually completed within a half hour or less in KCN *m*/100, while in *P. variegata* even the more apical regions live in the same concentration from half an hour to an hour, and the less susceptible parts die only after several hours.

Dasya elegans.—In both the polysiphonous axes of the thallus and the very numerous monosiphonous, dichotomously branched secondary branches, the susceptibility gradient is in general distinctly basipetal. In the secondary branches, however, irregularities appear, chiefly near the base. As in *Callithamnion*, the basal cell or the two most basal cells of the older branches very often die before those above them. The suggestion made in the case of *Callithamnion* may apply here also, namely, that these cells are more stimulated mechanically than those above them by the movements of the branch with the water currents. It may be, however, that some degree of physiological isolation of the basal region has occurred in the older branches.

A basipetal gradient also appears in the system of secondary branches on an axis as a whole, the most apical branches being most susceptible, those lower down less so, and so on. This is true even for the apical regions of the secondary branches, and indicates their differences in physiological age, corresponding in general to their level on the axis, the most apical being the youngest, the most basal being the oldest.

The elongated stichidia present on some of the plants examined also show a basipetal susceptibility gradient, and the gradient in development of the tetrasporangia in the stichidia is paralleled by a susceptibility gradient, the most basal and so most advanced tetraspores being the least susceptible parts of the plant on which they occur.

Discussion

The observations on these 13 species indicate, so far as they go, that in each definite vegetative axis of these forms, at least in plants that are in good condition and not too old, a more or less definite susceptibility gradient exists. To these may be appended some fragmentary observations on the blue-green alga *Calothrix*, which indicate that even in this simple form a basipetal susceptibility gradient exists. Although the observations are incomplete at many points and further work on other stages and species is necessary, the uniformity of results is striking and cannot but suggest that the order observable along these axes and the susceptibility gradients are in some way associated. If the susceptibility method is of any value as an indicator of general metabolic or oxidative activity, the susceptibility gradient represents a gradient in metabolic or oxidative activity, the most susceptible regions being the most active. If this be true, the axes of these simple plants possess primarily the same metabolic characteristics as the axes of animals in this respect. Such a fundamental resemblance or rather identity as this must possess some fundamental significance, and in the introductory section of this paper my views concerning this significance are briefly stated. Many lines of evidence have forced me to the conclusion that this axial metabolic gradient is the basis, the starting point of the order in space and time which is characteristic of the axes of organisms. In short, according to this point of view, the axis, whatever else it may become during development, is fundamentally a metabolic gradient. The order along the axis is primarily an expression of this gradient. The organic or physiological individual, as distinguished from other kinds of individuality which may exist in the organism and in the inorganic world, consists fundamentally of one or more metabolic gradients. The various aspects of this conception of the organic individual and their bearing on biological problems have been considered elsewhere (12), and it is impossible here to do more than note the complete agreement with the general conception of these observations on plants.

From this point of view there is a fundamental identity in the apical regions of plant and animal organisms and in their relations to other parts. I have suggested elsewhere (12, pp. 189–192) that the fact that the apical region of the animal gives rise to the highly specialized and physiologically stable cephalic nervous system or brain, while that of the plant remains indefinitely or for a long time more or less completely embryonic, must be connected with certain differences in the metabolic processes in the two groups. Morphological structure in the animal is to a large extent primarily colloid and protoplasmic, while in the plant the structural substances are mainly carbohydrates, and the protoplasm itself shows little permanent or persistent structure. Apparently in plant metabolism the proteids and other protoplasmic substances produced are less stable under physiological conditions than in the animal, and so do not persist as structure but are continually undergoing change and reconstitution, particularly in regions of high metabolic activity. In the animal, on the other hand, some relatively stable molecules arise even in regions of the highest activity; consequently morphological structure appears, even in these regions, and such structure is more stable than structure developed in regions of lower metabolic rate. Because of these differences the apical region of the animal undergoes differentiation, and its relations to other parts determine that this differentiation shall take the form of a transmitting system, the nervous system, which brings about a high degree of physiological integration, while in the plant the apical region usually remains embryonic or differentiates very slowly. Transmission exists only in relatively primitive form and the degree of physiological individuation in the plant remains low.

In the unicellular hairs of *Ceramium* and *Champia* the susceptibility gradient is usually acropetal in the full grown hairs, but apparently basipetal in the growing hairs and in the multicellular branching hairs of *Chondriopsis* the gradient of the hair as a whole is basipetal while that of single cells is acropetal in the full-grown hairs. In the multicellular hairs of *Castagnea* the gradient is usually basipetal, but a distinct intracellular gradient could not be distinguished. Further work is necessary for positive conclusions, but a suggestion is perhaps permissible. These hairs of course are incapable of photosynthesis and are dependent, therefore, on other parts of the plant for nutrition. The course of nutritive substances must therefore be acropetal in direction in them. In view of the facts available, it seems probable that during the growth of the hair the apical region has the highest metabolic rate and the gradient is basipetal. Under these conditions each level lives, so to speak, at the expense of the level or levels basal to it. At this stage the gradient is probably basipetal throughout, at least in some cases; but as senescence progresses and the activity of the apical region decreases, the nutritive factor may become more important in determining the metabolic rate at different levels. Since the direction of movement of nutritive substance must be acropetal in each cell, the basal portions of the cell are most favorably situated and perhaps therefore show a higher metabolic activity with an acropetal gradient, provided relations to more apical cells do not obliterate or reverse it. In this way a gradient in the cell opposite in direction to the gradient in the hair as a whole may arise, at least in the later stages of development. It may be that in some of the multicellular hairs this condition is present from the early stages of development, but the fact that in the unicellular hairs of *Ceramium* and *Champia* the gradient is basipetal in what appear to be the earlier stages of development, and acropetal in the fully developed hairs, indicates that such change may occur. It remains, however, for future investigation to determine whether these suggestions are correct.

Attention has been called incidentally to the differences in susceptibility which apparently indicate differences in physiological age, such, for example, as the lower susceptibility of fully developed as compared with small developing axes, and the low susceptibility of the carpospores. The basipetal axial gradient itself is in a sense an age gradient, for the physiological age of the cells increases from apex to base, the apical cell or cells remaining physiologically young because of continued division and cell reproduction, and the other cells differentiating and growing old more or less rapidly according as they divide less or more frequently. We may say in fact that the axial gradient is a more or less definite gradient in cell behavior, and it is this which must be accounted for. It is here, as I believe, that the physiological dominance of the apical region plays a rôle. This region is the first part of the axis to appear, and it originates as a region of high metabolic activity, either in consequence of physiological isolation from other apical regions or by the local action of external factors. After it has once arisen it becomes, as I have endeavored to show, the chief factor in determining the metabolic rate in the other regions of the axis to which it gives rise, so far as they are within the range of its influence. Since the changes transmitted from it to other regions undergo a decrement in energy or effectiveness in the course of transmission, a more or less definite gradient in metabolic activity and therefore in behavior of the cells results. The more active cells divide more frequently and differentiate and grow old less rapidly than the less active cells. The apical region itself, as the most active region of all, may remain embryonic and physiologically young indefinitely if the processes of senescence and rejuvenescence balance each other in it; but if this is not the case it may gradually grow old, though senescence is less rapid in it than in other parts because of the higher metabolic rate. According to this conception, therefore, an apical region arises wherever the metabolic activity of a cell or a cell group, or in unicellular forms of a part of the cell protoplasm, becomes high enough to enable it to dominate or control to some degree the metabolic activity of other cells within a certain range. When the apical region has arisen, the development of the plant or animal individual proceeds as the specific constitution of the protoplasm and the conditions determine.

As soon as differences between different parts along the gradient arise, chemical correlation, that is, the influence of substances produced in one part upon another, must begin to play a part, and this is unquestionably a factor of great importance in determining the further course of events along the axis. It is evident, however, that chemical correlation cannot occur in an orderly way unless orderly differences already exist at different points or levels, and I have endeavored to show how these differences may find their origin in the axial metabolic or irritability gradient (12). If my conclusions are correct, the physiological axes in both animals and plants consist in their simplest form of such a gradient, and it is the interrelation or correlation in this gradient which determines the orderly behavior in relation to this axis.

Summary

1. In 14 species of marine algae, including 10 species of Rhodophyceae, 2 of Phaeophyceae, 1 of Chlorophyceae, and 1 of Cyanophyceae, a more or less definite gradient in susceptibility to KCN exists in the vegetative axes, and in several species in other parts also; the susceptibility being greatest, at least during growth and development, in the apical region and decreasing basipetally.

2. Since extended experiment with the lower animals indicates that the degree of susceptibility to cyanides and to many other agents and conditions is in a general way, and within certain limits, a rough measure of metabolic activity or of certain fundamental metabolic processes, probably primarily the oxidations, these axial differences in susceptibility in the algae are regarded as indicating the existence of axial metabolic gradients. If this conclusion be correct, the axis in these simple plants is identical with the axis of the animal organism in this respect.

3. The relations of physiological dominance and subordination, of physiological isolation and of differentiation to metabolic gradients, are briefly considered.

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THE VASCULAR ANATOMY OF PIPER METHYSTICUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 218

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(WITH TWENTY-THREE FIGURES)

Historical

LINNAEUS placed the Piperaceae in the monocotyledons among the Aroidaceae; JUSSIEU held that they were near the Urticaceae; KUNTH (8) and BLUME ranked them with the monocotyledons. DE CANDOLLE (4), however, following JUSSIEU, stated that the embryo characters indicated their dicotyledonous character. ENGLER and PRANTL (5) put the tribe Piperales among the lower Archichlamydeae. Included in it are 4 families: Saururaceae, Piperaceae, Chloranthaceae, and Lacistemaceae. The genus *Piper* contains over 1,000 species. MIQUEL (10) separates 7 species from the original classification on the basis of the presence or absence of his so-called "stipula oppositifolia" and persistence of the petiole. Among these is *Piper methysticum* Forst., which he calls *Macropiper methysticum*. ENGLER and PRANTL (5), using as a basis of their classification of these two genera the method of branching in the floral regions, retain the name of FORSTER.

Piperaceae have attracted much attention because of their anomalies, both in anatomical and reproductive features. The former have been described in many species. MIQUEL (10) asserted that the internode of the stem ends blindly in the petiole, and the next internode develops from a bud between the petiole and the end of the foregoing node. He stated also that there are alternate, opposite, and whorled leaves on the same branch. The latter he concluded were atrophies, and the number of internodes which would have been produced would have been the same number as the whorls of leaves. SANIO (11) described in detail the structure and the course of the bundles in the stem of *Peperomia blanda*. SCHMITZ (12) verified the work of SANIO in *Peperomia*. He concluded that the peripheral bundles in *Piper* were joined at their base with the inner bundles of the stem. The small bundles of the leaf trace come from branches of the peripheral bundles. They, as well as the central bundles, go with or without branching into the petiole, which is merely a continuation of the stem.

In an elaborate discussion of the vascular anatomy of the Piperaceae, WEISS (19) deals with 11 species of *Peperomia* and 8 of *Piper*, and draws the conclusion that in the Piperaceae there is a transition from the monocotyledonous to the dicotyledonous type of bundle.

By far the most detailed account is that of DE BRAY (3). He used 4 species of Saururaceae, 16 species of *Piper*, 3 species of *Artanthe*, and 11 species of *Peperomia*. In *Piper* he found 2-4 rings of bundles arranged in two systems, the peripheral and the pith systems. The peripheral bundles are of two sizes; the outer ring is completed by an interfascicular cambium; the leaf trace comes from a peripheral ring, which in the node below is a branch of the central bundles; the vascular supply of the axillary buds is from both peripheral and central bundles. He compares the families of the group, but shows no relationships.

In VAN TIEGHEM'S (17) work on the mucilage canals of *Piper* he describes in detail the stem of *Piper nigrum*. He found that there are two circles of bundles; that the pericycle becomes lignified late in the growth of the stem; that the cambium for a long time cuts off segments only on the side toward the pith, and may cut off very late a few segments on the other side; that the mucilage canals vary in location with different forms and are not continuous through the nodes; and that the canal of the petiole is not continuous with that of the branch.

The present study of *Piper methysticum* was taken up with the idea of giving a modern interpretation to the vascular structure, and to ascertain whether the stem keeps pace with the gametophyte of the group.

Material

I am indebted to Dr. W. J. G. LAND for the material for this study. It was collected on the Island of Tutuila, Samoa (fig. 1). The plant is a native of the South Sea Islands, and is commonly called *Ava* or *Kava*, the natives extracting from its roots the national beverage bearing that name.

It becomes a shrub 1–1.5 m. in height and is propagated mostly by cuttings. No seedlings were obtainable. The leaves are ovate, 18–25 cm. in diameter, with a deep cordate base and a short accuminate apex. The blade is bright green, smooth above, and bears fine multicellular hairs on the veins beneath. It is distinctly petioled, the petiole having a sheathing base and a deciduous stipule. The leaves are alternate and the dense flower clusters are borne opposite them. The internodes are 7–10 cm. long, the nodes being swollen, smooth, and in the older ones irregularly spotted. Both old and young stems have an herbaceous appearance. The largest nodes examined were 2.75 cm. in diameter, and the smallest were 2 mm.

FIG. 1.—*Piper methysticum* growing in Samoa

For the sake of comparison *Piper umbellatum* (*Heckeria umbellata* Kunth) was used, the material being obtained from plants brought from Mexico. In shape and size the leaves resemble those of *Piper methysticum*. The petiole has a sheathing base;

the nodes are swollen and marked with several distinct rows of spots; the internodes are 5–9 cm. long and when young are covered with dense rows of hairs, giving a ridged appearance; the older internodes are smooth but spotted.

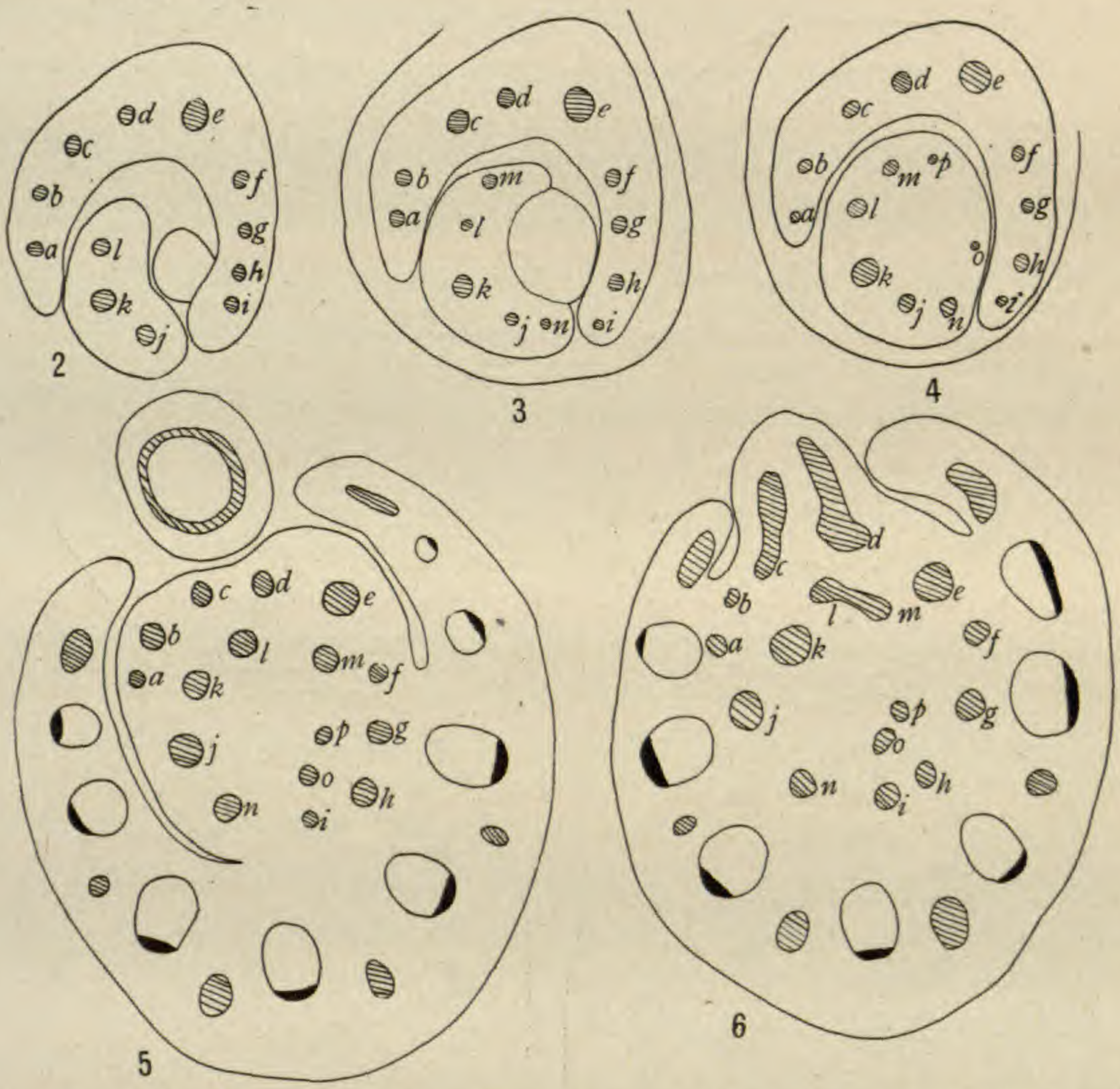
The younger stems and nodes were cut in paraffin at 25–30 μ and complete series obtained. The largest stems and nodes were treated with hydrofluoric acid for one week and then sectioned at 30–50 μ with a sliding microtome, in this case also the series being complete. The safranin Lichtgrün stain was used.

Origin and course of stem bundles

The stem of *Piper methysticum* contains at maturity three rings of bundles, one peripheral and two within the pith, the bundles being foliar. The terminal bud is enveloped in the sheathing base of the youngest leaf. In some cases a bud was found within the sheath of a single leaf with as many as 3 leaves, each of which was inclosed in the base of the petiole just beneath it.

The leaf primordium appears on the stem tip early and forms about it a semicircle, and 5–7 procambial strands are soon differentiated. These connect with the first strands of the stem (figs. 2–4, *j–p*). Strands *j–p* are the strands of the youngest leaf (node 1); *k* is the first to be differentiated; *j* and *l* follow quickly; *o* and *p* come last. The bundles of the second leaf (node 2) are represented by the series *a–i*. At node 1 the stem contains a single row of scattered bundles. These bundles (*j–p*) become the inner bundles of the stem at node 2, and *a–i* of leaf 2 become the peripheral bundles (fig. 5). Through the following internode these two sets of bundles run approximately parallel and become the inner rings of the next node (node 3, fig. 6). The union of the two inner rings takes place here. Five methods of joining were observed in the case of the undifferentiated strand: (1) two strands from the same ring may join (fig. 8, *lm*); (2) two strands from different rings (fig. 7, *ak*); (3) one strand may divide and join a strand of a different ring (fig. 7, g^1p); (4) two strands from the same or different rings may unite and then join a branch of a strand from either ring (fig. 9, *efg*, *ino*); (5) three strands may join, the result of this, for a short time, being a single inner ring (figs. 9, 10, *dlm*).

The peripheral bundles at any node may branch. In older nodes this branching may occur just before the peripheral bundles become pith bundles, and part remain in the peripheral ring; the latter will then connect with the bundles of the leaf at the node below (fig. 19).



FIGS. 2–6.—Transverse section through a terminal bud of *P. methysticum*, showing the course of the bundles; ×168.

The coalescence of the branches of these bundles does not always occur at the node, but may occur above or below it, as follows: (1) branches of the same bundles may join before passing through a node; (2) branches from different bundles may unite; (3) branches of a peripheral bundle of the internode above may join a peripheral bundle of the internode below. Not infrequently strands isolated in the anastomosing of the pith bundles at any node may join the peripheral bundles at that node. The peripheral bundles of any internode, therefore, when young will consist of one set of foliar bundles only; and when older may contain portions of the foliar bundles of the leaves of 3 nodes.

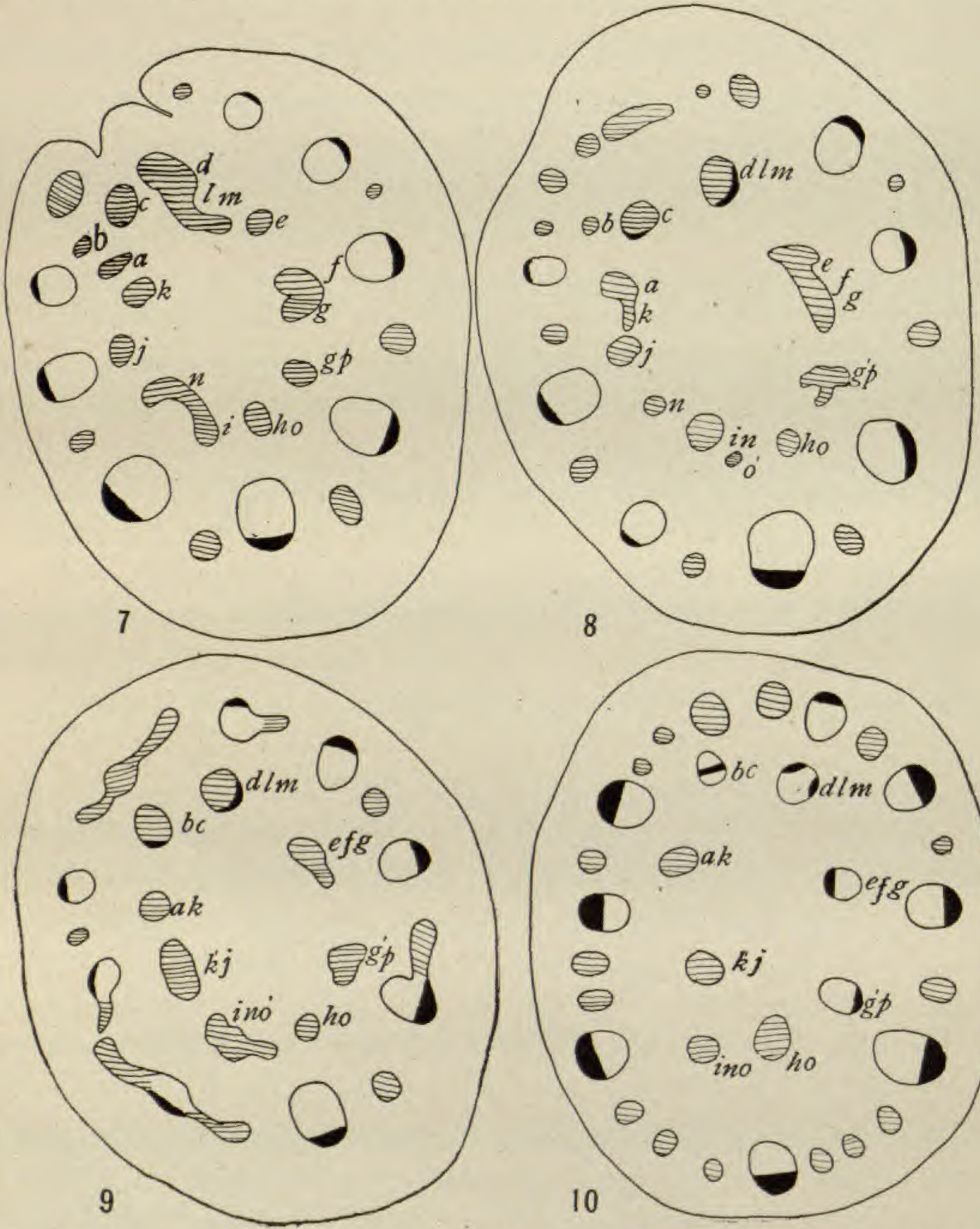


FIG. 7–10.—Transverse sections through a terminal bud of *P. methysticum* showing course of bundles; sections follow those of figs. 2–6; ×168.

The node in which the strands have become differentiated presents a most complicated appearance. One often finds for a short time xylem entirely surrounding phloem, and vice versa. Bundles may join with either the phloem or xylem together. They may lie side by side and union may occur either in the xylem or in the phloem. Not infrequently the xylem of two bundles may unite, and the phloem of a third join the phloem of either of the others. Strands become separated from bundles and several of these may coalesce to make a bundle, or they may join with other bundles. One often gets cross-sections and longitudinal sections of the same bundle in the same section.

The bundles in one internode are in the periphery, and through the following two internodes are pith bundles. The position in the pith often gives the appearance of two complete rings and one-half of a third ring, when the number of bundles is large; but careful study shows that there are really only two rings. The number of bundles reaches 45–50 in the pith.

Description of stem in detail

As previously stated, the stem of *Piper methysticum* has two sets of bundles which may be designated as the peripheral ring and pith or medullary rings, two in number, and an irregular half-ring which gives the appearance of a third ring at times. The bundle type is open collateral endarch, oriented for the most part in the usual manner. Sometimes, however, one finds the protoxylem points appearing in reverse orientation (fig. 10, *e–g*). Union is often incomplete and one finds double bundles persisting in the internode, and these may be of two types. The xylem points or the phloem points may be together (figs. 11, 12).

The peripheral ring of bundles is formed of two sizes: the primary, those differentiated first; and the secondary, termed supplementary by DE BRAY (**3**) (fig. 13). The secondary bundles are branches of the primary bundles and lie between them. More than one primary bundle may contribute to a secondary one (fig. 9); that is, two bundles may branch and the branches join. The number of secondary bundles varies from none to 3, according to the age and size of the stem. Near the tip there are none (fig. 4), as observed by SCHMITZ (**12**).

The inner rings of bundles are approximately the same size. This does not differ from the majority of the species of *Piper*.

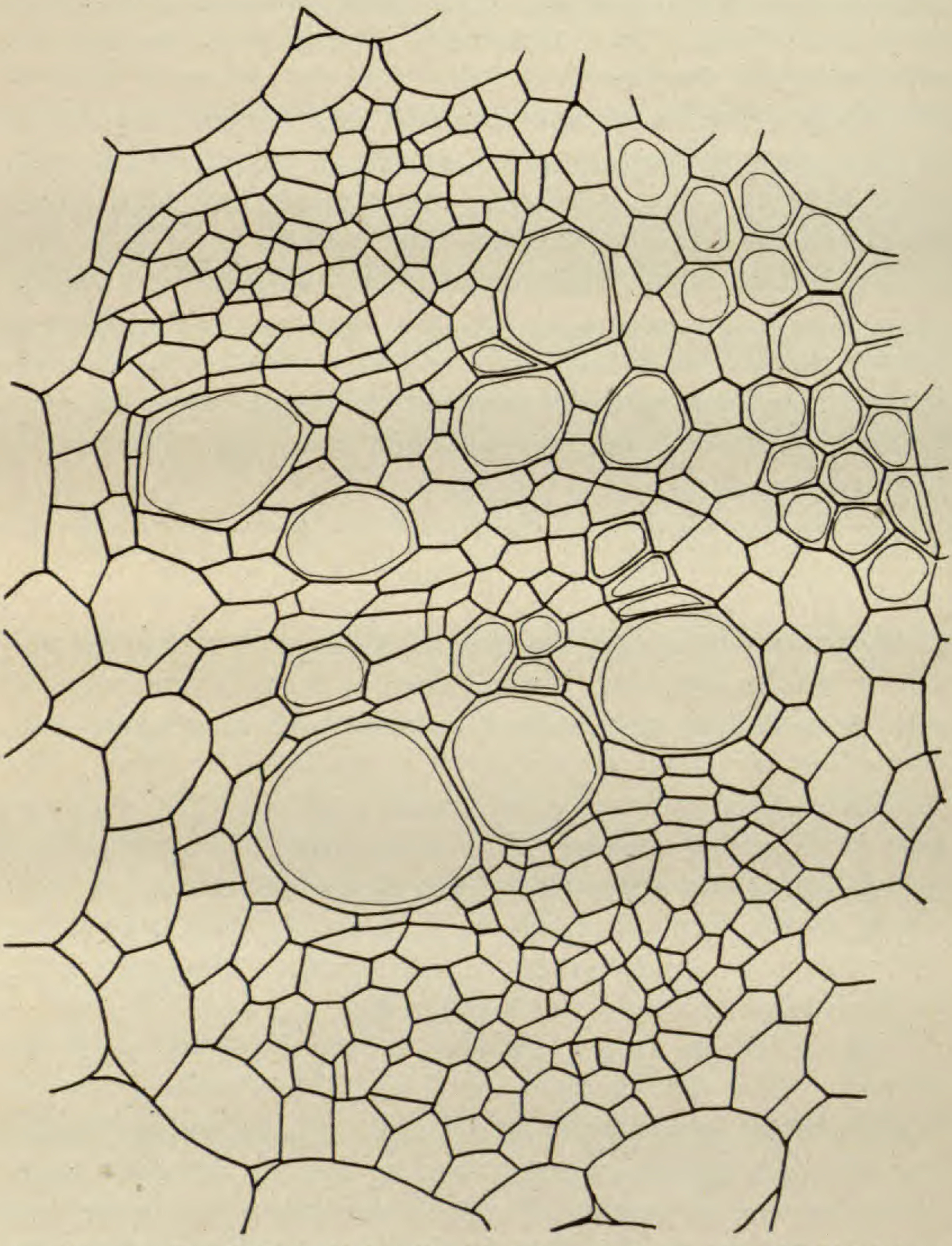


FIG. 11.—Detail of a double bundle with phloem on the outside and lignified tissue at the side; ×820.

Between the pith and the peripheral ring of bundles in the young stem is a continuous band of parenchyma, 4–6 rows thick, which early becomes lignified (figs. 13, 14). Later, owing to growth of the bundles, this band is broken and patches appear only at the inner portions of the bundles (fig. 18).

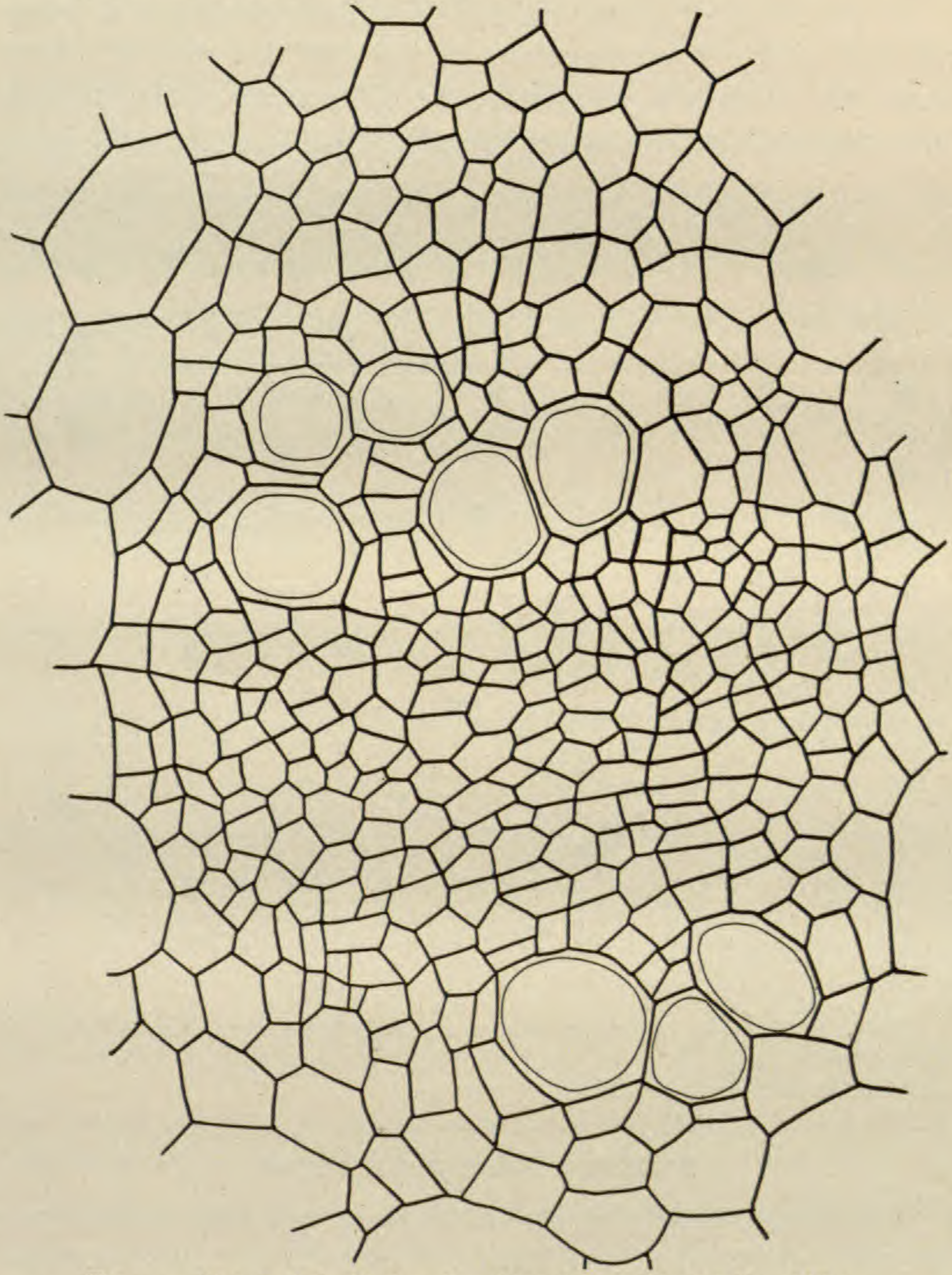
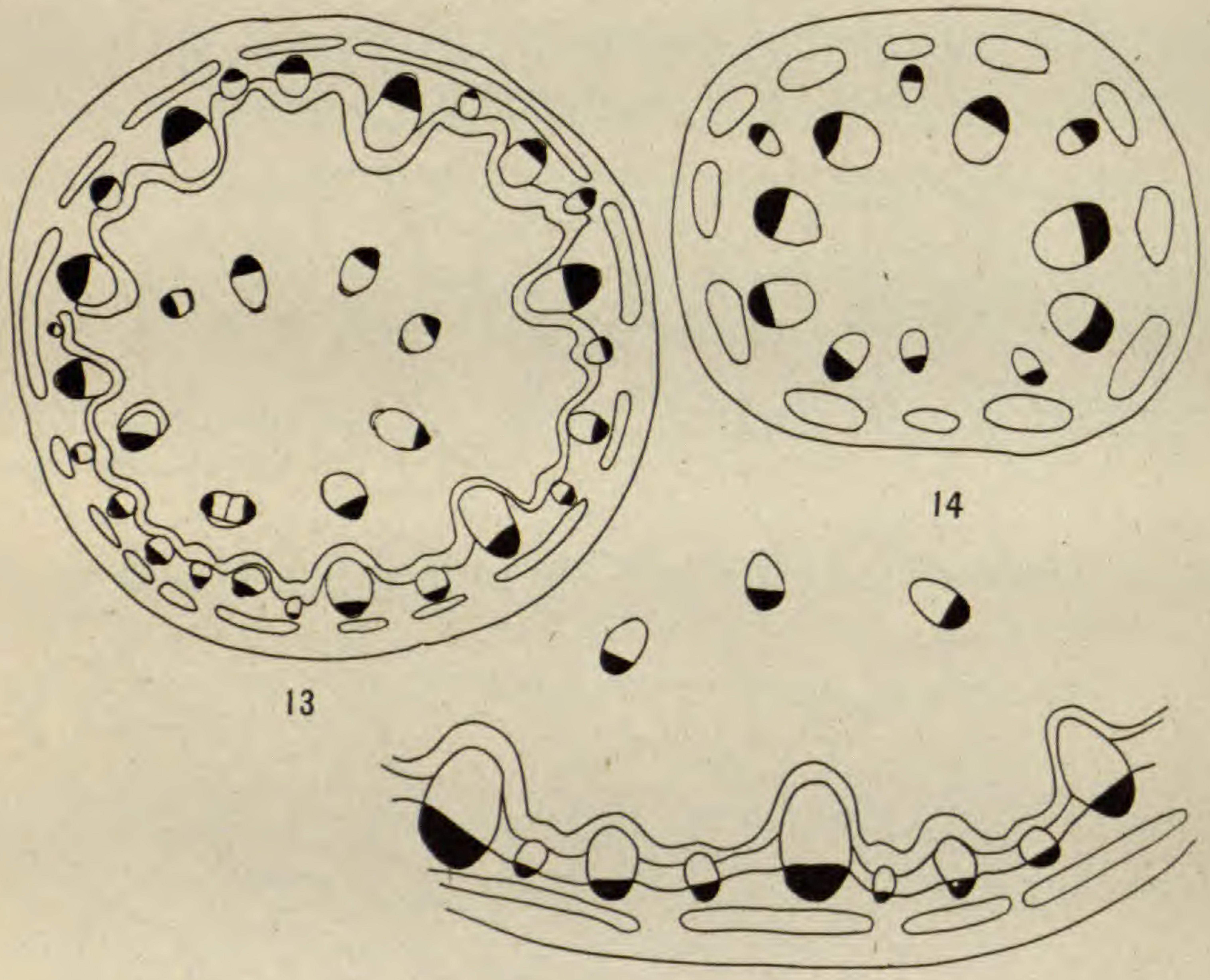


FIG. 12.—Detail of double bundle with xylem points outside; ×820

The pericycle consists of only a few cells (fig. 16) just outside the bundles in both the peripheral and pith systems, and nowhere else. It can be distinguished easily in the young stems by the dense cell contents. Late in the history of the plant the pericycle becomes lignified. There is no differentiated endodermis in either old or young stems.

In the cortex of the young stem is a continuous band of tissue 7–8 cells wide, which responds to the test for suberin (TUNMANN **16**). This is in patches in the older stems. The intercellular spaces of the pith are very large.



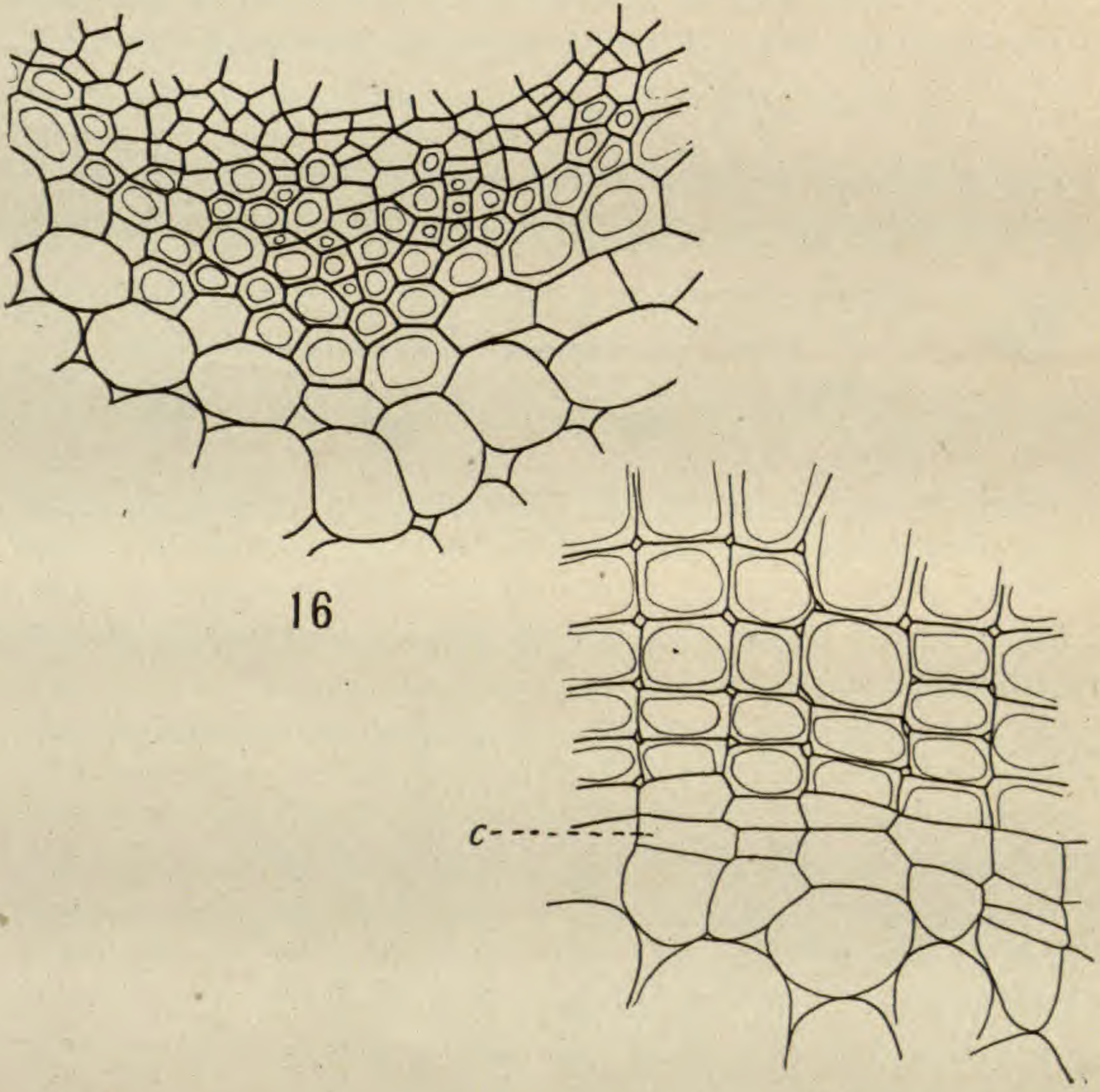
FIGS. 13–15.—Fig. 13, young stem of *P. methysticum* after differentiation has been completed, ×80; fig. 14, petiole, ×101; fig. 15, sector of stem after interfascicular cambium has been developed, ×101.

Secondary activity in the stem

There are two methods by which the stem of *Piper methysticum* enlarges: (1) by active division of pith cells; (2) by cambail activity.

1. In the center of and at irregular intervals throughout the pith, groups of cells will divide actively throughout the internode.

2. Each bundle of both the peripheral and pith rings has a cambium. The inner ones do not enlarge greatly. The outer or peripheral bundles lay down a ring of wood often 4–5 mm. in thickness. The proportion of xylem to phloem is about 8:1 (fig. 18).



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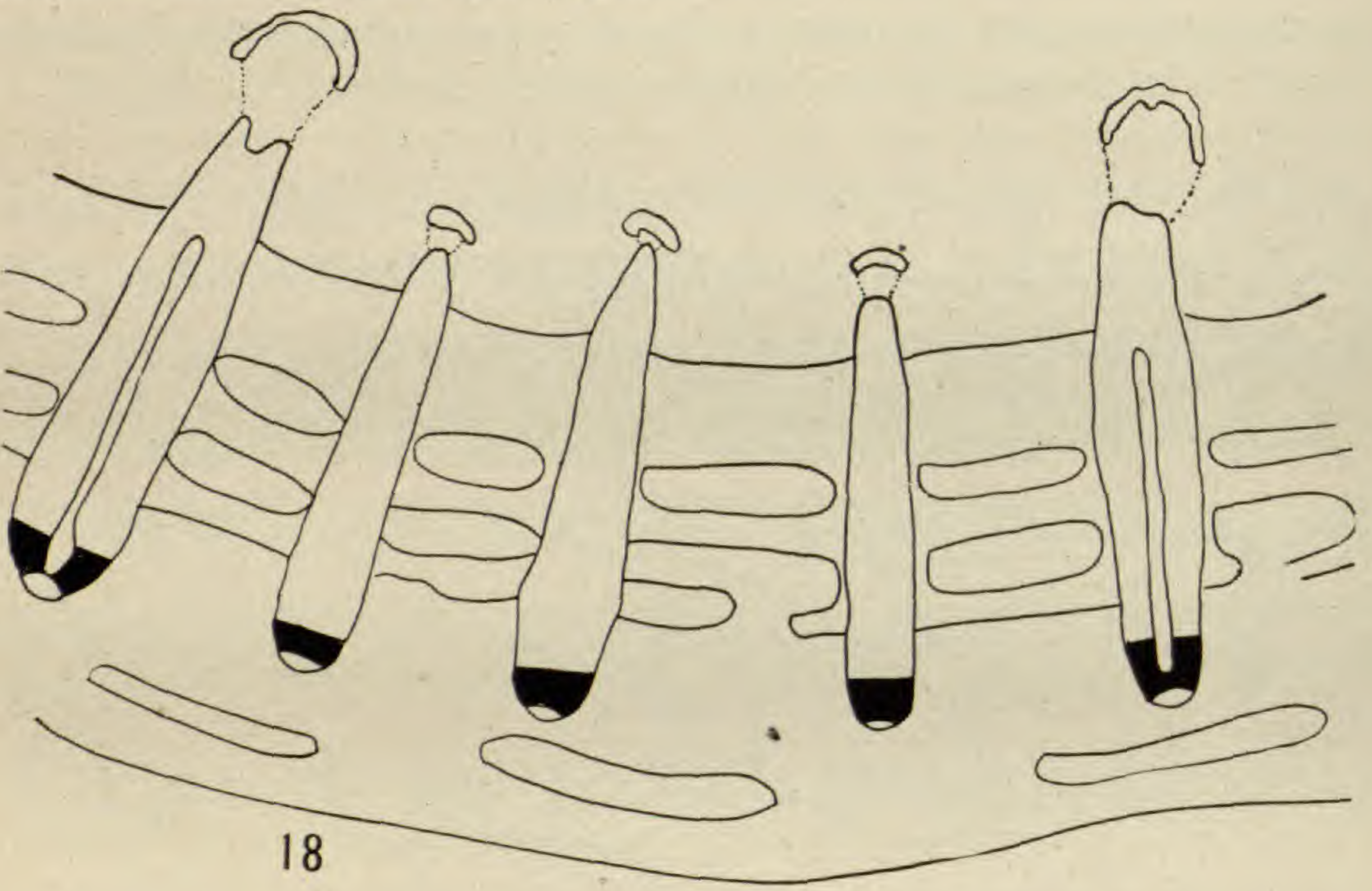
FIGS. 16, 17.—Fig. 16, detail of pericycle of *P. methysticum;* fig. 17, detail of medullary ray; *C*, cambium; ×820.

Late in the history of the stem an interfascicular cambium appears (fig. 15), which cuts off segments on the inside only; very late a few segments may be cut off on the other side (fig. 17). This closes the stem cylinder. The interfascicular cambium appears much later in the nodes and remains active much longer.

VAN TIEGHEM calls attention to the one-sided action of the cambium in his work on *Piper nigrum*.



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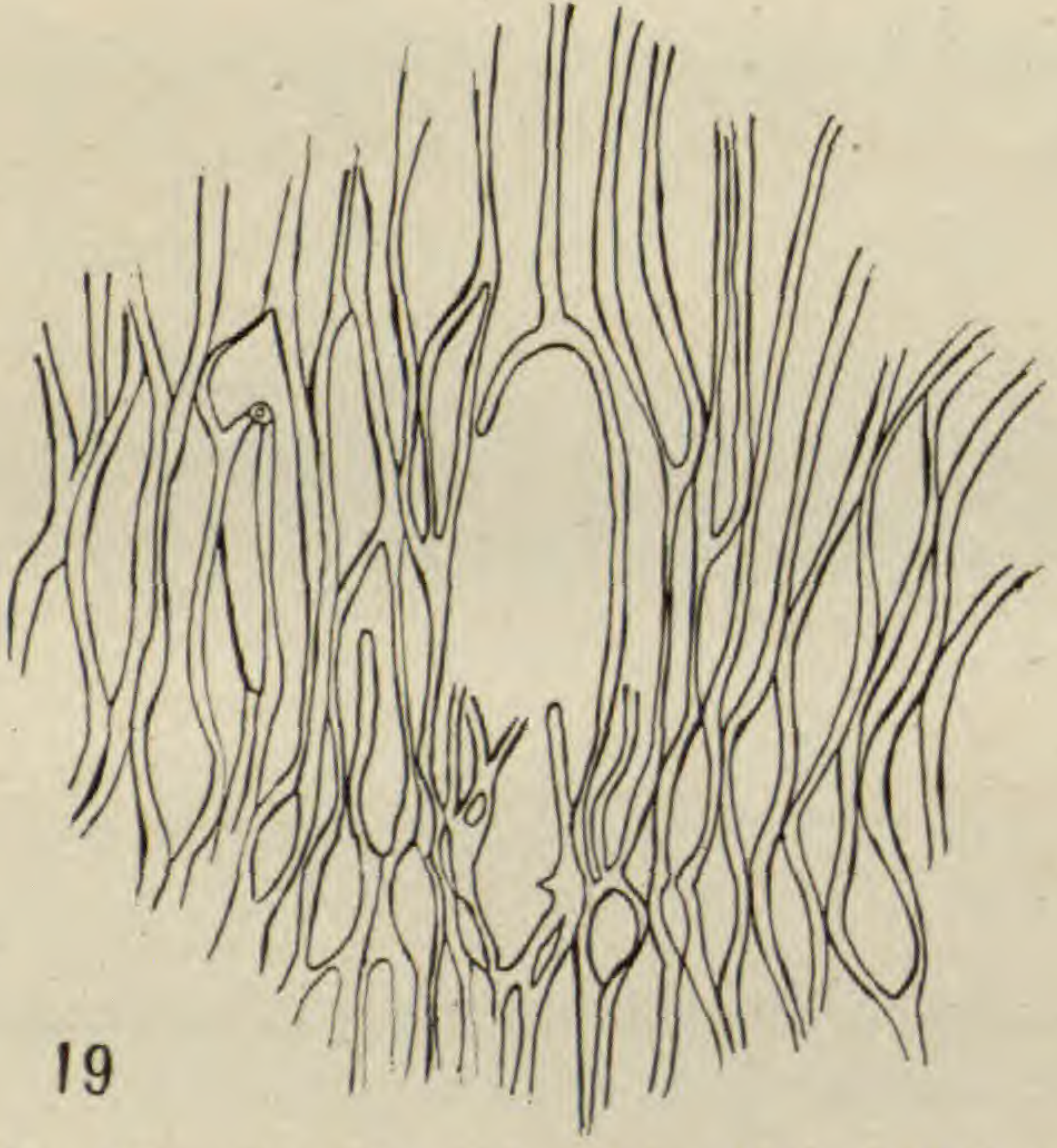

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FIGS. 18, 19.—Fig. 18, section of peripheral ring of the bundles of *P. methysticum* at a mature node, showing banded appearance of the rays, ×35; fig. 19, tangential section of peripheral bundles, showing branch gap; ×7.

The medullary ray becomes lignified and in longitudinal section shows pitted cells. At the node in the largest stems available, cells next to the cambium do not lignify and remain active. These are also scattered at irregular intervals throughout the ray. In section this gives the ray an irregular banded appearance (fig. 18). There are no growth rings. Much of the wood parenchyma touching the pith retains its activity at the node and divides long after secondary wood has been laid down. This pushes the primary vessels of the bundles apart at great distances, as well as the cells of mechanical tissue (fig. 18).

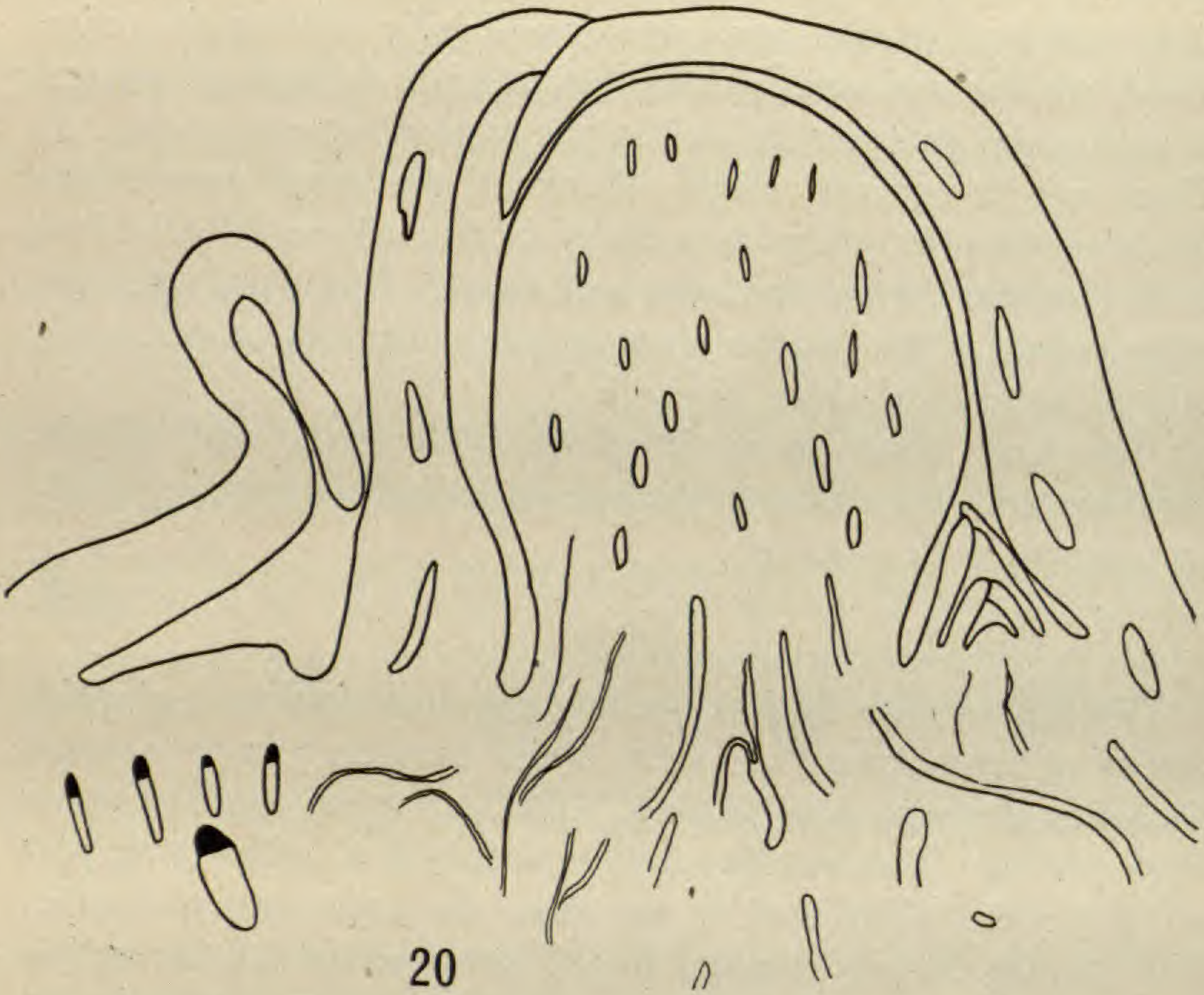
The stem when young is packed with starch and piperin. Mucilage cells are scattered throughout it, and the buds especially are covered with mucilage.

Leaves

The leaves have distinct petioles, the sheathing bases of which extend entirely around the stem, which becomes narrow opposite the point of insertion of the leaf. The leaf traces vary in number from 7 to 14. The bundles may be of two sizes. The lower part of the petiole is flattened on one side; the upper part is circular. The bundles form a crescent in the lower portion of the petiole (fig. 21), this crescent being made complete in the upper portion by the branching of the bundles at the points of the crescent (fig. 14). The secondary bundles form a more or less complete outer ring, are of the same type as those of the stem, and anastomose at any portion of the petiole. Union of the primary and secondary bundles, however, takes place before the traces enter the blade, which has 7–9 strong palmate veins, the 3 median ones running parallel for a while before they swing out laterally. The vernation of the leaf is involute.

Branching

Buds are borne in the bases of the sheathing petiole, the number varying from 1 to 3. The appearance of a single bud is often to one side instead of in the median position, which probably accounts for the appearance of branches coming from the side of the leaf. The buds are invested by a large amount of mucilage. The central bundles of the branch connect with those of the stem, and the peripheral ones connect with the peripheral bundles of the



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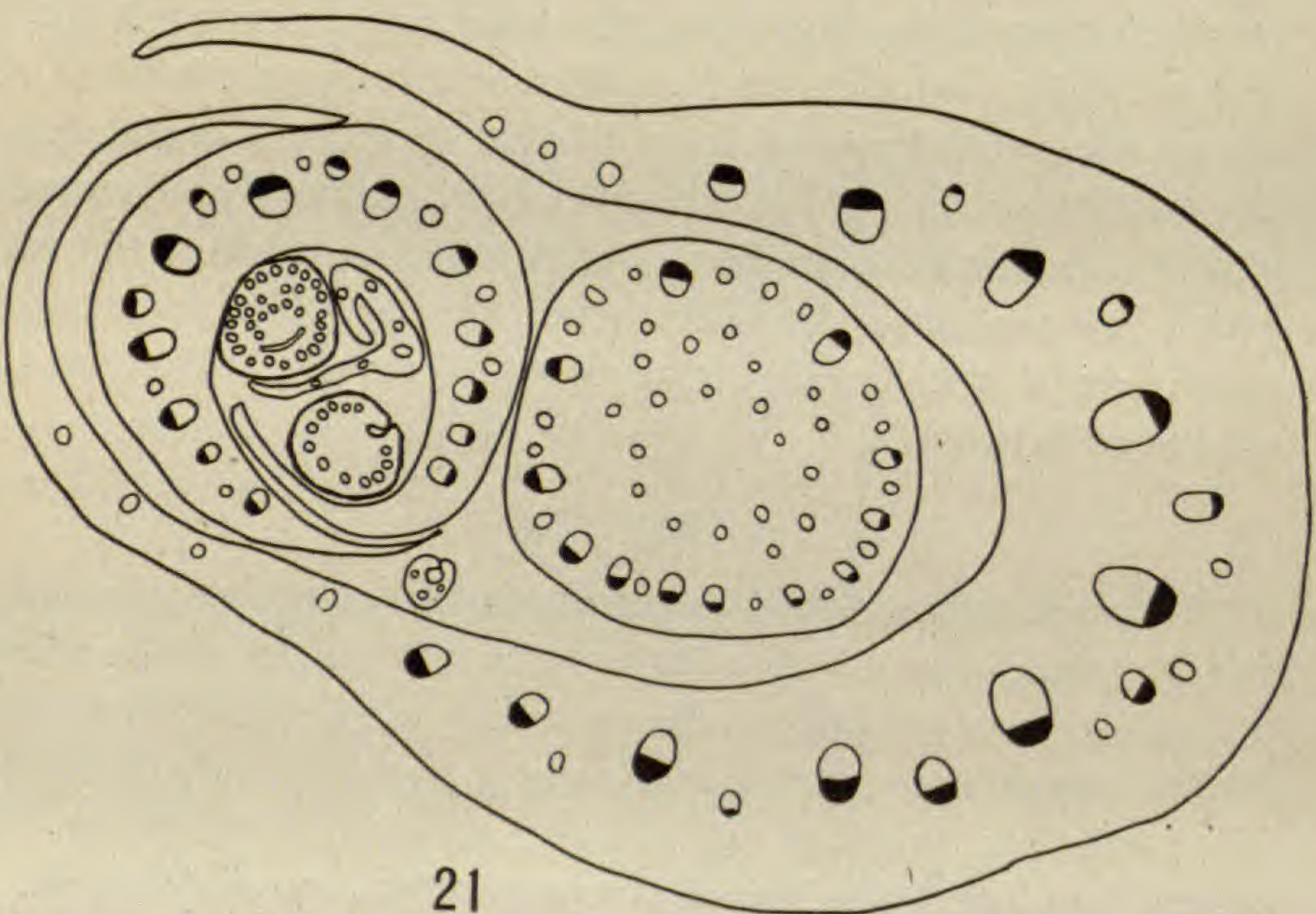

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FIGS. 20, 21.—Fig. 20, transverse section of mature and young buds, ×32; fig. 21, transverse section through the base of a petiole, showing branches arising in axils of abortive leaves, ×28.

stem (fig. 20). In two cases it was observed that the branch did not develop in the axil of a leaf where only the blade was developed (fig. 21), which was due probably to the pressure caused by the enveloping sheaths of the preceding leaves.

Piper umbellatum

The stem of *Piper umbellatum* is not essentially different from that of *Pipèr methysticum*. At maturity it has only one ring of pith bundles. The bundles, instead of traversing two nodes as internal bundles before fusing with the next group, traverse but one (fig. 22). The young plants are covered with hairs, both unicellular and multicellular. The number of leaf traces is 7–9. In the young stem there is an abundance of mucilage scattered in all regions. In the old stem a single mucilage canal appears in the center, which is continuous through the nodes and in the branches; VAN TIEGHEM found none that was continuous through the nodes (fig. 23).

Summary

1. The stem of *Piper methysticum* consists of two systems of bundles, peripheral and pith, the latter being in two rows.

2. The peripheral bundles are of two sizes: primary, the larger; secondary, the smaller. The latter are branches of the former.

3. The bundle type is collateral endarch.

4. The bundles are of foliar origin.

5. The bundles, after entering the stem, remain in the peripheral region through one internode and then traverse the pith for two internodes.

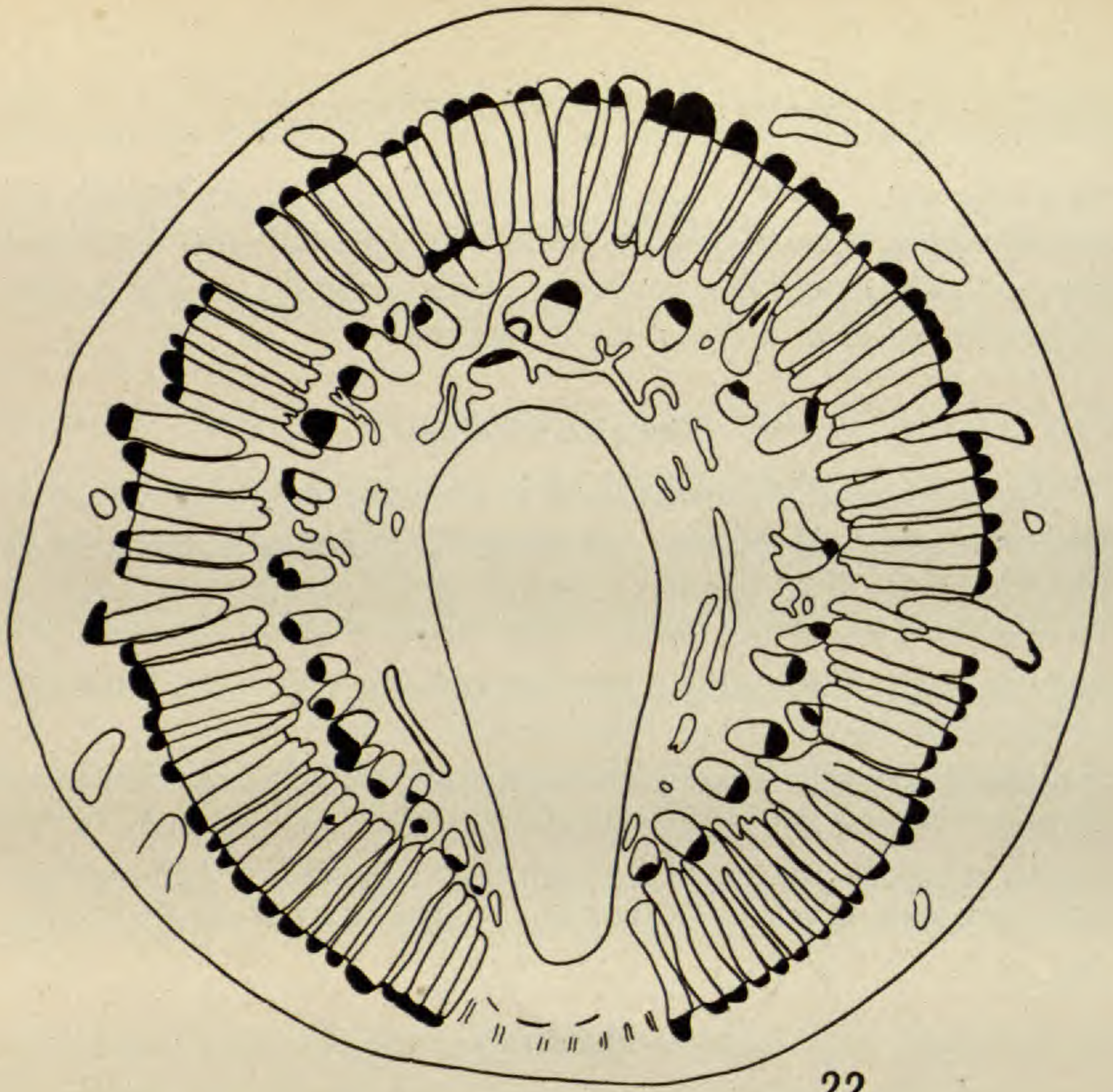
6. Both the peripheral and pith bundles show anastomoses.

7. The pericycle consists of only a few cells outside the bundles, which become lignified.

8. There is no differentiated endodermis.

9. The stem enlarges by cambial activity and divisions in the pith region.

10. An interfascicular cambium appears late in the internode and cuts off segments only on one side; it comes still later in the nodes.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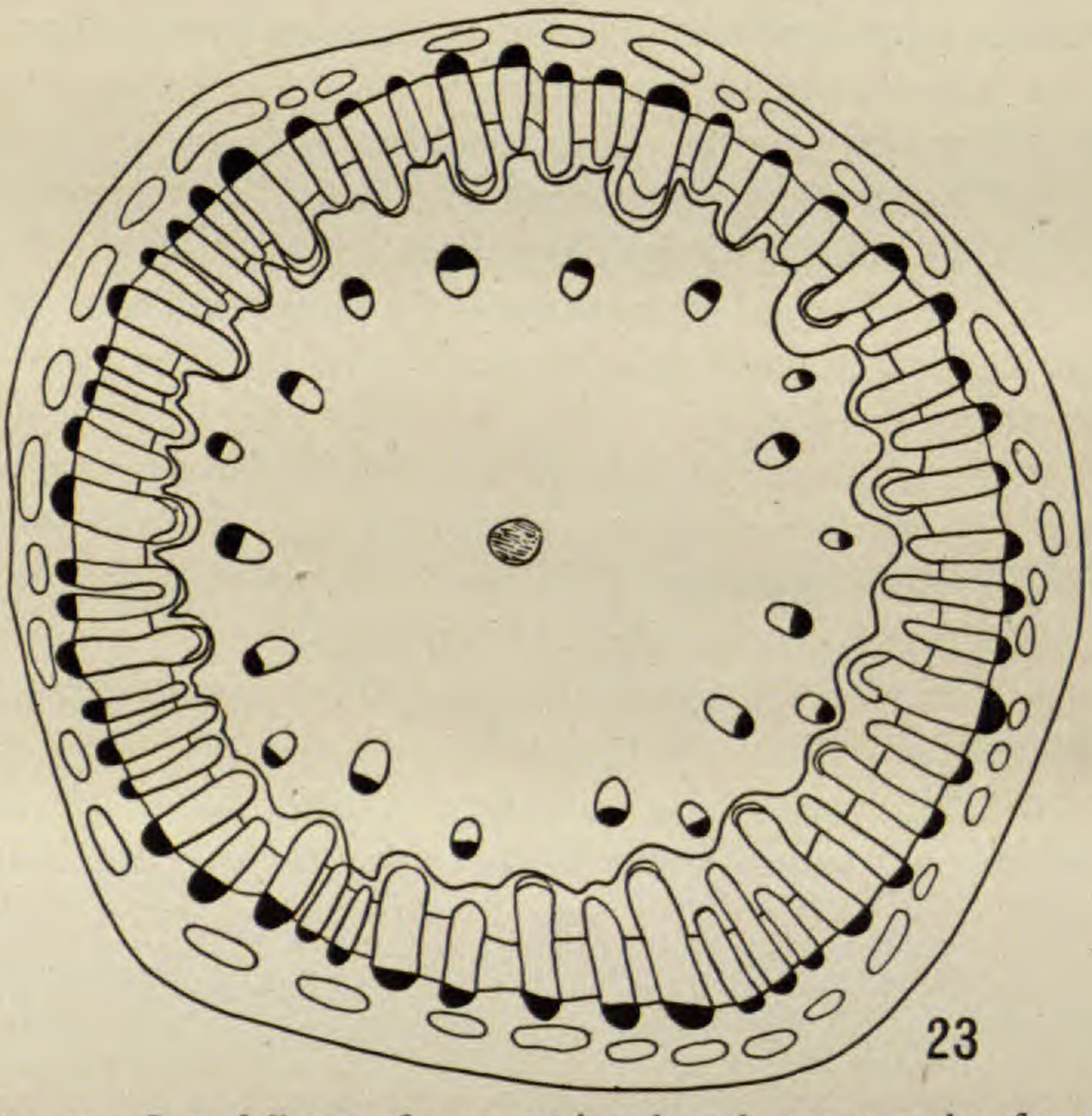
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FIGS. 22, 23.—*P. umbellatum:* fig. 22, section through mature node; fig. 23, section through internode; ×12.

11. Leaf traces are many.

12. The base of the leaf is sheathing and vernation is involute.

13. Buds vary in number and position.

14. *Piper umbellatum* differs from *Piper methysticum* in the following ways: (1) one ring of pith bundles only; (2) the mucilage canal runs through the center of stem and node; (3) the bundles run through one internode only in the periphery and one in the pith before fusing with those of the leaf above.

15. Both stems are packed, especially when young, with starch, piperin, and mucilage.

16. There are no growth rings.

Discussion and conclusions

The anatomy of *Piper methysticum* and of *Piper umbellatum* agree for the most part with the anatomy of the species of *Piper* already described; especially is this true in reference to the work of DE BRAY (**3**; see also DE BARY **2**). VAN TIEGHEM reports a differentiated endodermis in all species of *Piper*, but this was lacking in these forms.

The question of most importance is the relationship shown by these forms. SINNOTT and BAILEY (**13**, **14**) assert that the most primitive condition of angiosperms was woody, and that there is a tendency toward the herbaceous habit in the more recent groups. These forms show a tendency toward elimination of secondary growth and a breaking up of the woody cylinder in the following characters: (1) the pericycle is broken and all that remains is a small patch outside of each bundle; (2) the interfascicular cambium appears late in the internode and still later in the nodes; when it does occur the activity is primarily on one side; (3) there is a tendency toward the scattering of the bundles in the pith. These, together with the sheathing base of the petiole, the multilacunar (SINNOTT **13**) type of node, and the anastomosing of the bundles at the side, show a monocotyledonous tendency. Moreover, the embryo sac of the group shows the elimination of one division in oogenesis.

One may conclude from this combination of characteristics that *Piper methysticum* and *Piper umbellatum* lie in a group well toward the level of monocotyledons, and the conclusion is borne out by the female gametophyte.

The writer is indebted to Professor JOHN M. COULTER for suggestions and criticisms, and to Dr. W. J. G. LAND for the material used and for direction and encouragement during the investigation.

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THE STRUCTURE OF THE BORDERED PITS OF CONIFERS AND ITS BEARING UPON THE TENSION HYPOTHESIS OF THE ASCENT OF SAP IN PLANTS

IRVING W. BAILEY

(WITH PLATE I AND TWO FIGURES)

The structure and the function of bordered pits have been studied by a number of investigators, particularly in connection with various well known theories in regard to the "ascent of sap" in plants. The consensus of opinion among botanists[1] seems to be that these complex pits are valvelike structures which facilitate the flow of water from the lumen of one tracheid or vessel into those of the adjoining ones. By means of the embossed areas of the secondary walls, a comparatively large surface of the delicate permeable membrane is exposed without seriously impairing the strength of the cell (text fig. 1). The torus or central thickened portion of the membrane acts as a valve, which may be deflected from its median position until it rests over one of the openings in the secondary walls (figs. 2, 3). In this way the delicate, permeable membrane is prevented from being ruptured when excessive pressure is brought to bear upon it. Furthermore, it has been generally admitted by anatomists and physiologists that the membranes of the bordered pits in conifers form complete septa that are impervious to finely divided solids and undissolved gases.

STRASBURGER suggested, accordingly, that the bordered pits functioned, in the ascent of the sap, as filters for preventing the penetration of air into the water-conducting elements. This view is also held by DIXON,[2] who states: "Thus, from the point of view of the tension hypothesis, we regard the bordered pits as mechanisms to render the walls as permeable as possible to continuous water

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[1] See ELFVING, SCHWENDENER, PAPPENHEIM, RUSSOW, HARTIG, SCHACHT, DIPPEL, SANIO, DE BARY, STRASBURGER, EWART, DIXON, and others.

[2] DIXON, H. H., Transpiration and the ascent of sap in plants. London: Macmillan. 1915 (p. 99).

streams, while, when conditions require, they provide, by an automatic change, a rigid support to the tensile sap and oppose an impermeable barrier to undissolved gas."

In studying the fundamental factors which control the penetration of preservatives into green and seasoned timber, the writer[3] has had occasion to analyze critically the data upon which these conclusions in regard to the structure and function of the pit membranes are based.

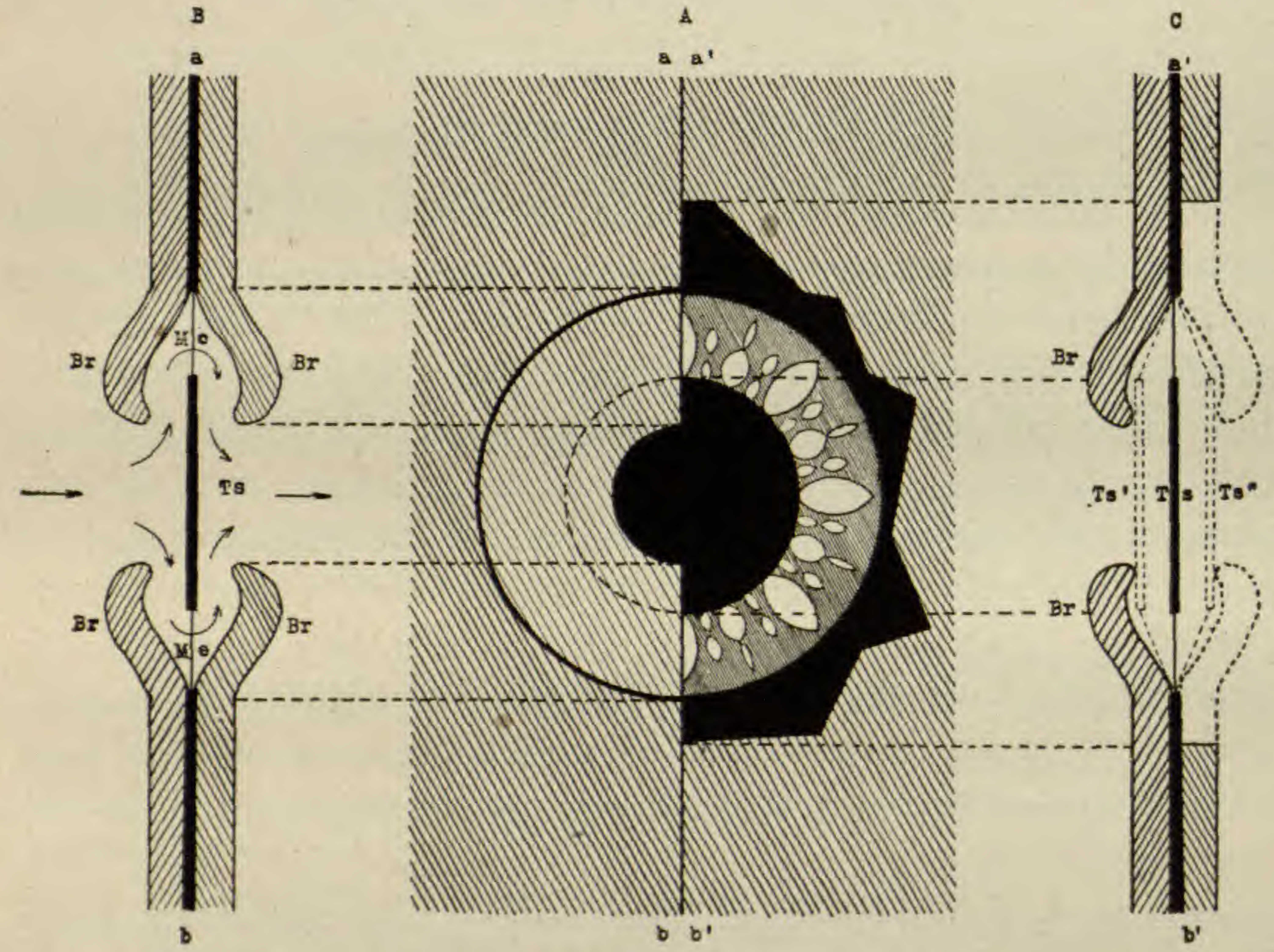

FIG 1.—*A*, piece of radial wall of a tracheid, showing a bordered pit in surface view; on the left, part of the dark colored *torus*, or thickened portion of pit membrane, may be seen through the *oriface* in the *embossed* secondary wall; on the right, part of this overlying wall has been removed, exposing a surface view of the *torus* and *perforated membrane; B*, view of section *a–b* through bordered pit, showing *embossed overlying and underlying secondary walls* (*Br*), *torus* (*Ts*), and *membrane* (*Me*); *C*, view of section *a′–b′*, showing how the torus acts as a *valve* by taking the positions *Ts′* or *Ts″*.

[3] BAILEY, I. W., The validity of certain theories concerning the penetration of gases and preservatives into seasoned wood. For. Quart. 11:5–11. 1913.

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The experimental evidence, which has been considered to demonstrate that the pit membranes are unperforated, consists of injection experiments, such as the well known tests of SACHS with mercury and powdered cinnabar. That absence of penetration in these experiments may have been due to other factors than the unperforated character of the pit membranes does not appear to have suggested itself to previous investigators. However, there are two possibilities that should be emphasized in this connection. In the first place, it is hardly to be expected, a priori, that mercury, alloys of low melting points, and similar substances should penetrate the pit membranes, even if they were porous or sievelike in structure. Owing to the small size of the tracheids and bordered pits in conifers, and the valvelike action of the tori, the very high surface tensions of heavy liquids would tend to prevent their penetration. In the second place, the structure of coniferous wood, particularly that of *Taxus*, is such that injection masses containing finely divided particles (for example, gamboge, India ink, cinnabar, etc.) would be very likely to clog the pits or to deflect the tori before they could succeed in passing through the minute openings in the pit membranes. This would be true even if the particles were considerably smaller than the perforations in the membranes, and is well illustrated by the frequent failure of India ink to penetrate the narrow cavities of "summer" tracheids when portions of the walls of the latter have been cut away.

In selecting an injection mass, therefore, it is very essential that its surface tension and viscosity should be similar to that of the sap in trees, and, if it contains a finely divided solid, the particles of the latter should be of such a size and in such numbers that clogging and deflection of the tori are reduced to a minimum.

That the failure of various investigators to secure penetration with suspensions may have been due to clogging rather than to the unperforated character of the pit membranes was suggested by the following experiments. Pieces of the wood of various conifers were immersed in India ink and subjected alternately to pressures of +10 and −10 lbs. In this way the tori were continually deflected from side to side to facilitate penetration. Figs. 1 and 4 show the dark colored injection mass passing from one tracheid into an adjacent one through the bordered pits.

Of course, the objection may be raised that the membranes were ruptured by the process of injection. This seems to be highly improbable, however, since in a special series of experiments, devised for the purpose, the writer was unable to rupture the membranes, even under a pressure of 250 lbs. per square inch. In order to obviate this difficulty entirely, the India ink was much diluted with distilled water, and allowed to percolate through sticks of freshly cut sap wood[4] under slight hydrostatic pressure (0.25 oz.). Under these conditions the India ink[5] passed through the bordered pits easily and rapidly.

By experimenting with various methods of cutting, staining, and mounting sections, the writer finally succeeded in making visible and photographing openings in the membranes (fig. 6) of the pits of *Larix laricina*. The reason that the perforations have escaped observation is undoubtedly due to the fact that the pit membranes are extremely minute and attenuated, and their structure is obscured by the thick overlying walls. Unless the embossed portions of the secondary walls are cut away, leaving the membranes exposed and uninjured, a somewhat difficult undertaking, or a necessary combination of light refraction phenomena is secured, the perforations remain invisible.

It should be noted in passing that a perforated structure of the pit membranes makes possible an entirely different interpretation of the penetration of gelatine, paraffin, cacao butter, and similar substances into the xylem of conifers. In discussing SACHS' imbibition hypothesis and the mobility of water in tracheid walls, the significance of ELFVING'S experiments with cacao butter and those of ERRERA and STRASBURGER with gelatine has been questioned upon the assumption that, because cacao butter and

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[4] Tests made with *Pinus Strobus* L., *P. rigida* Mill., *Picea rubra* (DuRoi) Dietr., *Larix laricina* (DuRoi) Koch., *Tsuga canadensis* (L.) Carr., *Juniperus virginiana* L., and *Taxus cuspidata* Sieb. and Zucc.

[5] The various commercial India inks vary considerably in the size of the carbon particles of which they are composed. Although the writer has succeeded in securing penetration with various Asiatic inks, they are usually composed of too coarse particles, and tend to clog quickly. Higgins' India ink contains much smaller and more uniform carbon particles and gives much easier and rapid penetration. In using India inks as injection masses the writer has been careful to make sure that that portion of the ink which passed through the membranes contained visible particles of carbon.

gelatine pass through the septa of the bordered pits, the walls of the tracheids must absorb them. In view of the perforated structure of the pit membranes in larch and in other conifers, and JÖNSSON's observations upon the sievelike membranes in the vessels of angiosperms, these criticisms of the work of ELFVING, ERRERA, and STRASBURGER do not appear to be entirely conclusive.

The presence of bubbles of undissolved gas in the wood of transpiring plants has been considered to be a serious objection to the cohesion hypothesis of the ascent of sap in plants; for it has been admitted by DIXON (*l.c.*) that a bubble, having a diameter of 0.02 mm. or more, would destroy the possibility of tension in any tracheid or vessel which is located more than a few feet from the ground. DIXON avoids this difficulty by stating that, although there is undoubtedly considerable undissolved air or gas in the sap wood of plants, not all of the tracheids or conducting elements contain bubbles; that is, a large number of tracheids may be blocked by air, yet all the continuous water columns in the wood need not be broken (text fig. 2). This is considered to be due to the fact that the membranes of the bordered pits prevent undissolved gas from passing from one element into adjoining ones.

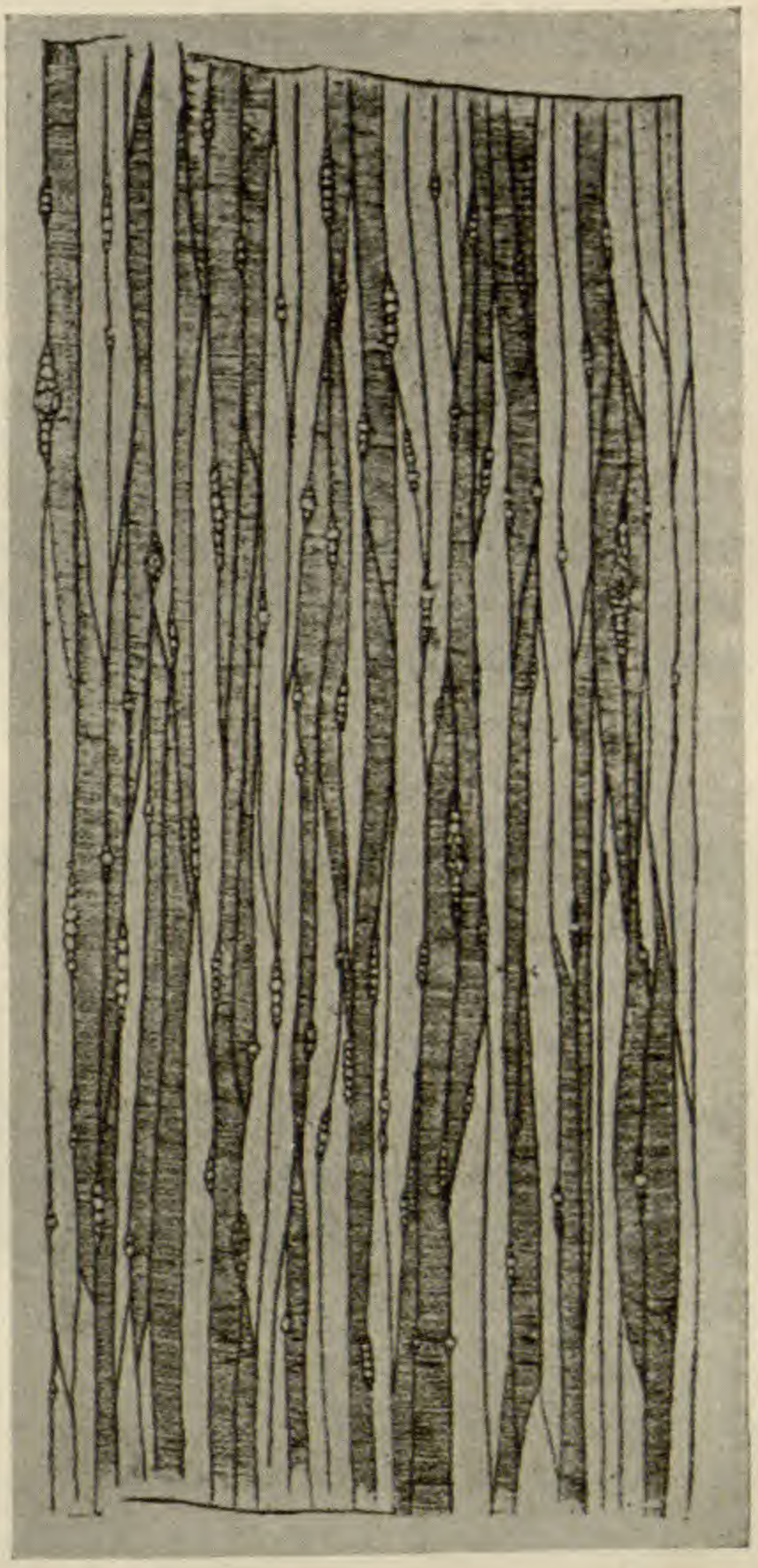

FIG 2.—Longitudinal tangential section of coniferous wood, showing continuous water columns in spite of the fact that nearly half of the tracheids are filled with air; after DIXON.

It is evident, therefore, that in a discussion of the tension hypothesis, the porous or sievelike structure of the pit membranes of conifers deserves careful consideration.

It has been estimated by DIXON (*l.c.*) that "the tension applied to the upper end of water columns, which will be able to raise the transpiration stream in a tree, must equal the pressure produced by a head of water twice the height of the tree." In trees 75–300 feet in height, therefore, there must be forces of 5–20 atmospheres, tending to draw air and other gases, through the perforations in the pit membranes, into the water-conducting passageways. This raises the question: Are the perforations in the membranes of such small size that the surface tension of the sap in them will be great enough to resist the penetration of gas under forces of 5–20 atmospheres?

Perforations of different shapes and sizes occur in each pit membrane (text fig. 1 and fig. 6). The larger openings generally are more or less elongated in outline, but many of the smaller ones are oval or circular. In the stem wood of *Larix laricina* the average diameter of the openings was found to vary somewhat as follows: largest perforations, 3.0 μ; medium perforations, 1.6 μ; small perforations, 0.5 μ.

In calculating the surface tension of liquids in circular openings, the expression of CANTOR[6] is commonly used. This is as follows:

$$y=\frac{rH}{2}-\tfrac{1}{3}\,dr^2-\frac{d^2r^2}{2H},$$

in which y = surface tension in dynes, r = radius of hole in centimeters, H = air pressure in dynes per square centimeter, and d = density of liquid. Assuming a temperature of 15° C., and that the pressure of one atmosphere equals 1,013,000 dynes, the pressure needed to overcome the surface tension of water in circular perforations of the foregoing diameters should be as follows: small perforations, 5.8 atmospheres; medium perforations, 1.8 atmospheres; large perforations, 1.0 atmosphere.

That the actual pressures required to overcome the surface tension of sap in the perforations of the pit membranes would differ

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[6] Wied. Ann. **47**:399. 1892.

from these figures seemed probable, since sap is not pure water, the membranes are elastic, the openings are not perfectly circular in most cases, and the formula is of questionable value in dealing with holes of such minute radii.

In order to check the matter experimentally, a series of tests were made to determine what pressure is required to force air through the membranes of conifers when they are saturated with sap, distilled water, glycerine, acetic acid, acetone, ethyl alcohol, and other liquids of varying surface tensions. A hemlock (*Tsuga canadensis*) 85 feet and a larch (*Larix laricina*) 60 feet in height were felled, and pieces of sap wood including the last formed layers were removed at various points along the stem. These specimens were cut (under water) into small cylinders and carefully tested to determine the location of tracheids which contained bubbles of air or gas. Microscopic examination of the material showed that, at the season when the wood was cut (November), very few of the tracheids of the last formed layers of growth of hemlock and larch contained bubbles of air or gas. Those tracheids which contained gas were conspicuous, owing to their lighter color, and their presence could be demonstrated, in cylinders of sap wood used for experimental purposes, by allowing a dilute solution of India ink to pass through the specimens. The ink traveled very rapidly in those elements that were filled with water, and comparatively slowly, or not at all, in cells which contained bubbles of gas.

In determining the pressures required to overcome the surface tension of the liquids in the perforations of the pit membranes, those pressures were recorded at which air succeeded in penetrating all of the water-filled tracheids.[7] As might be expected from the variation in the size of the perforations, the air penetrated many tracheids before these pressures were attained.

After testing a specimen with alcohol, acetic acid, acetone, etc., it was washed clean, thoroughly re-soaked with distilled water, and retested in order to determine whether the membranes had remained unaffected by the liquids.

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[7] That the air which passed through the specimens did not travel in intercellular spaces was determined in two ways: by direct observation with a binocular microscope, and by the fact that the lumens of the tracheids, which at the beginning of the tests were filled with liquid, contained air at the end of the experiments.

The following values are representative of many secured for hemlock, the specimen of sap wood being taken 75 feet from the ground.

Pressure required to force air through tracheids filled with—

1. Sap	30–40 lbs.
2. Distilled water	30–40 “
3. Glacial acetic acid	12–16 “
4. Distilled water	30–40 “
5. Ethyl alcohol 99+percentage	6–10 “
6. Distilled water	30–40 “

These figures indicate that the values obtained by the use of Cantor's equation are in all probability somewhat in excess of the actual values for the irregular openings in the elastic membranes of conifers; for the perforations in hemlock are considerably smaller than those which occur in larch, and in forcing air through the tracheids, other resistances, besides that of the surface tension of sap in the openings of the membranes, have to be neutralized.

In all of the specimens tested by the writer, which included sap wood of various pines and spruces, as well as larch and hemlock, the surface tension of the sap in the pit membranes could be overcome by air pressures of less than 3 atmospheres. In passing up the stem of a tall tree, the pressures required to overcome the surface tension of sap in the pit membranes do not appear to vary considerably in wood removed at similar distances from the pith.[8] On the other hand, the tension in the sap, according to the cohesion hypothesis, increases very rapidly. It is evident, accordingly, that in tall trees the forces tending to pull gas through the perforations in the pit membranes will greatly exceed the surface tension of the sap in the pores of the membranes. Furthermore, it is significant that in *Sequoia*, many specimens of which exceed 300 feet in height, the perforations are larger than they are in such small conifers as *Taxus*, *Juniperus*, *Pinus edulis*, etc.

In order to determine whether the valvelike action of the tori may be effective in sealing the pits and preventing the penetration

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[8] Through the courtesy of various members of the U.S. Forest Service, I have been able to secure pieces of *Sequoia* that were cut from the tops of trees over 200 ft. in height.

of gas, under forces of considerable magnitude, air pressures of 50–200 lbs. were applied to various conifers. The air passed through the membranes in all cases, whether the pressures were gradually or suddenly applied.

Summary and conclusions

Exception is taken to statements that the membranes in the bordered pits of conifers form complete septa that are entirely impervious to finely divided solids and undissolved gases.

Perforated pit membranes are clearly visible in properly stained sections of *Larix* and *Sequoia*. Owing to the minute size and tenuity of pit membranes, their detailed structure is more or less obscured by the thick, overhanging secondary walls.

Aqueous solutions, containing finely divided particles of carbon, can be made to pass through the membranes in the bordered pits of sap wood taken from the stems of large specimens of *Larix*, *Sequoia*, and other conifers.

The presence of perforations in the membranes is also indicated by the fact that large quantities of gases can be forced rapidly through the bordered pits of tracheids that are thoroughly saturated with sap.

The surface tension of sap in the pit of membranes of various conifers can be overcome by pressures of less than 3 atmospheres.

The tension hypothesis of the ascent of sap in plants, as interpreted by DIXON, requires continuous columns of water, which, at the tops of trees, 75–300 ft. in height, are subjected to tensions of 5–20 atmospheres. In other words, the bordered pits must be impervious to undissolved gases under pressures of 5–20 atmospheres.

It remains to be shown how continuous columns of water can be maintained in tall conifers when the membranes in the bordered pits may become permeable to undissolved gases at pressures of less than 3 atmospheres.

In conclusion, the writer wishes to express his sincere thanks to various members of the United States Forest Service, Mr. R. D. SWALES of the Union Lumber Company, and the Director of the

Harvard Forest for material of conifers, and to his colleagues, Professors T. W. RICHARDS, P. W. BRIDGMAN, and Dr. W. T. BOVIE for helpful suggestions.

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EXPLANATION OF PLATE I

FIG. 1.—*Pinus Strobus* L.: tangential section showing penetration of carbon suspension through bordered pits of freshly cut sap wood; ×700.

FIG. 2.—*Pinus* sp.: tangential section through bordered pit showing torus jammed into opening in secondary wall of left-hand tracheid; ×1000.

FIG. 3.—*Pinus* sp.: tangential section through 2 bordered pits showing usual valvelike action of tori; ×1000.

FIG. 4.—*Sequoia sempervirens* Endl.; tangential section showing penetration of carbon suspension through bordered pits; ×300.

FIG. 5.—*Dacrydium Franklinii* Hood: radial section showing peculiar strap-shaped tori; ×700.

FIG. 6.—*Larix laricina* (DuRoi) Koch: radial section showing perforations in pit membranes; ×520.

FIG. 7.—*Pinus palustris* Mill.: tangential section showing heavy tar oil passing through bordered pits; ×600.

BAILEY on BORDERED PITS

WESTERN PLANT STUDIES. IV

AVEN NELSON AND J. FRANCIS MACBRIDE

Plagiobothrys Harknessii (Greene), n. comb.—*Sonnea Harknessii* Greene, Pitt. **1**: 23. 1887.—Quite distinct from *P. Kingii*, its nearest relative, as GREENE has well shown (*l.c.*). The following representative collections indicate that its range is much greater than originally supposed. NEVADA: Eagle Valley, Ormsby County, June 10, 1902, *C. F. Baker* (1046); CALIFORNIA: Sierra County, 1875, *J. G. Lemmon* (794); OREGON: near Desert Well, 8 miles south of Big Springs, July 5, 1894, *J. B. Leiberg* (403).

Plagiobothrys foliaceus (Greene), n. comb.—*Sonnea foliacea* Greene, Pitt. **1**: 222. 1888.—This seems to be a well marked species, the dorsal depression of the nutlets being particularly unique, but it is apparently still known only from the original collection.

The maintenance of *Sonnea* Greene (*l.c.* 22) does not seem to be at all desirable. Although, as pointed out by GREENE, the character of the scar in this group is somewhat different from that of the other species of *Plagiobothrys*, the plants are habitally the same when one takes the genus into consideration in its entirety. Moreover, the scar character is not so characteristic in all the species as might be desired if they are to be removed from true *Plagiobothrys*. In this connection JONES has called attention to the fact that *P. Jonesii* (*S. Jonesii* [Gray] Greene) is an *Amsinckia* in everything but its white flowers (Contrib. West. Bot. **12**: 57. 1908). The pubescence of *P. Jonesii* and the tesellated nutlets surely suggest a relationship to *A. tessellata*, but the white and short corollas that are so widely at variance with the long yellow ones of *Amsinckia* are perfectly congeneric with the SONNEA section of *Plagiobothrys*. Altogether, *Sonnea* would seem superfluous in a group of genera already merging, but which, with *Sonnea* eliminated, seem well enough marked when dealt with in their aggregates.

Cryptantha vinctens, n. sp.—Annual, 10–15 cm. high, rather sparsely pilose or hispidulous, the leaves more or less papillose;

leaves essentially oblong, 1–2 cm. long, 2–3 mm. broad: inflorescence about 1 cm. long, peduncled, terminating the main stem and its lateral branches, mostly 2-forked, close even in age: corollas minute: calyx persistent and often in fruit very finely appressed-hispid, without a lens appearing silky; sepals ovate-lanceolate, 2.5–3.5 mm. long, the midrib pronounced but not cartilaginous: nutlets 3–4, nearly 2.5 mm. long, mainly ovate, lustrous brown, or gray-spotted with brown, very smooth, acutely angled, but not margined; groove closed or slightly open near the forked and closed base.

Rocky slopes Malheur Valley, near Harper Ranch, Oregon, alt. 1100 ft., June 10, 1896, *J. B. Leiberg* 2235 (type in Gray Herb.).

This specimen was distributed as *C. submollis* (Gray) Coville (*C. utahensis* [Gray] Greene), and that species is its nearest ally. However, the perfectly smooth, less sharply angled nutlets, with closed or nearly closed groove, and the larger sepals are some of the characters which forbid its being referred to *C. utahensis*. The larger calyces suggest *C. mohavensis* Greene, but in that species and in its near relative *C. oxygona* (Gray) Greene the flowers are conspicuous and the nutlets broader and acutely margined. *C. vinctens* and *C. utahensis* occupy positions analogous to those of *C. mohavensis* and *C. oxygona*, except that the two former are more distinct from each other than are the two latter. Furthermore, *C. vinctens* and *C. Bartolomaei* Greene (the only other member of the group) are (as pointed out by GREENE in regard to his species) connecting links between the smooth-fruited species in the deciduous calyx and persistent calyx groups. *C. Bartolomaei* is apparently confined to Lower California, and there it is unique in its pubescence and minute nutlets. None of the species, except *C. vinctens*, has been secured farther north than Utah or Nevada, the distribution of the group apparently centering in the desert areas of the southwest.

Oreocarya dura, n. sp.—Perennial, the single caudex densely clothed with leaf bases of many years: stems usually single, 1–1.5 dm. high, strigillose and densely hispid with widely spreading hairs: leaves oblanceolate or nearly oblong, about 3 cm. long and 5 mm. wide, not greatly reduced on the stem except in the inflorescence and there bractlike, densely shaggy with an indument of fine tangled hairs almost concealed by numerous pustulate-based spreading hirsute hairs: inflorescence a thrysoid glomerule: calyx densely hispid with greenish yellow hairs, the linear divisions about 4.5 mm. long, a little longer than the corolla tube: corolla white; appendages prominent: fruit unknown.

Although it is not possible to state definitely the relationship of this plant, since the fruit is not known, it is probably nearest those species characterized by muriculate not at all rugose nutlets, such as *O. Shantzii* and *O. nana*. However, its shaggy pubescence and simple perennial caudex mark it as distinct from any species of the short corolla section of the genus. The type, in the Rocky Mt. Herb., is *E. T. Johnson* 418, 1907, Central Colorado.

Oreocarya propria, n. sp.—Cespitose perennial: branches of the woody caudex densely clothed with the bases of past seasons' leaves: leaves numerous, 3–6.5 cm. long, half of this length gradually narrowed from the spatulate blade portion into a petiole which abruptly widens into a fibrosely woolly base; stem leaves mostly much reduced and bractlike; pubescence very appressed, finely strigose, on the upper leaf surfaces, also sparsely hispid, the appressed hispid hairs inconspicuously pustulate at base: stems 1–2 dm. high, floriferous above the basal leaves: inflorescence spikelike, consisting of axillary racemes or clusters, the lower often reduced to a single flower in a leaf axil: calyces on distinct pedicels, strigose and densely covered with spreading yellowish bristles; divisions linear, open in fruit, 3–5 mm. long: corolla white, the tube very slightly exceeding the calyx: nutlets ovate, 3–4 mm. long, evenly roughened with very fine intersecting and sinuous ridges, so as to appear irregularly foveolate; ventral groove enlarging toward the base.

This species is most closely related to *O. nana* Eastw. It is very distinct, however, in its racemose inflorescence, with different pubescence. The old leaves are covered with argillaceous soil in which the plant grew. OREGON: Vale, Malheur County, May 14, 1896, *J. B. Leiberg* 2049 (type in Gray Herb.); chalky hillsides, Malheur Valley, near Harper Ranch, June 8, 1896, *J. B. Leiberg* 2223.

Amsinckia carinata, n. sp.—Foliage, pubescence, calyx, corolla, etc., of *A. vernicosa* H. and A.: nutlets lustrous, smooth, very dark gray with a few darker markings, not less than 5 mm. long, not at all triquetrous, the angles obtuse (almost rounded), the back somewhat concave but distinctly carinate by reason of a raised but rounded ridge; ventral surface sharply keeled, the acute edge, including the linear scar, ending abruptly near the base where there are two distinct depressions, giving the impression of an open bifurcation.

OREGON: rocky soil, Malheur Valley, near Harper Ranch, alt. 1100 ft., June 10, 1896, *J. B. Leiberg* 2234 (type in Gray Herb.). It is surprising indeed to find a representative of this alliance so far north. *A. grandiflora* Kleeb, the species which *A. carinata* so closely simulates in habit, has not been collected north of San Francisco or Monterey. The Oregon species does not seem to differ from *A. vernicosa* except in its fruit; it is only related to *A. grandiflora* in a general way; its nutlets are radically different from both. Mature nutlets of *A. vernicosa* are bright gray, speckled with black, 4–4.5 mm. long, sharply triquetrous (like monster buckwheat grains), and with no obvious scar.

Mertensia Palmeri, n. sp.—Apparently tufted on a woody root: stems ascending, 2–3 dm. high, ciliate-hirsute: leaves ciliate-hirsute below, minutely hispid above; radical leaves ovate, obtusish, 2–5 cm. long, on stout petioles somewhat shorter; stem leaves broadly ovate, 5–10 cm. long, cuneately tapering to the acute apex, the stout margined petioles very short: inflorescence appressed-strigose, paniculate, few-flowered: calyx cleft to the base; the sepals linear-lanceolate, 6–7 mm. long: corolla with broad tube only as long as the calyx, the limb with rather strongly dilated throat and nearly 1 cm. long.

The type was collected somewhere in Arizona by *E. Palmer*, 1869 (no other data). It was distributed as *M. paniculata* Don.? It is deposited in the National Herbarium, the sheet bearing the accession no. 46975, and apparently is related to *M. pratensis*, but is remarkable because of the hirsute stems.

TETRACLEA COULTERI Gray, var. angustifolia (Wooton and Standley), n. comb.—*Tetraclea angustifolia* Wooton and Standley, Contrib. Nat. Herb. **16**:170. 1913.—Usually readily distinguished from the species by the narrowly oblong leaves. *T. Coulteri* varies in leaf shape considerably, however, and its leaves are often as strongly toothed as are those of the variety. The narrow leaved plants exhibit no other differences that are worthy of note and that may not be found in any representative series of the species.

At present known only from southern New Mexico, by *E. O. Wooton* (*l.c.*); and more recently by *A. Davidson*, of Los Angeles, who secured specimens at Summit, October 1, 1900, no. 352*a*, and at Duncan, September 1, 1902, no. 1078.

A new section of Pentstemon

In BOT. GAZ. **55**:381. 1913 we published a species of *Pentstemon* (*P. rex*) in which the anther cells remain closed and saccate at the apex. As stated there, this character seems to have been noted heretofore only in the red-flowered group, some of which have the anther cell saccate at base and some at apex. It is interesting, therefore, to find two more very distinct species having this unusual anther structure. It is of further interest that, so far, all the species of the section or subsection are from Idaho and have been evolved under similar environments.

The three species may be discriminated as follows:

Herbage more or less puberulent; corolla large (3–4 cm. long); sterile filament glabrous or nearly so . . . . *P. rex*
Herbage glabrous; sterile filament bearded
  Flowers about 12 mm. long, in a dense slender thyrse . . . . . . *P. minidokanus*
  Flowers about 18 mm. long, few, subsessile in axils of linear bracts *P. payetensis*

Pentstemon minidokanus, n. sp.—Glabrous throughout, pale: stems slender, 3–5 dm. high: basal leaves narrowly oblanceolate; stem leaves linear-oblong, passing into linear-lanceolate upward: flowers in a long narrow more or less drooping thyrse, deep blue, small for the *P. rex* section: corolla only 12–15 mm. long, gradually dilated, glabrous within and without: sepals ovate, acute, purplish, about 3 mm. long: anthers cells rather short and thick, not explanate, dehiscent from the base to a closed saccate apical portion of about one-fourth their length; sterile filament moderately bearded at the apex.

Based on specimens secured in the Minidoka, Idaho, National Forest, by *Geo. D. Crockett* of the Forest Service, July 17, 1913 (type in Rocky Mt. Herb.).

Pentstemon payetensis, n. sp.—Glabrous throughout, 3–5 dm. high: leaves rather few and remote; the basal lance-oblong, 4–6 cm. long, on slender petioles more than half as long; the cauline oblong, acuminate, passing into the linear bracts: flowers few, subsessile in the subsessile axillary cymes: sepals 6–9 mm. long, the very slender acumination about twice as long as the ovate oblong body: corolla blue, 20 mm. or less long, its tube about half as long as the moderately dilated limb, glabrous: anther cells as in the preceding; sterile filament scarcely dilated, sparsely bearded toward the apex with strongly refracted hairs.

Communicated to the Rocky Mt. Herb. by *G. B. Mains*, Supervisor of the Payette National Forest, being no. D-73 of the collection in the Supervisor's office at Payette; type in Rocky Mt. Herb. bearing accession no. 72941.

Machaeranthera rhizomata, n. sp.—Perennial from such a running rootstock as is often seen in *Solidago* and *Aster*, with numerous slender roots: stem strict and simple, very leafy, 3–5 dm. high, minutely glandular with some scattered moniliform hairs: leaves quite entire, oblong-lanceolate to broadly linear, up to 3 cm. long, rather densely scabrous: heads few to several, in a short compact corymb with foliar bracts, scabrous-glandular especially on the involucres; involucral bracts linear-lanceolate with long dark-colored slender reflexed or refracted acuminations: rays linear: pappus sordid.

The rootstock, the entire leaves (the bristly tipped teeth wanting), the strict stem, and the compact terminal corymb readily distinguish this species. The type is *C. L. Shear* 3461, West Cliff, Colorado, August 13, 1896, and in the National Herbarium bears accession no. 835200.

Machaeranthera inops, n. sp.—A depauperate perennial, from a tap root, with few to several short widely divaricate or ascending stems from its crown: stems slender, simple or branched, 5–12 cm. long, densely canescent, as are also the leaves: leaves small, from obovate to spatulate-lanceolate, some of them crenately toothed around the summit: heads few, medium size; involucral bracts linear-oblong, subacute, minutely pubescent, obscurely or not at all glandular, some of the tips refracted: rays wanting: pappus fuscous.

The type sheet is no. 417219 in the National Herbarium, collected by *F. A. Walpole* on Glacier Mountain, Oregon, in the Crater Lake region, August 21, 1902, no. 2288.

MACHAERANTHERA INOPS Nels. and Macbr., var. **atrata,** n. var.—Closely resembling the species but with fewer stems and fewer, larger heads: involucres broadly turbinate rather than hemispheric, and bracts with dark-purple bands or margins.

Crater Lake Park, on firm pumice gravel at the summit of Llao Rock. It is *F. V. Coville* 1470, September 14, 1902. The type sheet bears no. 415285 in the National Herbarium.

Macronema filifomis, n. sp.—Low freely branched undershrub with the aspect of *Chrysothamnus*, 2–3 dm. high; herbage from glabrate to softly lanulose: leaves numerous, linear-filiform, 2–4 cm. long: heads numerous, paniculate, 8–10 mm. high, 10–15-flowered;

involucral bracts in 3 unequal series, linear-acuminate, with delicate membranous ciliolose margins: ray flowers few (1–4 or wanting), ligule oblong, 6–8 mm. long; disk flowers slender, tube longer than the slightly dilated throat: linear-acuminate anthers and stigmas well exserted: achenes brownish, softly pubescent becoming glabrate.

The material seen by the writers consists of two fine sheets collected by *F. V. Coville* and *Elmer I. Applegate*, under nos. 544 and 545, August 16, 1897 in *Pinus Murrayana* forest, on the headwaters of the Deschutes River, Crook County, Oregon. These are the type sheets and bear accession nos. 380795 and 380796 in the National Herbarium.

Macronema glomerata, n. sp.—Low undershrub, freely branched, whitened throughout with short dense woolly pubescence, 2–3 dm. high: leaves linear, 2–4 cm. long, 1–2 mm. broad, callous-tipped: heads few (4–10), crowded at the summit of the stems, 10–14 mm. high; involucral bracts unequal, resembling the leaves into which they pass: rays conspicuous, 2–5, linear-oblong; disk flowers about twice as many as the rays, their slender tubes longer than the fusiform throat: fuscous pappus about as long as the corollas: achenes silky, less than half as long as the pappus.

The splendid sheet of this species, accession no. 401293 in National Herbarium, is named as the type. It is *F. A. Walpole* 370, secured at Ashland, Oregon, September 12, 1899.

Macronema Walpoliana, n. sp.—An undershrub but probably taller than the preceding, the stems simple up to the narrow thyrsoid panicle of numerous heads, glabrate or with a minute lanate pubescence persisting in the inflorescence: leaves linear, subcuspidate, glabrate and subglutinous, 2–4 cm. long: involucre turbinate, its bracts scarious, sublanate or ciliate, in 2 or 3 series; number of flowers as in the last: rays with conspicuous oblong-linear ligules 10–14 mm. long: achenes brownish, sparsely pubescent, a little shorter than the copious fuscous pappus.

Distributed under *Aplopappus* without specific determination. From Klamath County, Oregon, *F. A. Walpole* 387 and 406, collected September 19 and 27, respectively, 1899. These two sheets bear accession nos. 401312 and 401334 in the National Herbarium, the latter being designated as the type.

Macronema scoparia, n. sp.—A profusely and fasciculately branched undershrub; the old stems with a grayish-brown shreddy bark; the herbaceous stems slender, very leafy, greenish brown: leaves narrowly linear, glabrous and subglutinous, 1–3 cm. long: heads few, subracemose at the ends of the twigs; involucral bracts glabrous or obscurely ciliate on the scarious margins: rays none or only 1 or 2 in each head, the ligule oblong-linear and conspicuous; disk flowers not more than 5–8: the brown glabrous achenes not more than half as long as the pappus.

This was also secured by *Walpole* in Oregon. It bears his no. 386, Hunt's Ranch, Dead Indian Road, Jackson County, September 18, 1899. The type is on sheet no. 401311, National Herbarium.

Macronema pulvisculifera, n. sp.—Base evidently shrubby, the numerous branches 1–2 dm. high, naked below, very leafy toward the racemose-paniculate inflorescence, glabrous but covered with dustlike resinous or glandular particles even on the involucre: heads mostly terminal on the branchlets of the inflorescence, rather large (1 cm. or more), the narrowly linear foliar bracts passing into those of the involucre: rays conspicuous, 2–5, the ligule about 1 cm. long; disk flowers 10 or more, with slender achenes a little shorter than the pappus and slender corolla.

Based on a full sheet of specimens, no. 401010 in the National Herbarium collected by *F. A. Walpole*, on Mount Hood, Oregon, near Cloudleap Inn, no. 98, September 7, 1898.

Macronema imbricata, n. sp.—A pale green spreading shrub, glabrous but for a sparse glandular puberulence on leaves and involucres; main stems 2–3 dm. long, stout and prostrate, their numerous branches and the season's growth erect, about 1 dm. high, very leafy: leaves crowded and strongly overlapping, obovate-spatulate, the rounded apex tipped with a short tooth: heads solitary or very few at the ends of the branches, variable in size (7–12 mm. high); outer involucral bracts foliar, smaller but similar to the leaves with which they connect; inner bracts lanceolate, greenish with scarious margins: rays none, solitary, or very few, relatively short and broad; disk flowers many (15–25), the limb short and erect: achene brown, strigose, as long as the dingy pappus.

The type sheet is *Heller* 7182, "ridge south of Donner Pass, Nevada County, California, at about 8500 ft.," August 17, 1903. Deposited in the National Herbarium and bearing accession no. 467563. Originally distributed as *M. suffruticosa*.

CHAENACTIS MAINSIANA Nels. and Macbr. BOT. GAZ. **56**:478. 1913.—In the recent treatment of this genus in the *North American Flora*, the above species is reduced to *C. pumila* Greene (**34**:73. 1914). The latter is described as having the leaves "broadly obovate in outline," but in the key (*l.c.* 66) it is placed under the subdivision "leaves oblanceolate or ellipitic in outline." The subdivision parallel to this reads "leaves obovate cuneate or flabelliform in outline." Under this heading are placed *C. nevadensis* and *C. Evermannii*. The latter is scarcely more than a variety of the former, and in our publication we compared *C. Mainsiana* to these species, and according to the key referred to, it would have to be placed with these species because it has flabelliform leaves. But admitting that the key is fallacious (witness the disposition of *C. pumila*), let us compare *C. Mainsiana* with that species. *C. pumila* Greene, Leaflets **2**:221 (not 223 as in *North American Flora*) 1912, has "densely glandular-hirsute involucres" and "densely glandular peduncles" which are only 2–4 cm. long. These are characters which belong to *C. alpina* in greater or less degree, but are absolutely at variance with the characters of *C. Mainsiana*. The latter species, even when young, is greenish gray with a lepidote tomentum which is sprinkled with resinous atoms as in *Artemisia atomifera* Piper, and the peduncles, which usually bear more than one head, rise 5–10 cm. above the leaves. It is evident, therefore, that the nearest relative of this species is *C. nevadensis*, the species to which we originally compared it (*l.c.*); and it is very much more distinct from *C. nevadensis* than is *C. Evermannii* Greene, maintained in the *North American Flora*.

Evax breviflora (Gray), n. comb.—*E. caulescens* (Benth.) Gray, var. *brevifolia* Gray, Syn. Fl. **1**:229. 1888; *Hesperevax brevifolia* Greene, Fl. Fran. 402. 1897.—JEPSON has raised the var. *sparsiflora* Gray to specific rank, and it seems to us that the above variety deserves equal recognition. *E. brevifolia* has the heads in terminal clusters as in the species, but the leaves resemble those of *E. sparsiflora*. As a result, the aspect of the plant is distinctive. Furthermore, each species is confined to its own range; *E. brevifolia* grows in northern California and southwestern Oregon, and is the northernmost species of this group of curious composites.

LACTUCA SPICATA (Lam.) Hitch., var. **multifida** (Rydb.), n. comb.—*L. multifida* Rydb. Mem. N.Y. Bot. Gard. 484. 1900.—The salient character of this plant (linear or narrowly lanceolate leaf divisions) is a striking one, but except for this foliar difference the plant is referable to *L. spicata*. Accordingly, its relationship is better indicated if it is treated as a variety of that species. The variety is found throughout the Northwest, but it is not frequent.

ROCKY MOUNTAIN HERBARIUM
UNIVERSITY OF WYOMING, LARAMIE

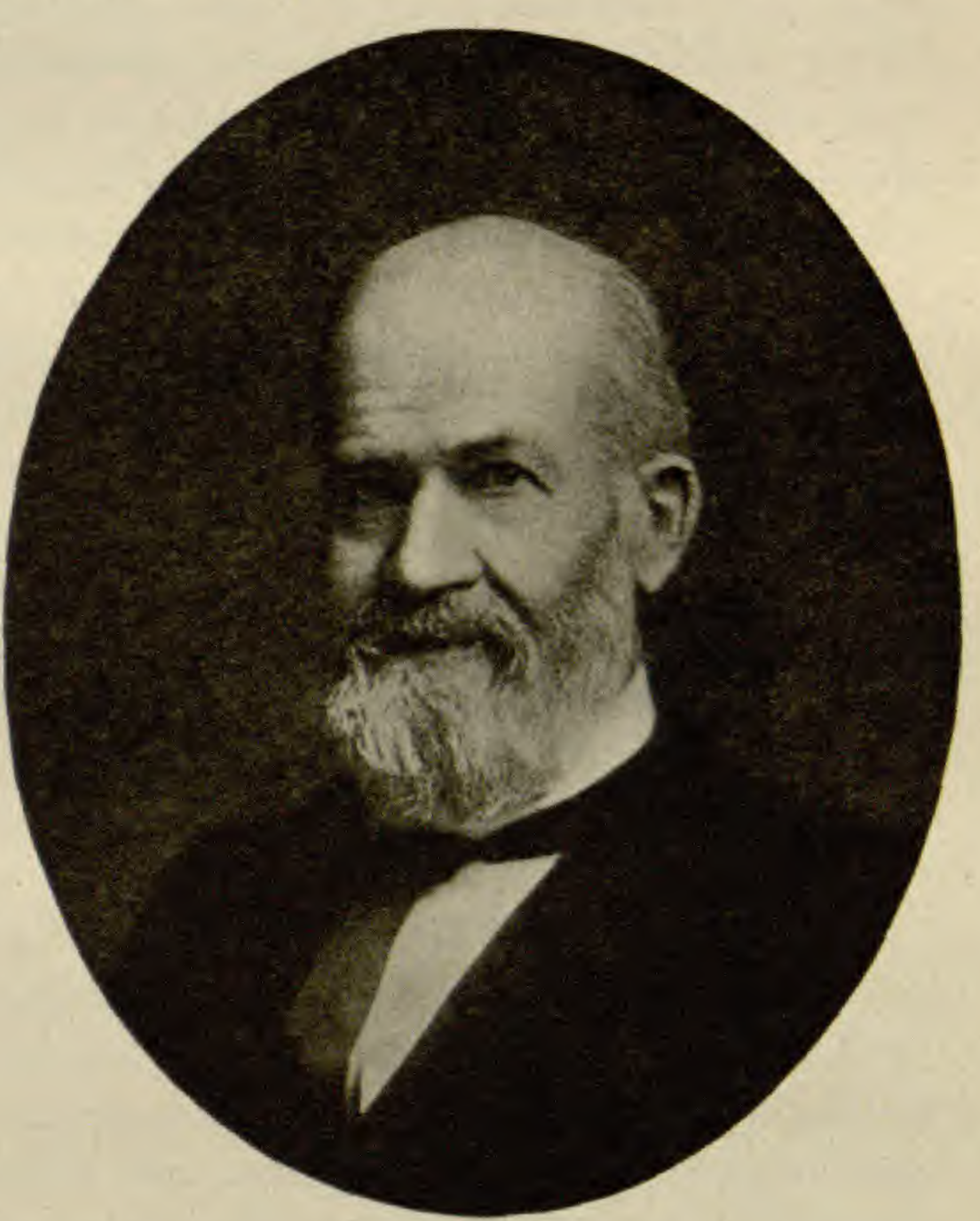
BRIEFER ARTICLES

THOMAS JONATHAN BURRILL

APRIL 25, 1839—APRIL 14, 1916

(WITH PORTRAIT)

From the moment of recognition of "the new botany," as one of them called it, three men of the middle west have stood out in prominence: BEAL in Michigan, BESSEY in Iowa and Nebraska, and BURRILL in Illinois. Each has exercised an influence on American botany further reaching than those of their generation have recognized while it was active, and each has been a force outside the field of his chosen profession.



Professor BURRILL was a microscopist, in the days when microscopy was finding itself. Only a few months ago we picked out the faces of the active workers in that near science as they appeared in a group photographed nearly half a century ago. The museum of the University of Illinois devotes one wall case to a collection of the cheap microscopes that enabled a young and poor institution to show its students the things lying beyond the range of the hand lens, and the long tube binoculars that made the smallest of living things visible to the investigator. It was thus natural that, while he knew the old botany, published a list of the higher plants of the State, and continued until his death impatient of a lessening ability in others to know them as he knew them, Professor BURRILL should have turned for his own productive work from these conspicuous beings to the smaller ones that grow as parasites on them. He has passed into the history of science as the discoverer, in the fire blight of fruit trees, that plant diseases, like animal diseases, may sometimes be caused by bacteria. Though it has given place long since to a more approved method, he introduced to the science a method of demonstrating the bacteria of tuberculosis in affected tissues, and up to the day of his short final illness he was working hopefully on a large unsolved problem in applied bacteriology.

My own contact with Professor BURRILL came over 30 years ago. I was working as a beginner on the parasitic fungi of Wisconsin; he, as a master, on those of Illinois. The correspondence was very helpful to me; it may have been helpful to him. His publications, though rendered less essential today through being replaced by more comprehensive or more readily accessible works, had the intrinsic value of originality and carefulness, and, as has been said by a friend since his death, one felt that he was writing on a subject of which he was a master.

It is only of late years that the University to which Professor BURRILL gave his life-energy has been stamping with its approval doctors majoring in his chosen field. To his inspiration, no doubt, the men who made pioneer mycological collections and studies in Illinois owe much: SEYMOUR, EARLE, WAITE, CLINTON, and others. A colleague has told me recently that, speaking approvingly of sabbatical year and similar provisions for study away from home, Professor BURRILL expressed his own sense of deprivation in never having been privileged to work in another man's laboratory. What he accomplished was doubly commendable because he did his work as a pioneer, breaking as well as blazing the trail to his goal.

Like most men of his type, Professor BURRILL was quite as useful outside his chosen profession as within its limits. Horticulture claimed his aid and was never refused. The "forestry" park of the University of Illinois is an enduring demonstration of his contention, long before the word ecology had been coined, that trees could be grown successfully on the prairie if the prairie sod was effectively broken up. The campus was planted by him, and he lived to see his saplings become large trees. Such a man could not escape administrative responsibilities. For most of his life he was Vice-President of the University; it was he to whom the trustees turned whenever an interregnum occurred, and his lasting affection for his chosen profession, and not their lack of appreciation of his administrative talent, explains the fact that he remained in the chair of botany and did not stay in the executive offices.

As a teacher he was inspiring and beloved; as head of an important department, he possessed a breadth far beyond his own scientific activity; acting as regent, he won and held esteem as a man of unselfishness, fairness, and firmness. He is mourned as few men in his walk of life are mourned; the University pays him the highest tribute of respect and affection; and that the younger men of his immediate profession esteemed him as of their own and not of a by-gone day and generation is shown by a banquet in his honor given by the horticultural society of his State only a few months ago, and by his election at about the same time to the presidency of the National Society of Bacteriologists, and the chairmanship of the botanical section of the American Association for the Advancement of Science. His passing leaves a void.—WILLIAM TRELEASE, *University of Illinois, Urbana, Ill.*

CURRENT LITERATURE

BOOK REVIEWS

Temperature and life processes

KANITZ[1] has written a monograph containing a critical discussion of the effect of temperature on life processes. He has brought together the literature from the fields of physical chemistry, physiology, pharmacology, bacteriology, botany, and zoology. The reader will find 363 citations of literature. Consideration is given first to the effect of temperature upon the rate of chemical processes. It is found that in general the latter follow the Van't Hoff law, that is, the rate at ordinary temperatures is approximately doubled or trebled for each increase of 10° C. in temperature. The coefficient for 10° C. rise in temperature (Q_{10}) is then 2 to 3. The formulae of BERTHELOT, ARRHENIUS, VON ESSEN, and VAN'T HOFF are derived and the relationships discussed. From these data equations are deduced by which the value of Q_{10} may be calculated from experimental results at any two temperatures. For this purpose either one of the two following equations may be used:

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

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

in which k_2 = rate observed at temperature t_2, and k_1 = rate observed at temperature t_1. While most chemical processes are in agreement with the Van't Hoff law, abnormal values of Q_{10} are found in certain types of reactions. High values of Q_{10} are common in monomolecular reactions, for example, in the inversion of cane sugar, $Q_{10} = 5$ to 6. Low values of Q_{10} are formed in heterogeneous system where two processes take part, one process slowing down the total rate, for example, when a process is conditioned by diffusion rate, $Q_{10} = 1.5$ to 1.2. In photochemical processes Q_{10}, with few exceptions, is not higher than 1.4, usually only 1.2 or even 1.0.

Many processes in living organisms show a temperature coefficient approximately that of the Van't Hoff law, within certain temperature limits. Among the plant processes for which this has been found to be the case the following may be mentioned: CO_2 assimilation (MATTHAEI) between 0° and 37° C.; respiration of seedlings (KUIJPER) between 0° and 35° C.; geotropic presentation time (RUTGERS) between 5° and 25° C.; phototropic presentation time

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[1] KANITZ, ARISTIDES, Temperatur und Lebensvorgänge. 8vo. pp. ix+175. *figs. 11.* Berlin: Gebrüder Borntraeger. 1915.

(M. S. De Vries) between 5° and 25° C.; protoplasmic streaming in *Elodea* (Velton) between 2°5 and 35° C.; permeability of plant cells and tissues (Rysselberghe) between 0° and 30° C.; intake of water by barley grains (Brown and Worley) between 3°8 and 34°6 C., etc. Among the processes in animals showing a Van't Hoff temperature coefficient are the following: heart beat frequency, pulsation of medusae, rhythmic muscular contraction, peristaltic movement of cat intestine, rhythm in frog stomach, breath frequency, propagation of nervous stimulus, latent time of muscle, rate of cell division in *Paramecium*, generation period of certain bacteria, pupation period, rate of oxygen consumption, etc.

Some high values of Q_{10} are found, especially in relation to life duration and coagulation effects of temperature. Thus, in life duration of sea urchin eggs, $Q_{10}=240$ to 1450; of *Tubularia crocea*, $Q_{10}=485$ to 3900; of barley grains, $Q_{10}=10$ to 16; of spores of certain bacteria, $Q_{10}=8$, 15, 30, 50, or even 320. In denaturing haemoglobin, $Q_{10}=14$; and in precipitation of egg white, $Q_{10}=635$. Some processes that give normal values of Q_{10} within a certain temperature interval show high values of Q_{10} at the lower or critical temperatures. Thus, in CO_2 assimilation, $Q_{10}=28.7$ from −6° to 0° C.; in heart frequency, $Q_{10}=13.7$ from 3°2 to 8°4; in geotropic presentation time, $Q_{10}=20$ from 0° to 5°; in protoplasmic streaming in *Vallisneria*, $Q_{10}=14.7$ from 1°25 to 3°75.

Kanitz brings out more clearly the relation of temperature to life processes by recalculating Q_{10} at the various temperature intervals, instead of indicating only the average coefficient for a long temperature range. When this is done, it is found that in many cases Q_{10} is not a constant at all intervals of temperature. Many processes show falling values of Q_{10} at higher temperatures, for example, CO_2 assimilation, respiration of seedlings, permeability of plant cells, etc. Some processes, however, show a temperature interval at which Q_{10} is constant. In general, in plants the range of temperature within which Q_{10} may be constant begins at 5° C. and ends at approximately 25° C. The rapidity of division of *B. coli* shows two temperature regions with constant coefficients, but with different coefficient values. Investigators should follow Kanitz in this regard, and should calculate Q_{10} for each temperature interval for which data are available.—F. E. Denny.

Heredity and environment

One of the notable recent books in the general field of genetics is that of Conklin,[2] which is a series of lectures delivered at Northwestern University on the "Norman W. Harris Foundation." The author has been unusually successful in maintaining a clear and popular style without any appreciable sacrifice of scientific values. The book departs from the usual type of textbook in genetics in several respects. In the first place, great emphasis is placed

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[2] Conklin, E. G., Heredity and environment in the development of men. 8vo. pp. xiv+533. Princeton University Press. 1915.

upon the environmental and functional factors of development as opposed to the heredity factors proper. In his zeal to point out the fact that geneticists have neglected the environmental factors, the author tends to overemphasize its relative value in development and heredity. Again, his point of view conflicts with the usual genetical conception of the physical basis of heredity in that he strongly emphasizes the cytoplasmic as opposed to the nuclear elements in germ cells. He points out "that at the time of fertilization the hereditary potencies of the two germ cells are not equal, all the early stages of development, including the polarity symmetry, type of cleavage, and pattern or relative positions of future organs, being foreshadowed *in the cytoplasm of the egg cell,* while only the differentiations of later development are influenced by the sperm. In short, the egg cytoplasm fixes the general type of development and the sperm and egg nuclei supply only the details." Has the author intended to deny to the sperm a cytoplasmic organization, which might conceivably have something to do with development and heredity?

Another distinctive feature of the book is the humanistic point of view that is maintained throughout. As the title indicates, *man* is the center of interest, and the author views man broadly, neglecting none of his salient qualities. Not only is the physical development and heredity of man discussed, but a similar attempt is made to correlate with the physical side that side which we usually term the mental and spiritual. With some boldness the author, in his final chapter entitled "Genetics and ethics," invades ground that even angels might fear to tread. Such questions as "the voluntaristic conception of nature and of human responsibility," "the determinism of environment," and "responsibility and will" are handled with confidence, and certain conclusions are reached and stated, in spite of our lack of a factual and experimental basis for any such conclusions.

The 6 chapters of the book may be characterized as follows. "Facts and factors of development" includes a discussion (1) of the phenomena of development of body and of mind, and (2) of the factors of development (preformation and epigenesis, heredity and environment). The second chapter, entitled "The cellular basis of heredity and development," and the first, previously cited, are decidedly the best in the book, as might be expected in view of the author's special attainments in this field. The reviewer knows no better treatment of these important matters, although he is unable to agree with some of the author's most fundamental positions. The third chapter, "The phenomena of inheritance," is a clear, though brief, statement of the facts, observation, statistical, and experimental, that make up the body of our modern knowledge of heredity. In the fourth chapter, entitled "Influence of environment," the author has an axe to grind and does it with thoroughness. Environment and functional activity as factors in development are given a place as important as heredity. While possibly somewhat overstated, this position is one that needs to be borne in mind, and this chapter will go far toward reinstating the factors of environment and functioning in the estimation of the student of development and heredity. The fifth chapter, dealing with the "Control of heredity: eugenics," is a somewhat conventional treatment of an important subject. The sixth chapter, entitled "Genetics and ethics," has been commented upon previously.

A useful bibliography, glossary, and index complete the volume, which, though possibly not so well adapted as some for textbook use, is, in the reviewer's opinion, probably the best presentation of genetics for the non-scientific reader.—H. H. NEWMAN.

Bacteria and plant diseases

The third volume of SMITH'S[3] *Bacteria in relation to plant diseases* continues the treatment of the vascular diseases which was begun in the second volume with a discussion of the wilt of cucurbits, the black rot of crucifers, and the yellow disease of hyacinths. In the present volume a full treatment is accorded Cobb's disease of sugar cane, Stewart's disease of sweet corn, the Grand Rapids tomato disease, the brown rot of the Solanaceae, and the wilt diseases of tobacco. These diseases have been extensively investigated by the author himself. The accounts, therefore, contain not only a complete and critical discussion of the literature, but also embody the author's own results and present his viewpoint at the time of the writing.

In addition to these diseases, to which the greater part of the volume is devoted, a number of diseases of minor importance, or such as have not been seen by the author, or whose right to be classed as bacterial diseases has not been definitely established, are considered. Among these may be mentioned several diseases of the sugar cane, some of which are probably identical with Cobb's disease, a disease of amaranths, a wilt of the peanut, a disease of orchard grass, various vascular diseases of the banana, and two diseases of the potato. The status of many of these is still obscure. That some of them, as the various diseases of the sugar cane and the many wilts of tobacco and other solanaceous plants, are not all distinct is the belief of the author, but, in accordance with his custom, each disease independently described is kept separate until its identity shall have been established.

The book may well be said to be a record of the present status of our knowledge of the diseases of which it treats. At the same time it bears internal evidence in the many changes during the making that this status is rapidly shifting. By bringing together and classifying the vast mass of scattered and fragmentary facts relating to the subject, the author has laid bare the gaps in our knowledge and has thus pointed the way for further research. Many gaps have been filled by the author's own work, but that much remains to be done before even the morphological and etiological phases of the subject shall have been cleared up is shown by a glance through the chapters on the brown

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[3] SMITH, ERWIN F., Bacteria in relation to plant diseases. Vol. III. pp. viii+309. *pls. 47. figs. 138.* Washington, D.C. 1914.

rots of the Solanaceae and on the tobacco wilts with their confusion of diseases described from different parts of the world and attributed to various organisms. It is the great merit of the author to have contributed so largely to the field of plant bacteriology. It is an almost equal merit to have clearly pointed out the lines for future progress.—H. HASSELBRING.

MINOR NOTICES

A popular guide to mosses.—Mrs. DUNHAM[4] has undertaken to present the mosses of the northeastern states in a non-technical way, so that the amateur may recognize at least their genera without using even a hand lens. The result is a very attractive little book, whose simple language and marginal illustrations should accomplish the purpose announced. If it succeeds, it will open up to the general student a group of plants present in every flora, and usually regarded as too difficult for even a speaking acquaintance.—J. M. C.

Plant diseases.—A second edition of MASSEE'S[5] very useful manual has just appeared, 5 years after the publication of the first edition. It differs from the former edition only in containing a supplement of 16 pages, giving statements concerning 20 diseases which are not included in the body of the text, or concerning which additional information is given.—J. M. C.

NOTES FOR STUDENTS

Evolution of species in Ceylon.—WILLIS[6] has followed his recent paper on the endemic flora of Ceylon[7] by developing still further his argument against natural selection as an explanation of the geographical distribution of species. His argument is based mainly upon statistics derived from TRIMEN'S *Flora of Ceylon*, in which the species are divided into 6 classes, ranging from "very common" to "very rare." He observes that in Ceylon the endemic species are the rarest, according to the foregoing classification, while species which are widespread outside of Ceylon are commonest there also. This not only appears from a consideration of the flora as a whole, but in every family the endemic species are the rarest. It also appears that within every family the groups of species into the rarity classes are remarkably alike. WILLIS regards these phenomena as the result of some natural cause working with practically even pressure throughout the whole plant kingdom, a cause entirely unlike natural selection, which is essentially differentiating in its results. This

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[4] DUNHAM, ELIZABETH MARIE, How to know the mosses; a popular guide to the mosses of the northeastern United States. 8vo. pp. xxv+287. Boston: Houghton Mifflin Co. 1916. $1.25.

[5] MASSEE, GEORGE, Diseases of cultivated plants and trees. 8vo. pp. xii+602. *figs. 173*. New York: Macmillan. 1915.

[6] WILLIS, J. C., The evolution of species in Ceylon, with reference to the dying out of species. Ann. Botany **30**:1–23. 1916.

[7] BOT. GAZ. **61**:82. 1916.

cause he claims is age, not the absolute age of a species, but its age in the locality. Thus endemic species are rare because they are "in the earlier stages of spreading."

WILLIS treats the two main objections to his theory as follows. The claim that the endemic species are really the oldest he answers with the evidence that frequently endemic species belong to widespread genera. These genera could not have originated in Ceylon; the endemic species must have arrived there late in the history of the genera in question. The other objection, which is merely the claim for natural selection, is that local species develop in response to local needs or conditions; but WILLIS finds that endemic species of a given rarity spread over an area of given diameter, although it may contain other kinds of soils, climates, and floras. Also the geographical boundaries of various endemic species do not coincide; that is, the species do not occupy jointly areas where special local conditions may exist. The arithmetical regularity of distribution of species within the various "rarity classes," and the geometrical regularity of the species boundaries, irrespective of physiographic or meteorologic conditions, must be explained by the mechanical, undifferentiating result of age.

In accordance with this theory, the figures given by WILLIS show that parasites, saprophytes, and climbers are "rarer" than independent plants, as should be expected, for they must have followed the others historically. It also appears that among water plants the dicotyledons are more common (therefore older) than the monocotyledons. Of angiosperms in general, WILLIS found no species that was "dying out." He believes that species die out only by accident, a more extensive accident being necessary for the disappearance of a "common" species than for a "rare one."—MERLE C. COULTER.

Securing complete germinations.—A difficulty often encountered by the geneticist is the failure of many seeds to germinate with desirable promptness. It is well known that in certain species of *Oenothera*, half or more of the seedlike structures contain no embryos, and that in many cases those seeds which do contain embryos are subject to delay in germination. DE VRIES[8] has found that in many cases he can more than double the number of prompt germinations by soaking the seeds for 2 days in water and then subjecting them submerged in water for 24 hours in an autoclave to a pressure of 6–8 atmospheres. The author assumes that the effect is due to the forcing of the water needed for germination through minute rifts in the hard portion of the seed coats. He gives a number of instances to show the contrast between seeds merely soaked, and those which have been subjected to pressure. In certain cases he found it advantageous to subject the seeds a second time to pressure. A very small percentage of the seeds resisted this treatment.

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[8] DE VRIES, H., Über künstliche Beschleunigung der Wasseraufnahme in Samen durch Druck. Biol. Centralbl. 35:161–176. 1915.

DAVIS[9] has also devised a method for increasing the rapidity and total percentage of germination in *Oenothera* seeds. He places the seeds on pads of filter paper in Petri dishes, adding water until a surplus remains about the edge of the pad. The dishes, covers, papers, and water are all sterilized before the seeds are inserted. The dishes, covered and kept in shaded portions of the greenhouse, showed much more prompt and complete germinations than samples of the same seeds sown in soil. The author notes that after periods of high temperature there followed a burst of germinations, and believes that the best results will be secured by subjecting the dishes of seeds to high temperatures in an incubator. DAVIS points out as one of the distinct advantages of his method that all seedlike structures which fail to germinate remain available for subsequent study.—GEO. H. SHULL.

Color inheritance in Oxalis.—Several wild forms of *Oxalis* growing in nature at Tokyo, Japan, are found by NOHARA[10] to constitute true breeding biotypes, and one wild form indicated its hybrid nature by producing 3 types of offspring in approximately a 1:2:1 ratio. The 4 homozygous biotypes were subjected to genetical analysis by controlled breeding. Reciprocal and double reciprocal combinations yielded only the same results as the single crosses, showing no differential effect of maternal and paternal germ cells. The 4 forms differed from each other in the presence and absence of a purple bar across the base of the petals forming an "eye," and in the occurrence of several degrees of purple coloration in the leaves. The leaf and flower pigmentation are associated, either by linkage or by the production of purple color in the leaves and the purple eye spot in the flowers by the same gene. As one of the true breeding forms has purple leaves and no eye spot, the relation of these characteristics seems to be more logically referable to linkage. The 4 forms differ from one another severally by single factors, thus presenting an instance of BAUR's "triangle," or multiple allelomorphism, athough the significance of this fact does not seem to have been appreciated by the author. In all crosses the heterozygotes are intermediate between the parents, the F_2 showing ratios approximating 1:2:1, and the F_3 behaviors are typical of Mendelian monohybrids. Three of NOHARA's forms are recognized by him as the taxonomic forms *O. corniculata* L., *O. stricta* L., and *O. corniculata tropaeoloides* (Schlachter) Makino, and the importance of such genetical studies in the solution of taxonomic problems is made clear.—GEO. H. SHULL.

Relation of leaves to climate.—BAILEY and SINNOTT,[11] in continuing their study of the phylogeny of angiosperms, have begun an investigation of the

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[9] DAVIS, B. M., A method of obtaining complete germination of seeds in *Oenothera*, and of recording the residue of sterile seedlike structures. Proc. Nat. Acad. Sci. **1**:360–363. 1915.

[10] NOHARA, S., Genetical studies on *Oxalis*. Jour. Coll. Agric. Imp. Univ. Tokyo **6**:165–182. *pl. 1.* 1915.

[11] BAILEY, I. W., and SINNOTT, E. W., The climatic distribution of certain types of angiosperm leaves. Amer. Jour. Bot. **3**:24–39. 1916.

distribution of various types of leaves in the principal phytogeographic regions, having in view "the question of the conservatism of foliar characters and their modification by environmental factors." The present paper summarizes the results from data obtained in reference to the distribution of entire and non-entire leaves and leaflets of dicotyledons. The authors conclude that there is a marked correlation between leaf margin and environment in the distribution of dicotyledons. Entire margins predominate in lowland tropical regions; while non-entire margins predominate in mesophytic cold-temperate regions. In the tropics non-entire margins are favored by "moist uplands, equitable environments, and protected, comparatively cool habitats." In the cold temperates, entire margins are favored by "arid environments and other physiologically dry habitats." These correlations are exhibited more strikingly by trees and large shrubs than by herbs. If these conclusions are tenable, "the determination of the percentages of the entire and non-entire leaves in Cretaceous and Tertiary dicotyledonous floras affords a simple and rapid means of gauging the general climatic conditions." Attention is called to the danger of inferring that leaves are conservative or inconstant because in some cases they remain unaltered through long periods, and in other cases they vary greatly among closely related forms. A very definite relation between the evolution of climate and the evolution of plants is becoming increasingly evident.—J. M. C.

Embryo sac of Onagraceae.—The embryo sac of this family, the subject of a good many investigations, has been reported as persistently 4-nucleate, with an occasional more or less doubtful exception. TÄCKHOLM[12] has investigated the situation and confirms the constancy of this feature, stating that he never observed an embryo sac that was not 4-nucleate, nor did he find any transition stages to the ordinary 8-nucleate type. Previous statements in reference to vanishing antipodals, thus reducing the number of nuclei to 4, are thought to be accounted for by confusing them with the remnants of the 3 lower megaspores, the uppermost megaspore in this family developing the embryo sac. In fact, it seems that one or more of the 3 lower megaspores often persist attached to the embryo sac, and this sometimes results in a striking resemblance to 3 antipodals in a linear series. This union of persistent megaspores with the embryo sac constitutes what TÄCKHOLM calls a gametophytic entity, sometimes intervening walls disappearing and the persistent megaspore nuclei left free in the antipodal region of the sac. As to the possible nutritive function of these persistent and occasionally proliferating megaspore nuclei, there has been much discussion, but no certain evidence. In any event, this behavior of the Onagraceae seems to be unique, and its possible phylogenetic significance must be left at present to the imagination.—J. M. C.

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[12] TÄCKHOLM, GUNNAR, Beobachtungen über die Samenentwicklung einiger Onagraceen. Svensk. Bot. Tidsk. **9**:294–361. *figs. 16.* 1915.

History of gymnosperms.—BERRY[13] has published his paper prepared for the symposium on gymnosperms at the meeting of the Botanical Society at the University of California last August. He outlines the paleobotanical evidence in reference to gymnosperms, which extends more or less continuously from the Devonian to the present time. A diagram not only summarizes the data as to the age and relative abundance of the groups, but also indicates the author's suggestions as to phylogeny. The cycadophyte phylum rises directly from the fern stock through Cycadofilicales, which group in turn gives rise to Williamsoniales, Cycadeoidales, and Cycadales. This splitting up of Bennettitales into two phyla seems to be justified by our increasing knowledge of the *Williamsonia* forms. The Cordaitales are represented as having an independent origin from the fern stock and developing the coniferophytes. The araucarians are credited with being the oldest conifer stock, the groups of Pinaceae being left in a more or less problematical position as to age of origin and ancestral group, and among them the Abietineae are regarded as more modern than the Taxodineae and Cupressineae. Taxaceae and the Ginkgoales are left unconnected, the latter beginning in the later Paleozoic, and the former recognized at the base of the Mesozoic.—J. M. C.

Morphology of Phylloglossum.—SAMPSON[14] has investigated this much discussed monotypic genus, chiefly with reference to its "annual storage tuber," the so-called "protocorm" of TREUB. As a result of detailed anatomical investigation, the author concludes that this tuber is "a specialized leafy axis, the terminal bud of which functions both as a means of vegetative reproduction and as an organ of perennation," comparable with the resting buds of *Lycopodium inundatum* and the "tubers" of certain Indian species of *Selaginella*. That in fertile plants the tuber is a modified branch is supported by the following facts: (1) a gap is left in the stele of the main axis by the exit of the vascular strand of the tuber; (2) the stele of the tuber often shows a corresponding gap; and (3) the tuber bears leaves, some of which are considerably reduced. The sterile plant consists of a simple axis, the apex of which has formed a storage tuber. The author concludes that the tuber of *Phylloglossum*, therefore, can no longer be compared with the protocorm of *Lycopodium cernuum*, but the two genera are found to be more nearly related by the fact that *Phylloglossum* is shown to be not characteristically an unbranched form.—J. M. C.

Flavone derivatives in plants.—SHIBATA and KISHIDA[15] find that aerial parts of high mountain plants are generally much richer in flavone bodies

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[13] BERRY, E. W., The geological history of gymnosperms. Plant World **19**:27–41. *figs. 2.* 1915.

[14] SAMPSON, K., The morphology of *Phylloglossum Drummondii* Kunze. Ann. Botany **30**:315–331. *figs. 5.* 1916.

[15] SHIBATA, K., und KISHIDA, M., Untersuchungen über das Vorkommen und physiologische Bedeutung der Flavorderivate in den Pflanzen. II Mitt. Bot Mag. Tokyo **29**:316–332. 1915.

(yellow pigments of the cell sap) than are plants grown on the plains. They believe that these pigments protect the mountain plants against the high insolation (especially actinic rays) of their habitat. Contrary to KERNER, they believe that anthocyanin in the foliage of mountain plants has little protective significance against light, for it develops mainly in the autumn after the light intensity has fallen. They consider the anthocyanin in foliage organs as originating by the reduction of the flavone bodies and thereby offering a problem in cell physiology, rather than being of physiological significance. They do not deny that anthocyanins of the brilliantly colored mountain flowers are of significance as light screens. They also emphasize the protective function of flavone bodies in the white and yellow flowers. The mountain plants show a great reduction in flavone content when they are grown on the plains. For a general statement of the chemical side of this problem the reader is referred to a recent review upon anthocyanins.[16]—WILLIAM CROCKER.

Morphology of Isoetes japonica.—WEST and TAKEDA[17] have investigated this species, rather widely distributed in Japan, and the largest species of the genus known, the stem ("caudex" of the authors) of an old plant often attaining 4 cm. in diameter, and in very large specimens a diameter of 8 cm. may be reached. The trilobed caudex consists of two distinct structures, stem and rhizophore, to which the leaves and roots are attached respectively, but on account of the stunted growth all external morphological differentiation between the two organs is lost. The stem apex is a conical mass of tissue at the base of the funnel-shaped depression in the cortex, and in this protuberance no apical cell can be distinguished. The primary vascular axis is "a non-medullated monostele," and no secondary xylem is formed in this species. The rhizophore, a distinct root-bearing organ, is regarded in *Isoetes* "as an organ *sui generis*." The anatomy of stem, rhizophore, and leaf is described in detail. The authors conclude that *Isoetes* "occupies an isolated position amongst recent vascular cryptogams, and is regarded as the sole living representative of the class Isoetales."—J. M. C.

Permeability.—FITTING[18] finds the plasmolytic method rather serviceable for studying the intake of salts by the cells of various plants, especially *Rhoeo discolor*, which was used mainly in the investigation. Permeability to salts of alkali metals (K, Na, and Li) is rather great. It varies with the leaf and is greatest in summer and very slight in winter. The permeability for sodium and potassium salts was about equal and for the lithium salts considerably lower. The permeability was largely determined by the anion, the sulphate

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[16] BOT GAZ. **61**:349–352. 1916.

[17] WEST, CYRIL, and TAKEDA, H., On *Isoetes japonica* A.Br. Trans. Linn. Soc. London **8**:333–376. *pls. 23–40. figs. 20.* 1915.

[18] FITTING, HANS, Untersuchungen über die Aufnahme von Salzen in lebende Zelle. Jahrb. Wiss. Bot. **56**:1–64. 1915.

entering less readily than the nitrate or the chloride. It is of great interest that a considerable sojourn in a hypotonic solution lowered the permeability of the cell to the salt and apparently to the water. FITTING says that this is not a toxic effect. The method shows no evidence that the salts of metals of alkaline earths (Mg, Ca, Ba, and Sr) enter the cells, so equilibrium between the inside and outside need not be reached even in a solution of an essential salt. One wonders whether the permeability in a plasmolytic concentration throws much light on permeability in natural conditions.—WILLIAM CROCKER.

Morphology of Peranema.—DAVIE[19] has investigated the development of the sorus, sporangium, and gametophyte of this Indian fern. The study was suggested by the possible intermediate character of the genus between Cyatheaceae and Polypodiaceae. The receptacle is of the Gradatae type, but the mature sorus is a mixed one. The sporangium also in its early segmentation sometimes follows the type of one family, and sometimes that of the other family. In comparing the features of the genus, the conclusion is reached that an intermediate series consisting of *Woodsia*, *Diacalpe*, and *Peranema* is probable, *Woodsia* coming nearest Cyatheaceae, and *Peranema* nearest Polypodiaceae. The mature sorus of *Peranema* is thought to be most related to that of *Nephrodium*, and a phyletic line is traced from the Cyatheaceae to the Aspidieae group of the Polypodiaceae.—J. M. C.

Two new terms.—TRELEASE[20] has proposed two new botanical terms to be applied to hitherto nameless morphological conditions. He points out that the old grouping of plants into thallophytes and cormophytes fails to include such plants as mosses, which are not cormophytes because, although "stem-like," they do not develop root and shoot. He proposes, therefore, a grouping of plants into 3 categories: thallophytes, "cormophytasters" (or pseudo-cormophytes), and cormophytes. The second term, "xeniophyte," is proposed for the so-called endosperm of angiosperms, which being neither an x nor a $2x$ generation is a third generation which has been "overlooked." The angiosperms, therefore, in addition to their other peculiar features, are unique in having 3 generations: sporophyte, gametophyte, and xeniophyte.—J. M. C.

Seed germination in Megarrhiza.—HILL[21] has investigated the peculiar seed germination of several species of this genus, which is also known as *Marah*. The petioles of the cotyledons are "fused together" to form a tube, which

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[19] DAVIE, R. C., The development of the sorus and sporangium and the prothallus of *Peranema cycathoides* D. Don. Ann. Botany **30**:101–110. *pl. 3. figs. 2.* 1916.

[20] TRELEASE, WILLIAM, Two new terms, cormophytaster and xeniophyte, axiomatically fundamental in botany. Proc. Amer. Phil. Soc. **55**:237–242. 1916.

[21] HILL, A. W., Studies in seed germination. The genus *Marah* (*Megarrhiza*), Cucurbitaceae. Ann. Botany **30**:215–222. *pl. 5. figs. 2.* 1916.

grows out of the seed carrying the plumule and hypocotyl with it. The length of the tube varies in different species, and in *M. horridus* the elongated tube bears "absorbent hairs." In emerging from the tube the hypocotyl breaks through at the base, and later the plumule pierces the side of the tube. Eventually a hypocotyledonary tuber is formed, which may become very large. In *M. horridus* the interesting discovery was made that in connection with the growth of this tuber the cotyledonary tube splits into 6 separate parts, each of which contains a vascular strand.—J. M. C.

An automatic transpiration scale.—The extensive work of BRIGGS and SHANTZ[22] upon the water relations of plants in arid and semi-arid regions, and more particularly their water requirement measurements, has necessitated the construction and use of an automatic scale of 200 kg. capacity, sensitive to 5 gm., in order to measure the transpiration of plants freely exposed to wind and weather. Such a scale is described in a recent publication.[23] In it steel balls are used as weights, and continuous records for periods of several weeks are obtained. The article also contains a review of other forms of transpiration balances, both of the step-by-step type, which includes the scale here described, and of the continuous record type.—GEO. D. FULLER.

Field rot of potato tubers.—PRATT[24] points out some interesting facts that he discovered in his studies of the potato rot situation in Idaho. "Stem end rot," "field rot," or "black rot" of potatoes of the round type, such as Rurals and Pearls, and jelly end rot of tubers of the Burbank group are induced by *Fusarium radicicola.* The organism is active at temperatures above 50° C. and can therefore be controlled in storage. Field control of the black rot situation is difficult. Seed pieces afflicted with black rot bring about infection of the following potato crop, and, interestingly enough, virgin soils produce heavier infection than lands that have been put to crops.—G. K. K. LINK.

Recent work in embryology.—SOUÈGES,[25] in continuing his embryological studies, has published an account of the Cruciferae, which traverses the classic work of HANSTEIN and FAMINTZIN. The figures are chiefly those of *Lepidium sativum*, although other species of *Lepidium* (*L. campestre* and *L. Draba*) and *Cochlearia officinalis* are included.

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[22] BOT. GAZ. **56**:514–515. 1913.

[23] BRIGGS, L. J., and SHANTZ, H. L., An automatic transpiration scale of large capacity for use with freely exposed plants. Jour. Agric. Research **5**:117–132. *pls. 9–11. figs. 18.* 1915.

[24] PRATT, O. A., A western field rot of the Irish potato tuber caused by *Fusarium radicicola.* Jour. Agric. Research, Dept. Agric. **6**:297–309. *pls. 34–37.* 1916.

[25] SOUÈGES, M. R., Développement de l'embryon chez les Crucifères. Ann. Sci. Nat. Bot. **19**:311–339. *pls. 11–14. figs. 76.* 1914.

CARANO has published the results of the investigation of the development of the embryo in Asteraceae,[26] *Senecio vulgaris*,[27] and *Poinsettia pulcherrima*.[28]—J. M. C.

Water absorption by epiphytes.—LIESKE,[29] studying certain epiphytes (*Epidendrum* and *Tillandsia*) in their natural habitats about Rio de Janiero, concludes that the velamen of the former and the scales of the latter do not condense physiologically significant amounts of water from the air. The water needs are supplied by rain, dew, and mist. The scales of *Tillandsia* seem important in trapping salts as dust from the air, which are later dissolved and absorbed.—WILLIAM CROCKER.

Toxicity of lithium salts.—FERKING[30] obtains some interesting results upon the toxicity of lithium salts. He finds that lithium salts, like magnesium salts, are highly toxic only to calcium-requiring plants and not injurious to the lower algae (*Chlorella* and *Scenedesmus*) and fungi (*Penicillium glaucum*) that do not require calcium. While calcium salts can fully antagonize the injurious effects of magnesium salts, they only reduce the toxicity of lithium salts.—WILLIAM CROCKER.

Morphology of Pittosporum.—BREMER[31] has investigated the ovule and embryo sac of two species of *Pittosporum*, and reports that in both cases the nucellus degenerates and has disappeared when the embryo sac is mature. In *P. ramiflorum* the third megaspore of the linear tetrad develops the embryo sac; and in *P. timorense* the tetrad is bilateral instead of linear.—J. M. C.

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[26] SOUÈGES, Ricerche sull'embriogenesi delle Asteracee. Annali di Botanica **13**: 251–301. *pls. 11–16.* 1915.

[27] CARANO, E., Sull'embriologia di *Senecio vulgaris* L. Rend. Roy. Accad. Lincei **24**:1244–1248. *figs. 10.* 1915.

[28] ———, Sull'embriologia di *Poinsettia pulcherrima* R. Grah. Annali di Botanica **13**:343–350. *pl. 17.* 1915.

[29] LIESKE, R., Beiträge zur Kenntnis der Ernährungsphysiologie extrem atmosphärischer Epiphyten. Jahrb. Wiss. Bot. **56**:112–122. 1915. PFEFFER's Festschrift.

[30] FERKING, H., Über die Giftwirkung Lithiumsalze auf Pflanzen. Flora **108**: 449–453. 1915.

[31] BREMER, G., Reliquiae Treubianae II. The development of the ovule and embryo sac of *Pittosporum ramiflorum* Zoll. and *Pittosporum timerense* Blume. Ann. Jard. Bot. Buitenzorg II. **14**:161–164. *pls. 23–26.* 1915.

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A PHYSIOLOGICAL STUDY OF TWO STRAINS OF FUSARIUM IN THEIR CAUSAL RELATION TO TUBER ROT AND WILT OF POTATO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 219[1]

GEORGE K. K. LINK

(WITH THIRTEEN FIGURES)

There is little doubt among phytopathologists that members of the genus *Fusarium* play an important rôle in producing diseased conditions in many plants, both wild and cultivated. According to WOLLENWEBER (41), *Fusarium* spp. produce wilt in members of the following families: Liliaceae, Bromeliaceae, Musaceae, Solanaceae, Convolvulaceae, Leguminosae, Malvaceae, Linaceae, Cucurbitaceae, Cruciferae, Compositae, Araliaceae, Caryophyllaceae, and Pedaliaceae.

History

The genus *Fusarium* was established by LINK (20, 21) in 1809, and *Fusarium* species were reported on rotted and ring-discolored tubers by MARTIUS in 1842, HARTIG in 1846, and SCHACHT in 1856. PIZZIGONI (29) and WEHMER (38, 39) demonstrated by experimental inoculation that *Fusarium* species can bring about tuber rot. They referred to the *Fusarium* in question as *F. solani*. Others, however, among them FRANK (11, 12) repeating their work, obtained negative results so far as *Fusarium* species were concerned; while DE BARY (6) and many others regarded the *Fusarium* spp. as nothing more than obligate saprophytes.

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[1] In cooperation with the Nebraska Agricultural Experiment Station.

The credit of first demonstrating experimentally the relation of *Fusarium* spp. to certain plant wilts belongs to SMITH (34), who found a *Fusarium* responsible for watermelon wilt. SMITH and SWINGLE (35) reported a potato wilt and tuber rot which they considered due to a *Fusarium* which they called *F. oxysporum*. They considered this organism identical with *F. solani* of PIZZIGONI and WEHMER, and used the oldest name available, *F. oxysporum* (SCHLECHTENDAHL, 1824); however, they reported no experimental inoculations. PETHYBRIDGE and BOWERS (28) reported a dry rot due to *F. solani*, and LONGMAN (22) also reported a dry rot due to a *Fusarium*.

Many pathologists and mycologists entertained considerable doubt as to the parasitic nature of *Fusarium* spp., while others were quite convinced of their parasitic nature. SORAUER (36) was quite positive in his decision, while MASSEE (25) wavered. LINDAU (18) remained skeptical and referred to the *F. oxysporum* of SMITH and SWINGLE as a "Mischart." DUGGAR (10) was quite positive in his decision. Much of this difference of opinion undoubtedly was due to the confusion that prevailed as to the status of *F. solani*, *F. oxysporum*, and the genus in general, since no basis for extended morphological study of the genus had been established, and even the genus itself had not been sharply defined. MASSEE (25) considered *F. solani* to be the conidial form of *Nectria solani;* while REINKE and BERTHOLDT (30) considered it the conidial form of *Hypomyces solani*. LOUNSBURY (23) tried to arbitrate the matter by suggesting that *F. solani* and *F. oxysporum* are one and the conidial stage of *Nectria solani*. APPEL and WOLLENWEBER (5) published a monograph in which they defined the genus and brought some order into the chaos of species. Among other radical changes they dropped *F. oxysporum* and established *F. orthoceras* in its place.

MANNS (24) demonstrated by experimental inoculation that a *Fusarium*, which he designated (following SMITH and SWINGLE) as *F. oxysporum*, could produce tuber rot and wilt. He made no morphological studies, however, and undoubtedly had not had access to APPEL and WOLLENWEBER's monograph.

JAMIESON and WOLLENWEBER (16) published an account of a dry rot of tubers induced by a *Fusarium* which they described as a new species (*F. trichothecioides* Wr.). They refer to it as "a wound parasite capable of destroying potato tubers" and say "this disease is clearly differentiated from the wilt and dry rot ascribed by SMITH and SWINGLE to *F. oxysporum.*"

Later, the writer (**19**) submitted his studies of a dry rot occurring among Nebraska potatoes as a thesis to the Graduate Faculty of the University of Nebraska. The work was done at the request of Dr. E. MEAD WILCOX, and consisted in part of a study of the morphology of a *Fusarium* that had been isolated from dry rotted tubers in 1908 by Miss VENUS W. POOL from potatoes that farmers had sent in from throughout the state during the season 1907–1908. Miss POOL established the causal relation of this *Fusarium* to the dry rot by experimental infection, and named the organism in manuscript *F. pulverulentum,* because of its powdery habit of growth. Both field and laboratory work were carried on for several years, and it was found that this organism caused primarily a dry rot of the tuber, and that it was not the *F. oxysporum* of SMITH and SWINGLE, a culture of *F. oxysporum* having been furnished the laboratory for comparative work through the courtesy of Dr. SMITH. The results were to have been published in 1911, and the organism was to be named *F. pulverulentum,* but upon the appearance of APPEL and WOLLENWEBER'S monograph Dr. WILCOX proposed to the writer that he reinvestigate the organism along the lines suggested by these authors. This was especially desirable since *F. oxysporum* had been dropped and several new species established. Not only was this carried out, but the whole etiology was gone over again and all of Miss POOL'S results verified. It was found that APPEL and WOLLENWEBER (**5**) had not described the species, and consequently it was described as *F. tuberivorum* Wilcox and Link (**40**). It was so named because of the apparent restriction of its activity to tubers.

A comparison of this paper and the paper of JAMIESON and WOLLENWEBER (**16**) made it seem quite likely that both were dealing with the same organism. The organism was isolated in the Washington laboratories from potatoes sent in from Washington, Nebraska, and other states in 1910, and WOLLENWEBER upon his arrival in the laboratory, using his monograph as the basis, described it as a new species. He told the writer in 1913 that he felt convinced that we had described one and the same thing. Comparative studies made by the writer during the past year verify this point of view, and since the Nebraska publication by WILCOX, LINK, and POOL (40) did not appear in print until 1913, the name *F. trichothecioides* should be adopted.

WOLLENWEBER (41, 42) published a further paper in which he categorized the *Fusarium* spp. very sharply, dividing the genus into sections on the basis of physiological (that is, pathogenicity) and morphological (that is, conidia and chlamydospores) characters. *F. oxysporum* was again established and taken as the representative of the section ELEGANS, which comprises vascular parasites; and *F. trichothecioides* was put into the section DISCOLOR, which comprises parenchyma destroyers. He distinguished sharply between these and also between the vascular ring-discoloring *Fusarium* species of section ELEGANS and the tuber-rotting *Fusarium* species of sections DISCOLOR, GIBBOSUM, MARTIELLA, etc.

Referring to the papers by SMITH and SWINGLE (35), MANNS (24), and others, particularly to that by MANNS, he writes: "They do not separate fusarioses causing tuber rot from those causing both the wilt diseases of the plant and ring discoloration of the tuber, so that the reader might conclude that both wilt disease and tuber rot are caused by the same organism." Referring to his own experiments, he writes: "It also brings out the striking fact that the fungus, a typical xylem inhabitant, does not entirely destroy the tuber without the help of tuber rot *Fusarium* or bacteria," and "the fact that *F. oxysporum* causes the wilt of growing potato plants and only uses the xylem of the stem end of tubers for over-wintering, without producing a rot of the parenchyma, leads to interesting comparisons with the following 4 species which are able to destroy the tuber entirely from artificial wounds, namely, *F. coeruleum* (Lib.), *F. trichothecioides* Wr.," etc., and finally "the fact that the latter (*F. oxysporum*) cannot produce a tuber rot gives a biological contrast to the wound parasites of the tuber, and the fact that they cause the wilt disease of the growing plant presents a contrast to the saprophytes."

Problem and method of attack

In the spring and summer of 1914 the writer discussed the *Fusarium* situation as outlined by WOLLENWEBER with Dr. E. M. WILCOX and Dr. WILLIAM CROCKER. The former suggested that the whole situation ought to be gone over, and the latter that it would be of interest to search for the physiological basis of this alleged biological contrast. It is clear that, if the strict categories of WOLLENWEBER exist, then potato parenchyma must possess either an absolute or an effective immunity toward *Fusarium* spp. of the ELEGANS section, and that *Fusarium* spp. of the DISCOLOR section are either absolutely or practically unable to produce vascular mycoses or wilts.

The purpose of this research was twofold: (1) to determine whether such a sharp biological contrast exists; and (2) to determine what is the physiological basis for such a contrast. Experimental infections of potato plants and tubers were used for the first phase of the problem. It was clear that the second phase might involve a great many considerations, such as the structural, compositional, and metabolic nature of both host and parasite, as well as the relation of environmental factors to these. The important rôle played by the structural and compositional peculiarities of the potato and the influence of external factors upon these is well illustrated by the studies of APPEL and KREITZ (1, 3) on the efficacy of the cork layer in checking bacterial invasions of the tuber. Considerations of time and equipment limitations made it obligatory that the scope of the work be limited to a study of a few representative strains of the groups.

The writer is under obligation to the Departments of Agricultural Chemistry, Horticulture, and Experimental Agronomy of the University of Nebraska Experiment Station for the use of materials and equipment; to Miss ETHEL BEATY for help in much of the laborious routine; to Dr. FLORENCE A. McCORMICK for valuable help in the anatomical and microtechnical phases of the problem; and to Mr. R. A. DAWSON for help in preparing the photographs.

The writer decided to work with *F. oxysporum* as representative of the vascular parasite section (ELEGANS), and with

F. trichothecioides or *F. tuberivorum* as representative of the parenchyma-invading section (DISCOLOR). Since it was desirable that the identity of the organisms be well established, the writer asked Mr. W. A. ORTON, in whose laboratories Dr. WOLLENWEBER had carried out his recent work, for cultures of the organisms. It was impossible to get cultures which had been authenticated by Dr. WOLLENWEBER, since he had gone to war, but through the courtesy of Mr. ORTON, MR. CARPENTER (Dr. WOLLENWEBER'S assistant) furnished a strain of *F. trichothecioides* (no. 41, 1916) and a strain of *F. oxysporum* (no. 3345A). The other strains of *F. trichothecioides* used had been isolated by the writer in 1911, and were described as *F. tuberivorum*. Several strains of *F. oxysporum* isolated from Nebraska potatoes were also used.

Pure cultures of these organisms were maintained on sterilized rice in plugged Erlenmeyer flasks, and these were used as a point of departure for all the work recorded.

I. Infection experiments

(1) EXPERIMENTAL INFECTION OF TUBERS

Tubers of the Early Ohio and Red Cobbler varieties were used in these experiments. Only sound tubers were selected, and these were thoroughly cleansed and sterilized before infection. At first they were sterilized by the formaldehyde gas method recommended by WOLLENWEBER (41). Several difficulties were encountered in using this method. It was found very difficult to remove the last traces of the gas without contaminating the chamber, and the tubers often showed the characteristic formaldehyde vapor injuries that have been discussed in bulletins of the New York Experiment Station (13, 37). Consequently, the writer abandoned the first method and sterilized tubers by immersing in 1:1000 $HgCl_2$ solution for 1.5 hours. Inoculation was carried out by removing a piece of the cortex with a sterile cork borer, placing an infected grain of rice into the hole, and then replacing the piece of tuber tissue. The wound was then sealed with sterile grafting wax and the tuber placed into sterile chambers. This proved an efficient and convenient way of carrying out the great number of experimental inoculations made.

The first inoculations were made in December 1914. The cut ends of 20 Early Ohio tubers were wetted with spore suspensions of *F. trichothecioides* and 5 tubers were kept as controls. Four inoculated and one control potato were kept in each compartment at a temperature of 25° C. in an almost saturated atmosphere. After 4 weeks all of the inoculated tubers were in advanced stages of rot.

On January 31, 3 potatoes were inoculated according to the second method with *F. oxysporum*, and 3 with *F. trichothecioides*, and kept at 20° C. until February 17. Two of the former set were slightly rotted and one totally, while the entire latter set was rotted severely. The controls showed no rot (fig. 1).

On January 15 another series was started which was kept at a temperature ranging from 15–20° C. until February 15. Six sets of 3 tubers each were started and each set was kept in a separate sterile chamber, 2 tubers of each set being inoculated by smearing cut surfaces with agar grown inoculum. Sets I, II, and III were inoculated with *F. oxysporum*, and sets IV, V, and VI with *F. trichothecioides*. In set I, one inoculated tuber was rotted, while the other and the control were sound; in set II, one was deeply rotted and the others sound; in set III, one was deeply rotted and the others sound; in set IV, two were rotted and the control sound; in set V, two tubers were rotted slightly and the control sound; and in set VI, two tubers were rotted and the control sound.

F. oxysporum and *F. trichothecioides* were re-isolated from these rotted tubers by placing tissue cut from such tubers on plated glucose agar. Nothing other than the organism with which the tuber had been inoculated developed. Inoculum from these plates was used in infecting tubers again with the same results.

Since these results were at variance with the statements of WOLLENWEBER the experiments were repeated with hundreds of tubers, and the results were verified.

DISCUSSION.—Tubers inoculated with *F. oxysporum* did not develop the ring discoloration that is considered characteristic of the activity of *F. oxysporum*, but a general rot of the whole tuber. Generally, however, this was not a dry rot, but a rot that resembles more the soft rots of bacterial origin, although it is not accompanied by the offensive odors of bacterial rots, producing a blackening and softening of tissue which extends a considerable distance beyond the actual site of the organism. This was demonstrated microscopically and culturally. At times, however, especially in cold, dry conditions, a dry rot as typical as that produced by *F. trichothecioides* was produced. *F. trichothecioides* invariably produced a dry rot with only a very limited darkened zone extending beyond the destroyed zone, made up of large cavities and a mixture of disintegrated, dry, shrivelled tissue and fungus tissue. No darkening extended beyond the actual site of the fungus and no softening of tissue occurred. Microscopic examination revealed the fact that *F. trichothecioides* attacked the tissue intracellularly and destroyed each cell completely before it proceeded to the neighboring cell, while *F. oxysporum* attacked the tissue intercellularly at first, and then attacked the cells intracellularly, but not until the tissue had been blackened and disorganized. In this way a softened tissue without cavities was produced. These rots produced experimentally with pure cultures of *F. oxysporum* lend support to the observations and conclusions of SMITH and SWINGLE (35), MANNS (24), who reports the occasional appearance of black specks in the parenchyma of tubers infected with *F. oxysporum*, and JONES (17), who attributes stem end rot of tubers to the activity of this organism, although they may have dealt with "Mischarten." SHERBAKOFF (33) reports certain strains of species of ELEGANS (using the section as a morphological group) to be tuber rotters. He distinguishes between *Fusarium* spp. that are tuber rotters and such as are vascular element inhabitants.[1]

(2) EXPERIMENTAL INFECTION OF LIVING PLANTS

A series of experimental inoculations of healthy potato plants with *F. oxysporum* and *F. trichothecioides* were carried out, in an attempt to determine whether or not *F. trichothecioides* is unable to

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[1] After these experiments had been concluded and this paper written, a paper by CARPENTER (7) has appeared. This represents a wholly independent although simultaneously conducted piece of work. The results of CARPENTER make it quite probable that the observations made by the writer on a few strains of *F. oxysporum* are of quite general application, since he arrives at the same conclusions for numerous though different strains of *F. oxysporum*. His conclusions as to the method of attack by the fungus and the nature of the rot are practically identical with the writer's.

produce wilt, or whether the potato plant enjoys an effective or practical immunity rather than an absolute one. Even though Wollenweber (41) did not consider *F. trichothecioides* a wilt

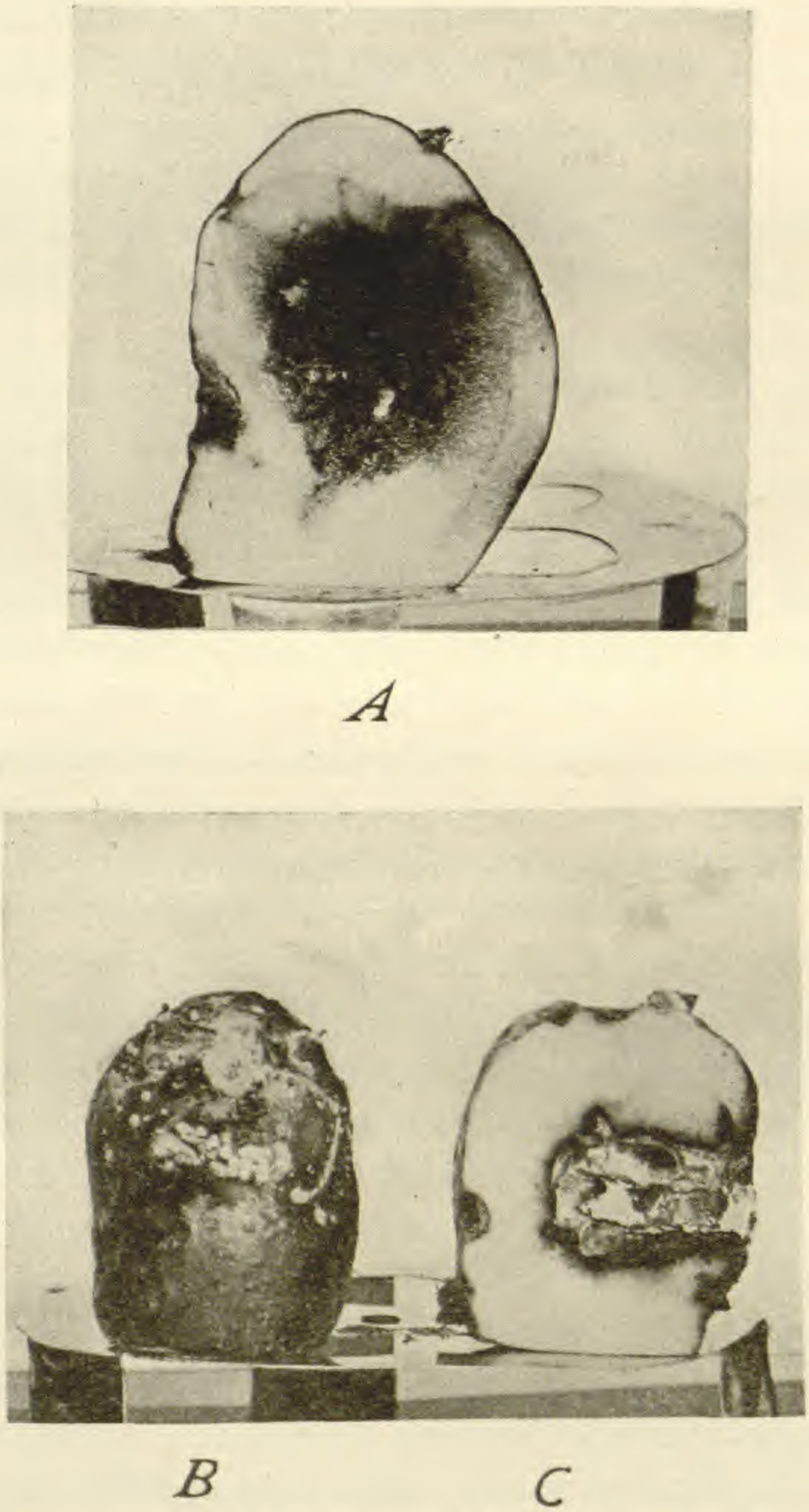

Fig. 1.—Tuber rot produced in laboratory with *Fusarium oxysporum*, and *F. trichothecioides; A*, soft rot produced by *F. oxysporum*, incubated at 20° C. for 17 days, Early Ohio variety; *B*, exterior of tuber rotted by *F. trichothecioides*, incubated at 20° C. for 17 days, Early Ohio variety; *C*, dry rot produced by *F. trichothecioides*, incubated at 20° C. for 17 days, Early Ohio variety.

producer in his 1913 paper, there is a reference in the 1912 paper by JAMIESON and WOLLENWEBER (16) to a wilt produced by *F. trichothecioides*. They referred to inoculation experiments, and report wilting in 12 days, "accompanied by a yellowing of the leaves and a discoloration of the tissue." The results of all of the writer's attempts of 1911–1912 to produce wilting of potato plants with *F. trichothecioides*, excepting one, were negative. During the past winter, however, it was noticed again and again that sprouts of tubers experimentally infected with this organism were dying. Microscopic and cultural studies left no doubt that this organism was responsible for the death of the sprouts.

Encouraged by these observations, the writer carried out some preliminary experiments on potato plants. Quartz was sterilized in 6 inch flower pots in the autoclave, and 8 plants that were about 10 cm. high were transplanted into these, the stems of some being smeared with rice infected with *F. trichothecioides*, and those of others with rice infected with *F. oxysporum*. The plants so inoculated and the controls were kept under bell jars. In three days the three plants smeared with *F. oxysporum* and two smeared with *F. trichothecioides* were dead, while the third one of the latter set and the controls remained healthy. The experiment was also conducted with potato plants growing in the open bench in the greenhouse, with similar results. The soil in this case was not sterilized.

The potato plants used in the following experiments were grown from sterilized tubers of the Early Ohio and Red Cobbler varieties in soil in 6 inch pots which had been thoroughly sterilized by heating in an autoclave for 4 hours on two consecutive days at 15 lb. pressure. The soil was watered with sterile water throughout the experiments.

On February 15, fifteen pots were planted with Early Ohio tubers and the soil of one set of 5 was infected with rice infected with *F. oxysporum*, of another with rice infected with *F. trichothecioides*, while the third set was left as a control. The controls came up in due time, while not a single one of the others came up. This experiment was repeated several times, but in no case was so striking a result obtained, although it often happened that some sprouts showed lesions, that some failed to come up, and that some were tardy in coming up. *Fusarium* spp. were isolated from such lesions. These lesions are identical in appearance with lesions found on potato stems and roots in the field which often are designated as "foot disease" and ascribed to the activity of *Rhizoctonia*. Late in May other series were started and the soil was infected with rice and spore suspensions. No infections resulted at all, even though the inoculum was derived from the same source as that used in earlier experiments.

On March 12, sprouts that were just breaking through the ground were uncovered and smeared with rice infected either with *F. oxysporum* or with *F. trichothecioides*, 6 sprouts being used in each set. The plants were wounded no more than was inevitable in removing the soil. The soil was then replaced. The soil in the controls was removed in the same way, but no inoculum was applied. The 12 sprouts to which inoculum had been applied were killed, while the controls remained healthy. There was no spreading of the disease to other sprouts, even where an abundance of inoculum was applied.

The affected sprouts reminded one forcibly of affected sprouts in potato fields in the spring. Here and there in the fields one finds sprouts that look sickly and small, which usually wilt and die or remain sickly and small. Upon examination of such sprouts, prominent brownish, watery lesions are found. At times such sprouts overcome the trouble and make a fair growth, at least until transpiration becomes excessive. These lesions also account for many of the "poor stands" or failures of potatoes to come up evenly. If one digs in where a sprout ought to have come up, one can often find a tuber that has sprouted, but whose sprouts have been cut off entirely by such lesions. Often lateral buds develop into branches on such decapitated sprouts, only to be cut off again. If such a tuber finally manages to get a shoot above the ground, the shoot is sickly and backward. In 1912, 1913, and 1914 the writer plated the inner tissue of many such sprouts and almost invariably obtained cultures of various *Fusarium* spp., although often associated with *Rhizoctonia* and bacteria. Infection experiments conducted with *Rhizoctonia* in 1912–1913 gave almost uniformly negative results. The writer was at first inclined to refer the major part of the potato troubles to the activity of this organism. Even though it is not the sole or even the main cause of Nebraska potato troubles it may play an important rôle. The work of APPEL (**2**), CORSAULT (**8**), DRAYTON (**9**), and MORSE and SCHAPOVALOV (**26**) gave results similar to those obtained by ROLFS (**31, 32**).

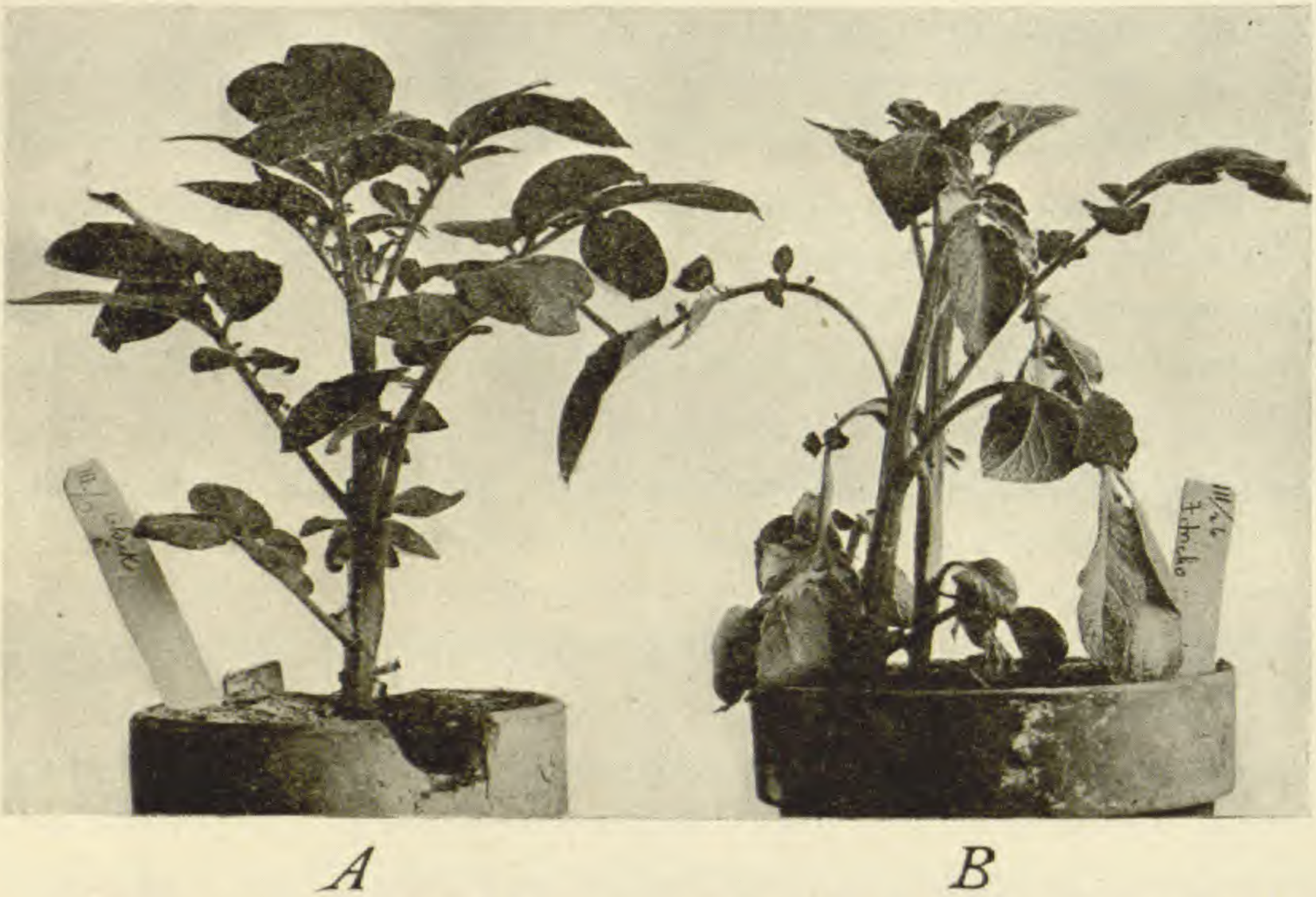

FIG. 2.—Wilt produced in laboratory with *Fusarium trichothecioides*, and control plant; *A*, control, Early Ohio variety; *B*, wilting and drying of leaves, 4 days after inoculation, Early Ohio variety.

On March 13, 24 plants grown in sterile soil were used in another experiment. These plants were about 10 cm. high at the time. The soil was removed from one shoot in each pot and the pots were arranged in 6 series. In series *A* the shoots were wounded and the wound smeared with *F. oxysporum* infected rice; in series *B* the sound stem was smeared with *F. oxysporum* infected rice; in series *C* the wounded shoots were smeared with *F. trichothecioides* infected rice; in series *D* the sound stems were smeared with *F. trichothecioides* infected rice; in series *E* no inoculum was applied to the wounded shoots; in series *F* the soil was merely removed and replaced (figs. 2 and 3).

On March 15 the following notes were taken. Series *A:* plant 1, slight curling of leaves; 2, apparently sound; 3, curling of leaves; 4, curling of leaves. Series *B:* plant 1, drooping leaves; 2, lower leaves drooping, upper leaves drying; 3, apparently sound; 4, apparently sound. Series *C:* plant 1, apparently sound; 2, some wilting; 3, some wilting; 4, some wilting. Series *D:* plants 1, 2, and 3 apparently sound; 4, wilting. By March 21 the plants infected with *F. oxysporum* showed a pronounced folding upward of leaves on the midrib, wilting and rolling on the margins of the leaves, the folding being most pronounced in the tips of the plants. The plants affected least showed discoloration on the margins, which at times was of a yellowish tint, at times purplish to violet. The leaves of plants most severely affected showed a yellowing and burning of the leaf margins. One plant, inoculated with *F. oxysporum*, developed a pronounced rosette, but overcame this later, growing into quite a normal plant (figs. 4 and 5). These symptoms remind one forcibly of certain symptoms of the leaf-roll disease which has received so much attention, and which has been made the subject

FIG. 3.—Wilt produced in laboratory with *Fusarium oxysporum*, and control plant; *A*, control, Early Ohio variety; *B*, wilting of lower leaves and curling of upper leaves, 4 days after inoculation, Early Ohio variety.

of thorough study by APPEL and his co-workers (**2**, **4**). Eventually the plants infected with *F. trichothecioides* showed much severer symptoms than those inoculated with *F. oxysporum* (fig. 6). Eight plants died in the former sets, and 3 in the latter. Plants infected with *F. trichothecioides* showed such severe and rapid burning and drying up of leaves, that the typical wilting phenomena were scarcely realized. The vascular bundles were blackened and the blackening extended even into the petiole and the leaf veins. This rapid killing was at first strictly localized on that side of the plant to which the inoculum had been applied, even in the leaf, where the leaflets on one side of the midrib would be affected, and those on the other side not. Eventually in those cases in which killing of the

FIG. 4.—Leaf roll and rosette of field plant of the Pearl variety; August 1912, at the U.S. Substation at Mitchell, Neb.

whole plant took place, the fungus girdled the whole stem, while plants that were not girdled lived on, even though one side was entirely destroyed. There was little lateral and subsequent vertical spreading of the fungus from one vascular strand to the other. These experiments were repeated with 25 other plants and in most cases the same symptoms were observed. These symptoms have been repeatedly observed in the dry land areas of Nebraska, but have always been looked upon as cases of "sun scald," and and in previous experiments with wilting due to *F. trichothecioides* such cases were ignored.

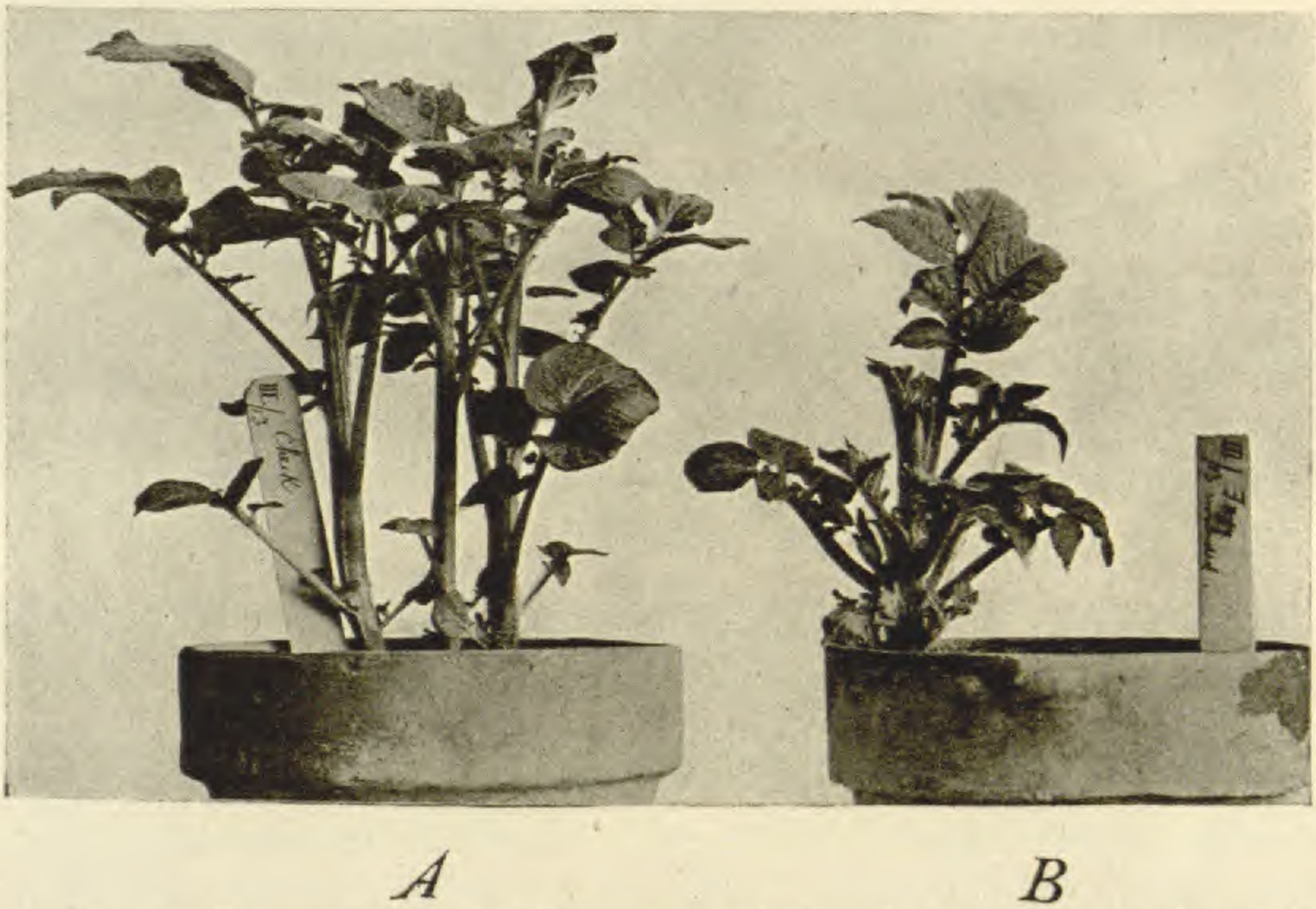

FIG. 5.—Rosette produced in laboratory with *Fusarium oxysporum*, and control plant; *A*, control, Early Ohio variety; *B*, rosetted plant, 10 days after inoculation, Early Ohio variety.

Plants grown in soil infected with *F. oxysporum* and *F. trichothecioides* showed severe lesions of root and stolons. Examination of roots affected with either organism showed that the cortical regions are first and most severely attacked, not only intercellularly, but also intracellularly, the cells being packed full with hyphae. In most cases the cortex could be sloughed off with exceeding ease. From the cortex the organisms invaded the stelar regions, where

F. oxysporum makes greater headway than the other and there causes a vascular mycosis more frequently, which accounts for its designation as a vascular parasite (figs. 7 and 8).

Discussion.—If plants, experimentally inoculated, showed only light symptoms to begin with, most of them continued their growth with symptoms less severe than those shown in the field. If they showed severe symptoms early, these proved more severe and more rapidly fatal than those in the field. The organisms in the field work much more insidiously, attacking the roots of the plant slowly but progressively, and permitting the plant, except in extreme cases, to readjust for its water requirements. These readjustments manifest themselves in the curling and rolling phenomena (figs. 4 and 9).

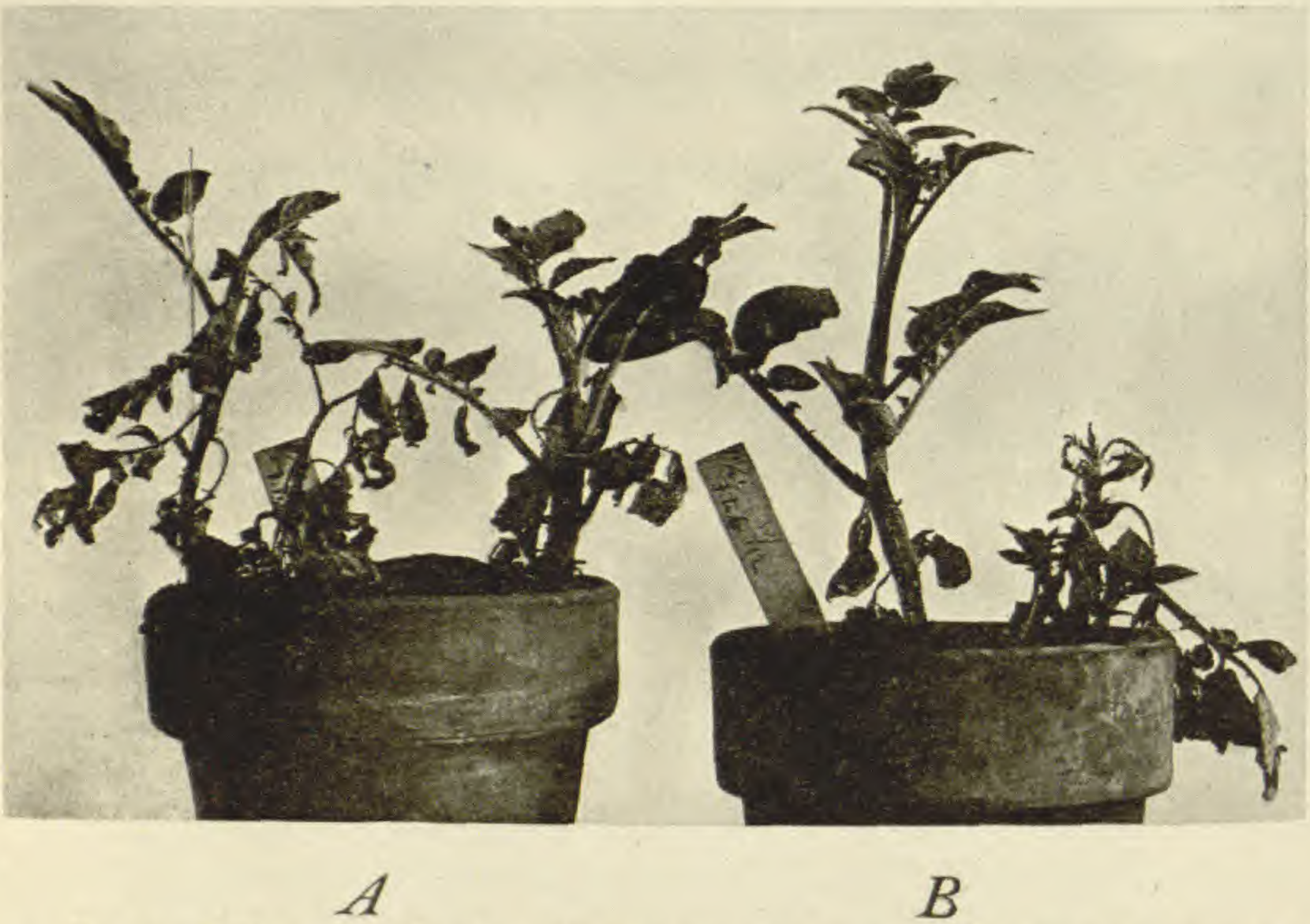

Fig. 6.—Wilt and death of potato plants produced in laboratory with *Fusarium trichothecioides*, 12 days after inoculation; Early Ohio variety; wilting is restricted to the side to which inoculum was applied.

Potato plants in the irrigated sections show this phenomenon nicely. As long as cultivation and irrigation are maintained, the plant develops new roots progressively higher up, and the infected plants get along fairly well, showing slight curling and wilting, although tuber development occurs. When in midsummer

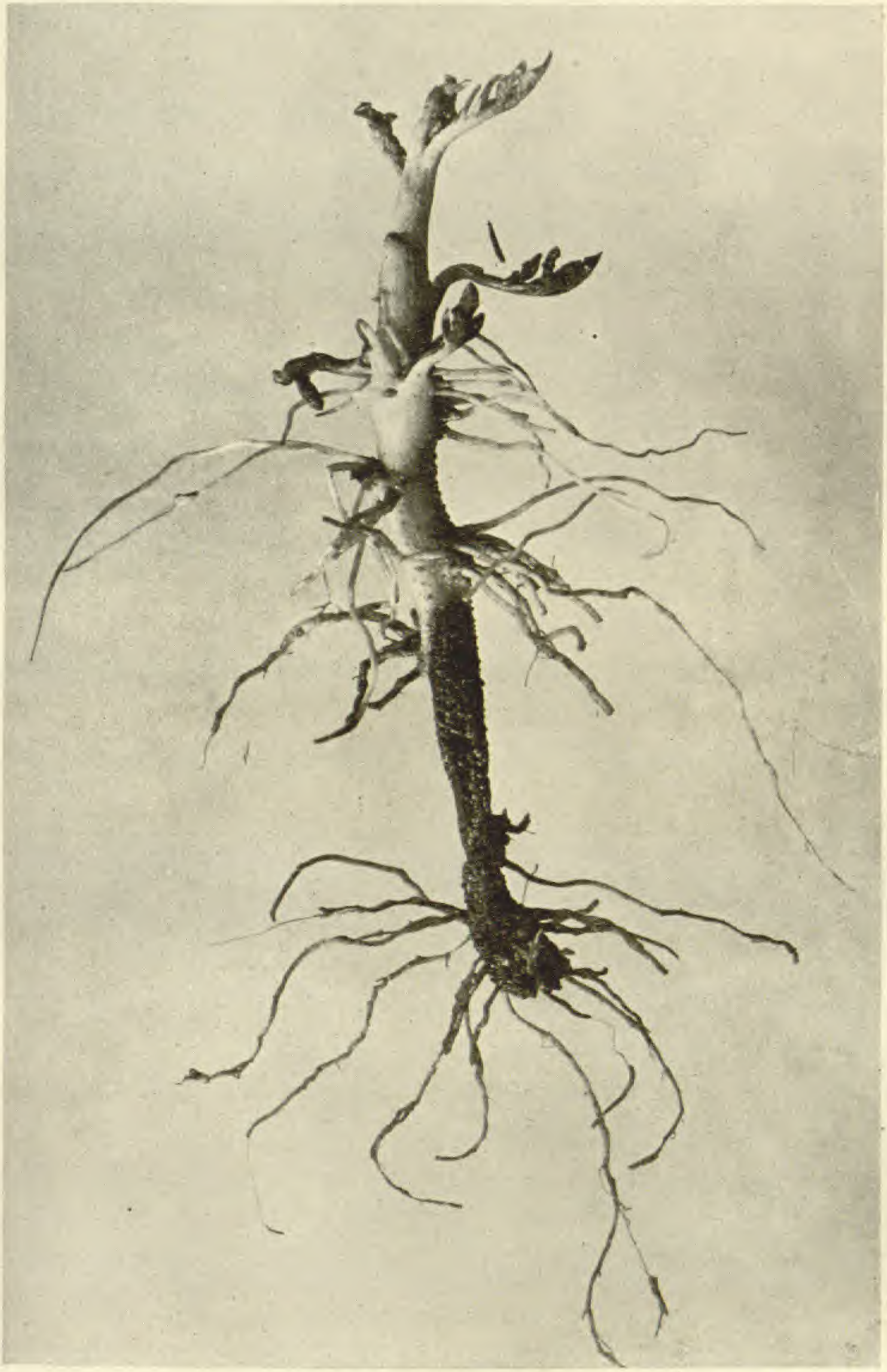

FIG. 7.—Lesions on stem and roots produced in laboratory with *Fusarium oxysporum*, 2 weeks after inoculation; Early Ohio variety.

FIG. 8.—Root lesions produced in laboratory with *Fusarium trichothecioides*, curling and rolling of leaves, 2 weeks after inoculation; Early Ohio variety.

irrigation ceases and no more soil is heaped about the crown of the plant and transpiration requirements must be met by badly infected lower roots and a few healthy upper ones without the possibility of developing new roots, the plant soon succumbs. In this way we get the exceedingly frequent phenomenon of large plants, usually

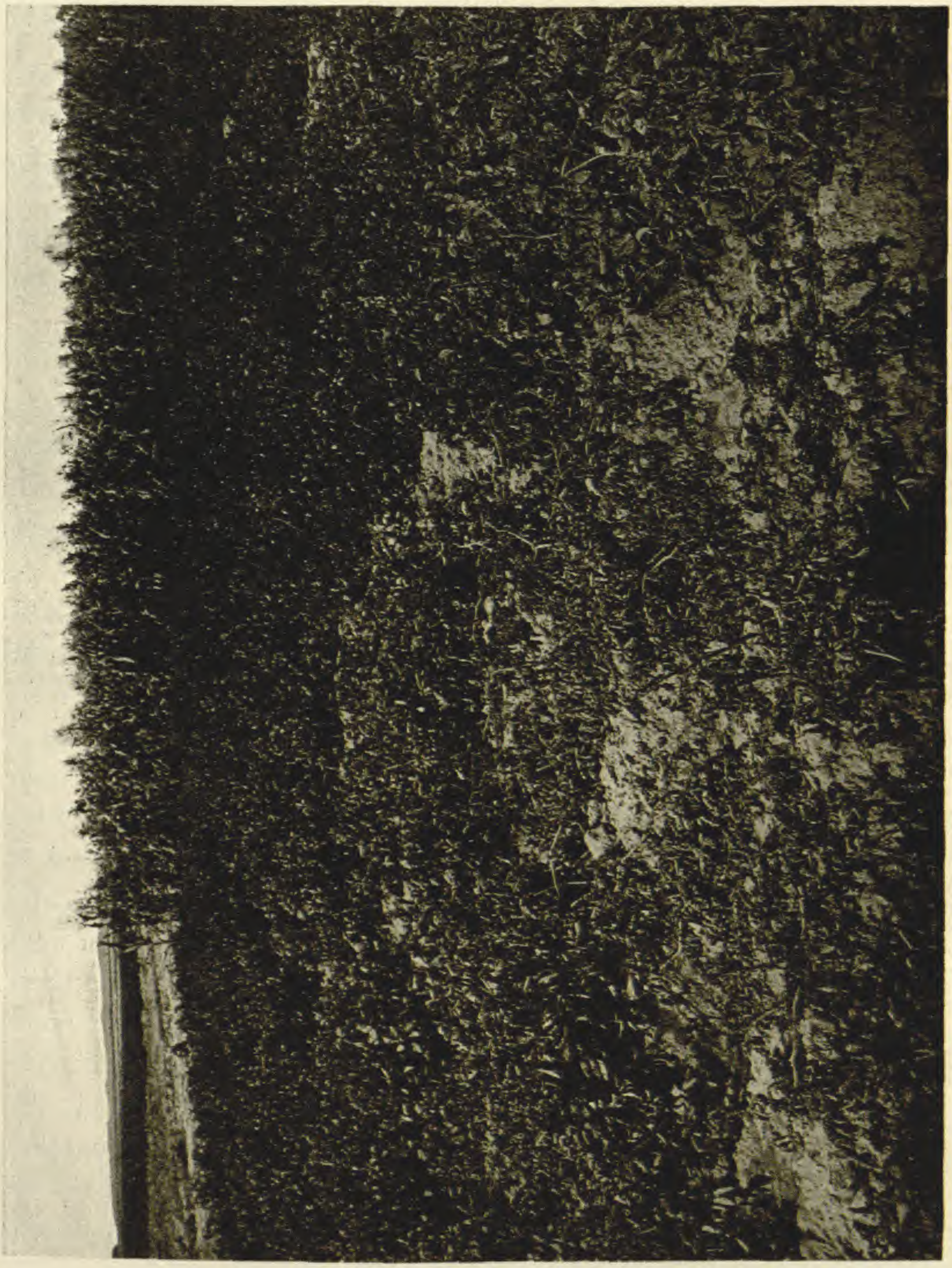

FIG. 9.—Potato wilt and little potatoes in a field of the Pearl variety, August 1912, at Mitchell, Neb.

with many small tubers, wilting down suddenly after the last irrigation. The frequent occurrence of aerial tubers, the prevalence of excessive numbers of small tubers, and the occurrence of few abnormally large tubers on such plants is also attributable to the insidious manner of attack. The organisms attack the stolons and main stem as well as the roots. Stolons with tubers in all stages of maturity can be found partially or completely cut off by lesions. As the balance between the photosynthetic and storage centers in such plants is disturbed, new stolons are developed nearer and nearer the surface and the stolons that are not attacked develop abnormally large tubers. Often the plant responds to this disturbance in the assimilation-storage balance by producing swellings of the aerial parts of the plant, the so-called aerial tubers. Many large plants can be pulled up with ease, because lesions make separation of the tops from the roots or even the basal portion of the stem easy. Such plants may show a comparatively sound main axis (fig. 10).

Infection carried over by the mother tuber, which is frequent, rarely permits the growth of stems more than 20 cm. high, and seldom allows the development of tubers. An early attack from without upon the main stem leads to equally disastrous results.

The wilts of the potato plant induced by *Fusarium* spp. have generally been considered vascular mycoses due to a clogging of the vascular elements. In fact, however, the symptoms are due to killing of the root system as much as to clogging of the vascular elements. It is true that members of the ELEGANS section, such as *F. oxysporum*, frequent the vascular elements, spreading in these rather than clogging them, but it is true also that they destroy roots in numbers. Again, even though some have referred to this disease as a root disease (SMITH and SWINGLE 35), it is stated that the fungus enters a root, then spreads to the stelar part, and from there enters other roots and stolons. Just as much damage is done by the persistent attack from without upon roots and stolons, as noted by MANNS (24).

In the course of these experiments several questions were raised. The soil in these experiments surely was more severely infected with the organisms than soil under field conditions can

FIG. 10.—A plant of the field shown in fig. 9; after showing slight curling and discoloration of leaves and formation of aerial tubers in earlier part of summer this plant suddenly wilted down and died; such a plant as this usually bears a great number of small and a few abnormally large tubers which are infected in the stem end with the fungus that killed the mother plant.

be, yet there were many plants grown in such soil that showed no infection whatsoever. Less success in producing wilt was observed as the season progressed. It remains a question whether this is due to a loss in vitality or to a gain in resistance in the plants, due to a change in the soil, tubers, or the organism.

Whether the success in producing wilt with *F. trichothecioides* and the apparent waning of this power is due to a gain or regain of virulency and a subsequent loss again is also an unanswered question. SHERBAKOFF (33), working with *Fusarium* spp., got uniformly negative results so far as producing wilt is concerned, and concluded that the results were due to a loss of virulency of the cultures or to some other important factor that had escaped attention.

Summary

It is quite apparent that some of the strains of *F. oxysporum* can cause tuber rot; that they can destroy tubers entirely without the aid of other *Fusarium* spp. or bacteria; that at least one *Fusarium* of the DISCOLOR section (*F. trichothecioides*) can produce wilt of stem; and that the biological contrast drawn by WOLLENWEBER between the *Fusarium* spp. is not as sharp as one would infer from his paper. It is possible that these strains of *Fusarium* spp. are morphologically identical with those described by WOLLENWEBER, but physiologically unlike them. That this rule, if it exists, is not so rigid generally, however, is noted by SHERBAKOFF (33), who found that no correlation exists between morphological relationship and pathogenicity.

Although *F. oxysporum* is not absolutely unable to attack potato parenchyma, the potato tuber, in which usually only the xylem elements are invaded, enjoys an effective immunity from its attacks; and although *F. trichothecioides* can attack any subterranean part of the living potato plant, generally all parts excepting the mature tuber enjoy an effective immunity from its attacks.

The data given in the second part of this paper may furnish a partial explanation of these phenomena.

II. Ecology and physiology of the organisms

METHOD AND DATA

1. TEMPERATURE RELATIONS.—Observations of cultures grown at ordinary temperatures showed that there is a striking difference in the rate of growth of the two organisms. Potato cylinder, rice, liquid potato, and glucose media, and glucose and potato agar cultures all showed that *F. oxysporum* makes a considerably greater initial growth at temperatures above 20° C. than does *F. trichothecioides*. At temperatures in the vicinity of 10–15° C., however, *F. trichothecioides* makes the greater initial growth, although these temperatures lie below its optimum. The same difference was noted in cultures on neutral and acid potato agar. This point was also tested with cultures on sterile slabs of potato tubers kept in Petri dishes. At 25° C. *F. oxysporum* covered such slabs completely when *F. trichothecioides* barely had made a start, while at 12° C. the situation was reversed.

When 1 per cent liquid glucose media were inoculated with spore suspensions of *F. oxysporum*, visible growth was made in 16 hours; when *F. trichothecioides* was used, 30–42 hours elapsed before visible growth was made. This holds for temperatures above 20° C. The optimum temperature for *F. oxysporum* was about 30° C., and for *F. trichothecioides* about 20–22° C., both varying slightly with the medium used. The maximum for *F. oxysporum* lay between 38 and 40° C. The optima and maxima were higher for cultures in potato extract than for glucose media cultures. The writer has not been able to determine the minima accurately because of inadequate apparatus. HUMPHREY (15) gives 4° C. as the minimum growth temperature for a certain strain of *F. oxysporum*.

Potato agar cultures of *F. oxysporum* and *F. trichothecioides* could endure a temperature of 40° C. for 5 and for 20 hours respectively and remain viable. Exposure at 50° C. for 5 hours killed *F. trichothecioides*, but not *F. oxysporum;* while exposure for 20 hours killed both. Some *F. oxysporum* cultures survived 5 hours exposure at 57° C.

The growth relations were also checked up quantitatively. In these experiments, as well as in all the following ones, the method suggested by HASSELBRING (14) was followed. Erlenmeyer flasks of 200 cc. capacity were used with 50 cc. of solution per flask. The solutions in the flasks were autoclaved for 10 minutes at 7 lb. pressure, and then inoculated by means of sterile pipettes with a drop or two of spore suspension. The cultures were killed by adding 10 cc. of 10 per cent HCl to each flask. The cultures were then filtered off on tared Gooch crucibles prepared with asbestos, washed until acid free, and brought to constant weight in a Freas electric oven at 100° C., and the dry weight determined. It was found impossible at times to filter luxuriant cultures of *F. oxysporum* by this method, because of the tenacity with which this organism holds water. Consequently they were filtered on soft filter paper, transferred to tared Gooch crucibles, dried, and weighed. The other organism holds water with little tenacity and filters with ease.

In all of experiments given below the following stock mineral solution was used: 20 gm. NH_4NO_3; 10 gm. KH_2PO_4; 5 gm. $MgSO_4$ per 1000 cc. H_2O. When carbohydrates were employed,

TABLE I

DRY WEIGHT (IN MILLIGRAMS) AFTER 20 DAYS' GROWTH IN POTATO EXTRACT MEDIUM; ROOM TEMPERATURE

	FUSARIUM OXYSPORUM					
	Temperature					
	35°	30°	25°	12°	1°.11*	—1°.11*
Flask 1	40	55	63	64	62	86
Flask 2	47	78	68	66	....	....
Flask 3	61	86	80	68	....	....
Average	49	73	70	66	62	86

	FUSARIUM TRICHOTHECIOIDES					
	Temperature					
	35°	30°	25°	12°	1°.11*	—1°.11*
Flask 1	0	0	60	87	146	83
Flask 2	0	0	64	100	....	....
Flask 3	0	0	65	147	....	....
Average	0	0	63	111	146	83

*For 20 days (no growth), then at 25° C. for 25 days.

these were added at the rate of 10 gm. per liter. Potato extract medium was made up by extracting 500 gm. ground potato tuber with 500 cc. H_2O, and then adding 500 cc. of the foregoing solution to the extract.

A series of cultures (table II) was run at 12° C. and the amount of dry weight formed determined at 2 day intervals for 10 days. In this series the medium was at 12° C. at the time of inoculation.

TABLE II

Dry weight (in milligrams) in potato extract medium; temperature 12° C.

	Fusarium oxysporum					Fusarium trichothecioides				
	Number of days					Number of days				
	2	4	6	8	10	2	4	6	8	10
I	0.2	0.4	5.2	......	13.2	1.4	4.0	9.4	......	27.0
II	0.6	0.4	5.6	......	13.0	1.8	4.4	12.2	......	44.6
Average	0.4	0.4	5.4	......	13.1	1.6	4.2	10.8	......	35.8

Table III shows the growth by day intervals made for 10 days when levulose was used as the carbon source. The solutions were at the temperatures indicated at the time of inoculation.

TABLE III

Dry weight (in milligrams) formed by day intervals

	Fusarium trichothecioides at 25°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I	0.2	0.6	4.2	12.8	29.0	19.2	25.4	19.4	34.0	43.6
II	0.2	2.4	18.0	31.6	32.4	34.2	27.2	41.9	47.7	50.8
Average	0.2	1.5	11.1	22.2	30.7	26.7	26.3	30.6	40.8	47.2

	Fusarium oxysporum at 25°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I	0.4	1.8	9.2	16.0	23.6	19.6	24.0	24.1	30.9	37.0
II	0.8	3.2	10.8	29.2	33.4	21.2	27.5	26.0	34.8	38.8
Average	0.6	2.5	10.0	22.6	28.5	20.4	25.7	25.0	32.8	37.9

TABLE III—*Continued*

	FUSARIUM OXYSPORUM AT 30°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I.........	1.0	3.6	15.4	17.8	37.6	45.8	55.8	66.0	78.2	62.2
II........	8.9	5.2	17.6	19.0	40.2	77.8	62.6	69.6	78.6	66.6
Average.	4.9	4.4	16.5	18.4	38.9	61.8	59.2	67.8	78.4	64.4

	FUSARIUM TRICHOTHECIOIDES AT 25°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
Average...	4.4	19	67.4	......	......	......	......	262	......	......

	FUSARIUM OXYSPORUM AT 30°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
Average...	11.2	48	108.6	......	......	......	......	240.2	......	......

These tables show a tendency of *F. trichothecioides* to make a greater initial growth at low temperatures. At higher temperatures, however, unless above the optimum of *F. trichothecioides*, *F. oxysporum*, even though it made the greater initial growth, was soon overtaken and passed by *F. trichothecioides*. This was especially marked when dextrose and levulose were used as carbon source. This may be the result of a faster though more superficial feeding of *F. oxysporum*, which makes it unable to use materials as thoroughly as the other organism. This phenomenon is hardly a case of more rapid intoxication on the part of *F. oxysporum*.

The results obtained with artificial media were verified by infection experiments conducted with potato tubers kept at various temperatures. Tubers of the Red Cobbler variety were used. These were inoculated on April 1, and examined on May 27 (table IV). See figs. 11 and 12.

It should be noted here that *F. trichothecioides* when inoculated into a tuber can grow at 30° C., while it cannot do so in artificial media; and that *F. oxysporum* can survive a temperature of 1° C. in artificial media, but not in the tuber.

TABLE IV

CONDITION OF TUBERS AT CLOSE OF EXPERIMENT

Temperature	Fusarium oxysporum	Fusarium trichothecioides
30° C.	All completely rotted; sprouts killed	Slight rot in some
25° C.	All completely rotted; sprouts killed	All completely rotted; some sprouts killed
12° C.	All with very slight rot	All completely rotted
1° C.	No rot	All with slight rot
1° C. for two weeks, then 25° C. for two weeks. .	Slight rot in one tuber	All completely rotted
−1° C.	No rot	No rot
−1° C. for two weeks, then 25° C. for two weeks. .	No rot	All completely rotted

DISCUSSION.—These results may, in part at least, explain why *F. oxysporum*, even though it can attack parenchyma and rot tubers, usually is not found in rotted tubers, while *F. trichothecioides* is. The ability of the latter to make a faster initial growth at the temperatures which prevail in the soil about digging time and in well kept storage places is probably the determining factor in this phenomenon. The experiments with tubers showed that *F. trichothecioides* made a great increase in growth rate when transferred from a low to a higher temperature.

These temperature relations may also explain in part the fact that we usually find *F. oxysporum* producing wilt under field conditions, and lend support to the observations made by ORTON (27), who reports potato wilt induced by *Fusarium* spp. to be pre-eminently a warm climate disease. *F. trichothecioides* can produce wilt, but the temperature conditions in the soil are such as to favor *F. oxysporum*, the maximum temperature of the former being the optimum of the latter. HUMPHREY (15), working in Washington on the tomato wilt induced by *F. oxysporum*, came to the conclusion that temperature differences in various parts of the state were determining factors for the appearance and non-appearance and severity of the disease.

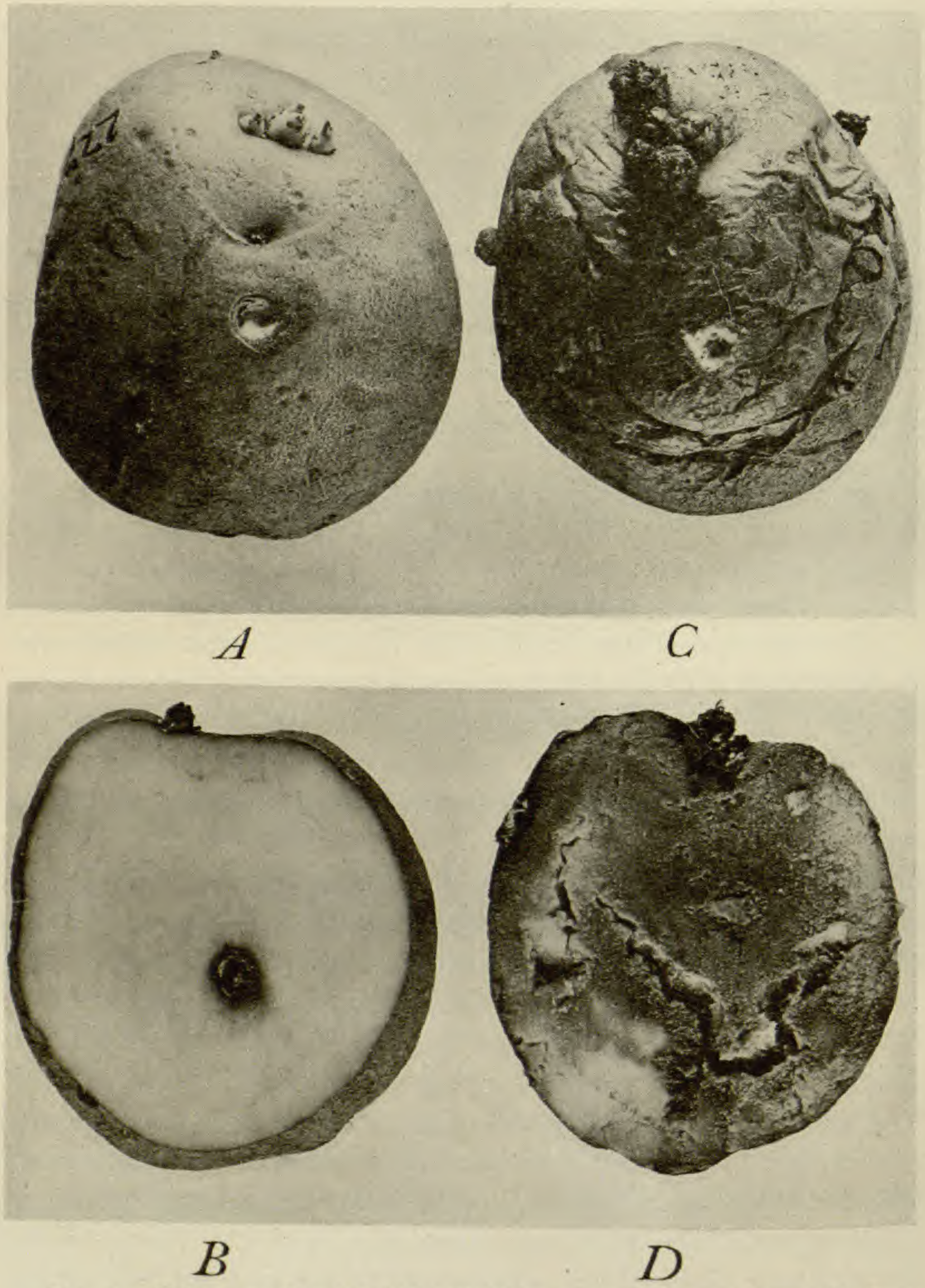

FIG. 11.—Tuber rot of Red Cobbler variety produced by inoculation with *Fusarium oxysporum*; *A*, *B*, external and sectional view of same tuber, incubated for 30 days 12° C.; *C*, *D*, external and sectional view of same tuber, incubated for 30 days at 25° C.

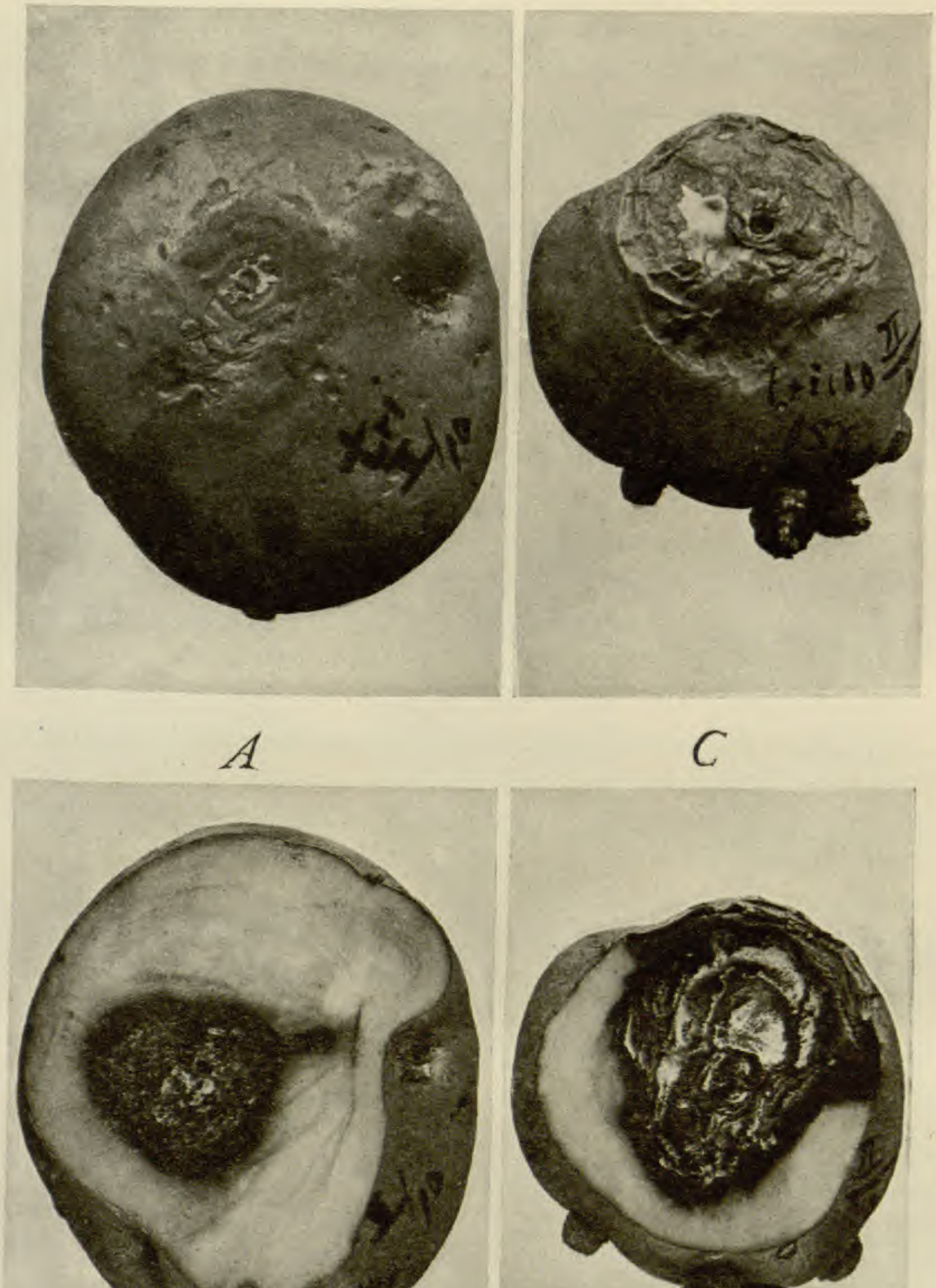

FIG. 12.—Tuber rot of Red Cobbler variety produced by inoculation with *Fusarium trichothecioides*; *A*, *B*, external and sectional view of same tuber, incubated for 30 days at 25° C.; *C*, *D*, external and sectional view of same tuber, incubated for 30 days at 12° C.

2. GROWTH HABIT.—It was observed in nearly all cultures that *F. oxysporum* not only made a greater initial growth at ordinary temperatures, but that it was at all temperatures much more of a surface grower than *F. trichothecioides*, making a superficial spreading growth, rather than the penetrating restricted intensive growth of the latter. Early sporulation was associated with the restricted growth habit of the latter (fig. 13). These habits were especially clearly marked on solid substrata, but even in liquid media *F. oxysporum* made a much less compact growth than the other species. It may be that the more spreading and extensive growth habit of *F. oxysporum* at all temperatures and its more rapid initial growth at temperatures above 10–15° C. are associated with a greater oxygen requirement than that possessed by *F. trichothecioides*. This would explain in part the frequenting of intercellular spaces and xylem elements by the former, and its consequent greater efficiency in causing vascular mycosis and wilt, as well as its tendency to cause bundle discoloration. The xylem elements of the stem end are undoubtedly infected while the tuber is yet in the soil, where temperature conditions are such as to favor the growth of *F. oxysporum*. Storage temperatures check the growth of this organism and the cells bordering the infected vascular elements shut the infected area off by suberizing their walls. Cultural experiments and microscopical studies show that cork is not absolutely impenetrable to these organisms, although it provides under normal conditions an effective barrier to the progress of both of these species. Because of the slower growth of *F. trichothecioides* at higher temperatures, the potato plant undoubtedly has a much better opportunity to guard itself by cork formation against this organism than against the other.

3. THE CARBON SOURCES OF THE TWO ORGANISMS.—A difference in the metabolic requirements of two organisms, a difference in their ability to utilize various substances, or a difference in their ability to tolerate the presence of substances may be factors of critical importance in determining which of the two will attack a given tissue or a given plant. These factors may determine also the modes of attack of an organism upon a tissue or a plant. Thus an organism that can digest pectinaceous material and not cellulose

would have to destroy a tissue whose walls are mainly cellulose by intercellular activity, while one that could digest cellulose might

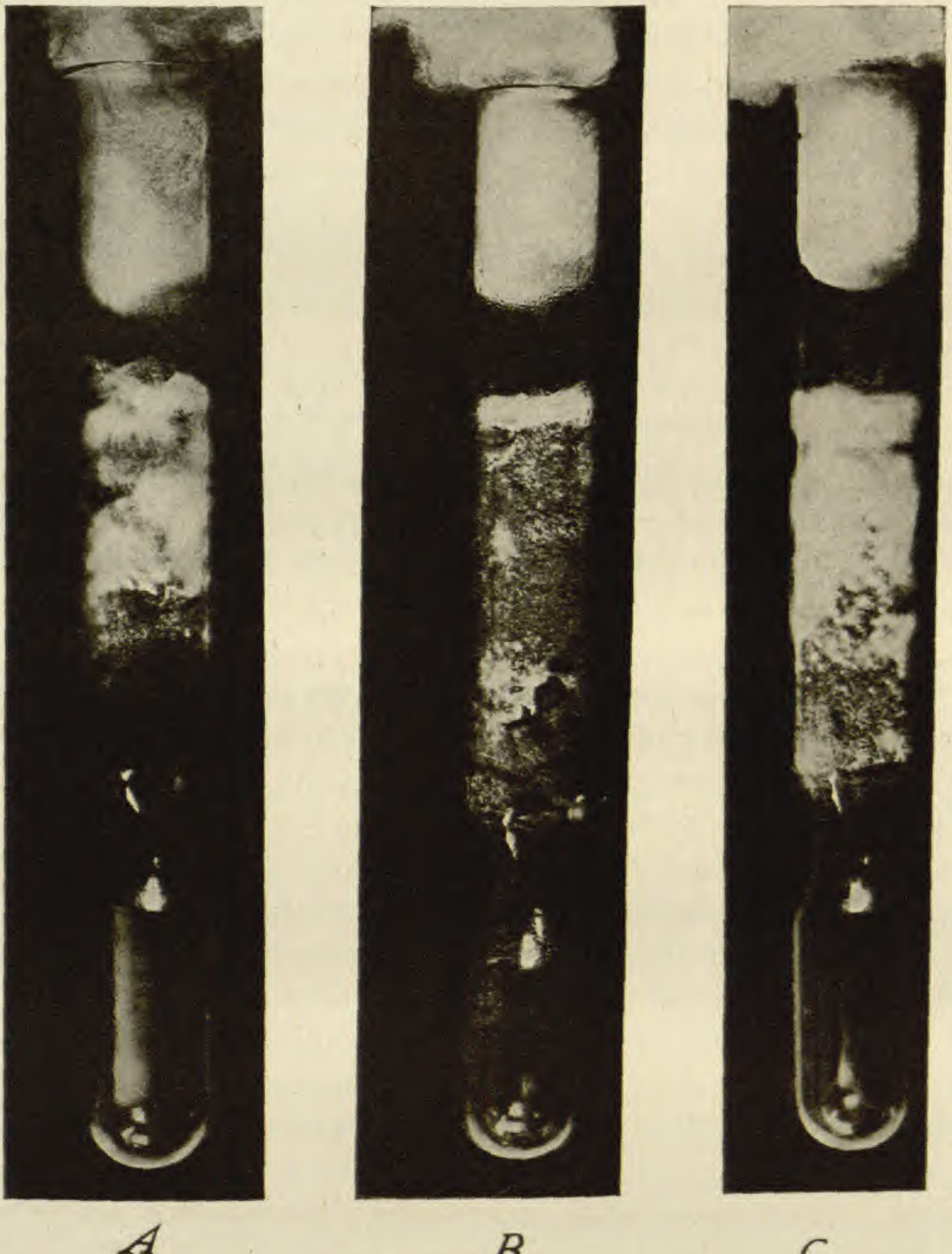

FIG. 13.—*Fusarium trichothecioides* and *F. oxysporum* on sterile potato cylinders; *A*, *C*, cylinders inoculated with *F. oxysporum*, incubated for 2 days at 25° C.; *B*, cylinder inoculated with *F. trichothecioides*, incubated for 2 days at 25° C.

destroy this tissue by a primary cell invasion. Again, a greater ability on the part of an organism to digest suberin, other things being equal, would render it a much more formidable enemy of the potato plant than an organism without this ability, or possessing it to a less degree. This problem was attacked by making a study of the carbon sources of the organisms. The data reported here are only a beginning of this phase of the problem.

Fifty cc. of nutrient solution were measured quantitatively into 200 cc. Erlenmeyer flasks with a pipette. The flasks were then plugged with cotton, covered with tinfoil, and autoclaved. After cooling, 0.5 gm. of carbohydrate material was transferred quantitatively into each flask, and the flasks covered again with tinfoil and sterilized in a Freas oven by heating at 85° C. for one hour every 12 hours, for 6 consecutive days. The solutions were then incubated at 25° C. for 48 hours, so as to allow any contaminations present to appear. Low sterilization temperature was used to reduce hydrolysis of carbohydrates to a minimum.

The dry weight determinations were made by the methods outlined above. It was found advisable to kill two cultures of each set after 6 days, for the striking differences in rate of growth between the two organisms that were observed during the first 48–120 hours were obliterated by prolonged growth. The other 3 cultures were killed after 12 days' growth. The dry weight values do not show the differences in habit and rate of growth in the cultures as strikingly as they appeared to the eye. In many cases a visible growth was not determinable as dry weight. This is readily appreciated when we consider that moisture determinations indicated that the dry weight varied between 10 and 20 per cent of the wet weight.

In the controls, consisting of the plain mineral medium without carbon material, *F. oxysporum* made a weighable growth in 12 days, though not in 6 days, while *F. trichothecioides* made no weighable growth even after 12 days. Another important observation was made. In no case was it necessary to reinoculate with *F. oxysporum*, while many *F. trichothecioides* inoculations failed. The latter undoubtedly is the slower starter and much more poorly equipped for sure and quick infection than the former.

The figures in tables V–VIII represent milligrams of dry weight of material formed, except in those cases in which per cent is written. In such cases (cork, cellulose, and hemicellulose), the figures represent the percentage of decrease in dry weight of material. The last weighing in these cases unavoidably included the dry weight of fungus material formed, so that the figures are higher than they ought to be. The differences in weight in these cases give only comparative values of the amounts of material respired by the organisms. The filter paper used was the best Swedish paper, and the cork was obtained by skinning steamed potatoes, scrubbing the skin thoroughly, boiling it for 48 hours in distilled water, extracting for 48 hours in ether, and then boiling again with water. All figures represent averages, the composition of these figures being shown in tables VI and VII. In many cases there was a fair coincidence of the values, while in others a great disparity appeared. The averages probably would more nearly approximate the true value if a greater number of figures were available.

TABLE V

DRY WEIGHT (IN MILLIGRAMS) FORMED IN 6 AND 12 DAYS BY *Fusarium trichothecioides* AND *F. oxysporum*

	FUSARIUM TRICHOTHECIOIDES		FUSARIUM OXYSPORUM	
	Number of days		Number of days	
	6	12	6	12
Ethyl alcohol	....	1.2	15.0	18.5
Glycerine	12.0	20.5	17.0	92.0
Mannit	108.5	136.3	109.5	112.0
Arabinose	5.2	35.0	78.7	43.0
Glucose	11.5	42.3	43.5	44.0
Mannose	2.0	52.0	49.0	62.0
Galactose	28.8	43.3	39.0	73.0
Fructose	56.5	81.0	74.5	77.6
Saccharose	9.5	45.6	36.5	35.6
Maltose	13.0	61.0	35.5	50.3
Lactose	1.3	20.0	4.0	21.0
Raffinose	12.4	47.3	44.5	54.6
Potato starch	....	45.0 per cent	81.0	103.0
Wheat starch	22.6 per cent	42.1 per cent	54.3 per cent	127.0
Corn starch	19.7 per cent	33.4 per cent	37.9 per cent	67.2 per cent
Soluble starch	45.2 per cent	49.0 per cent	110.5	210.3
Dextrine	4.4	37.3	42.0	56.3
Inulin	63.6	123.0	102.0	90.0
Gum arabic	1.5	18.6	37.0	58.3
Gum tragacanth	67.5 per cent	14.1 per cent	27.1 per cent	25.7
Hemicellulose	10.1 per cent	7.3 per cent	10.1 per cent	6.7 per cent
Cellulose	4.0 per cent	1.0 per cent	4.25 per cent	6.7 per cent
Cork	10.1 per cent	5.9 per cent	12.8 per cent	3.9 per cent
No carbon source	....	....	....	0.5

Table V shows that qualitatively the two organisms behave alike in their ability to use all the carbon compounds tested. Quantitatively there is considerable difference, both as to rate of consumption and total growth after 12 days. *F. oxysporum* in general shows the greater speed of growth and greater growth after twelve days. In some cases *F. trichothecioides* shows the greater growth after 12 days.

TABLE VI

DRY WEIGHT (IN MILLIGRAMS) FORMED WHEN EACH FLASK RECEIVED 0.5 GM. OF MANNIT; CONCENTRATION 1 PER CENT

NUMBER OF FLASK	FUSARIUM OXYSPORUM		FUSARIUM TRICHOTHECIOIDES	
	6 days	12 days	6 days	12 days
I	93	....	107	....
II	126	....	110	....
III	....	108	....	130
IV	....	112	....	135
V	....	116	....	141
Average	109.5	112	108.5	136.3

TABLE VII

HEMICELLULOSE (IN GM.) USED IN 12 DAYS

NO. OF FLASK	FUSARIUM OXYSPORUM			FUSARIUM TRICHOTHECIOIDES		
	6 days			6 days		
	Grams of material	Decrease in weight	Percentage of decrease	Grams of material	Decrease in weight	Percentage of decrease
I	0.5055	0.0455	9.0	0.5213	0.0513	9.8
II	0.5154	0.0584	11.3	0.4964	0.0524	10.5
Average	....	....	10.1	....	....	10.1
	12 days			12 days		
III	0.5185	0.0355	6.1	0.501	0.028	5.5
IV	0.5192	0.0382	7.3	0.5058	0.0488	9.4
Average	....	....	6.7	....	....	7.3

Table VIII gives the dry weight formed by the organisms when carbon acids were furnished as carbon sources. N/100 solutions were used, excepting for asparagin and asparagenic acid, whose solubility permitted only N/200 solutions. In the case of the higher fatty acids and oils, the material was weighed out as though N/100 solutions were being prepared. With these no weighings of the material formed were attempted, but merely differences in luxuriance of growth were recorded. To those acids which showed no growth with N/100 solutions, 5 cc. of 10 per cent glucose solution was added, making the sugar concentration 1 per cent, so as to determine whether the acid was merely non-usable, or whether it was toxic. Since it was found that some were toxic at N/100 concentration, lower concentrations were made up also. The results are given in table IX.

TABLE VIII

DRY WEIGHT (IN MILLIGRAMS) FORMED WITH THE FOLLOWING CARBON COMPOUNDS AS CARBON SOURCES

	FUSARIUM TRICHOTHECIOIDES		FUSARIUM OXYSPORUM	
	Number of days		Number of days	
	6	12	6	12
Formic acid N/100....	None	None	None	None
+5 cc. 10 per cent glucose solution.....	"	"	"	85.5
Acetic acid N/100.....	"	"	1.2	10.6
+5 cc. 10 per cent glucose solution......	"	106	None	None
Proprionic acid N/100..	"	None	"	"
+5 cc. 10 per cent glucose solution......	"	"	"	"
Butyric acid N/100....	"	"	"	"
+5 cc. 10 per cent glucose solution......	"	"	"	"
Glycocollic acid N/100.	2.5	7.3	20.0	8.6
Lactic acid N/100.....	0.3	0.6	8.0	23.0
Oxalic acid N/100.....	Non-weighable	Non-weighable	Non-weighable	Non-weighable
Succinic acid N/100...	Non-weighable	Non-weighable	8.5	9.3
Malic acid N/100.....	Non-weighable	Non-weighable	4.5	8.0
Tartaric acid N/100...	1.0	0.93	6.0	4.3
Citric acid N/100.....	2.6	6.6	8.0	6.6
Aspartic acid N/200...	1.6	....	5.5	6.5
Asparagin N/200......	3.5	3.8	5.5	5.3
Tannic acid 1 per cent.	1.0	31.0	0.2	42.0
Tannic acid 0.5 per cent +5 cc. 10 per cent glucose.............	3.5	32.0	5.0	47.0
Control 0.5 gm. levulose..............	....	82.0	....	116.0

TABLE VIII—*Continued*

	FUSARIUM TRICHOTHECIOIDES	FUSARIUM OXYSPORUM
	12 days	12 days
Palmitic acid I	No growth	Good growth
“ “ II	Slight “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “
+5 cc. 10 per cent dextrose, V	Excellent “	“ “
Stearic acid, I	Slight “	“ “
“ “ II	No “	“ “
“ “ II	No “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “
+ 5 cc. 10 per cent dextrose, V	Excellent “	“ “
Oleic acid, I	No “	“ “
“ “ II	Poor “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “
+5 cc. 10 per cent dextrose, V	Slight “	“ “
Palm oil, I	No “	No “
“ “ II	“ “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “
+5 cc. 10 per cent dextrose, V	“ “	“ “
Olive oil, I	Fair “	Fair “
“ “ II	“ “	“ “
“ “ III	“ “	“ “
“ “ IV	“ “	“ “

A marked difference was found in the ability of the two organisms to use the fatty acids, *F. trichothecioides* being much more restricted in its ability. The experiments with alcohol and the acids also showed that the former organism was much more readily poisoned and inhibited in its growth. It was found that *F. oxysporum* grew well in 1 per cent ethyl alcohol, and that *F. trichothecioides* made no growth. The solution was then diluted one-half, whereupon *F. trichothecioides* made a good growth. This was clearly a case of inhibition. The growth of *F. trichothecioides* was inhibited by N/100 acetic acid, as can be seen by the fact that it grew in N/125 concentration and that it grew in N/100 when glucose was added, while *F. oxysporum* grew well in N/100 acetic acid. N/100 formic acid was toxic to *F. trichothecioides*, while it merely inhibited growth with the other organism. The latter grew in N/125 formic acid, while *F. trichothecioides* did not grow in N/500 solution. N/100 proprionic acid was toxic to both, while both grew in N/250 solution. N/250 butyric acid was toxic to *F. trichothecioides*, while *F. oxysporum* grew in it.

TABLE IX

DRY WEIGHT (IN MILLIGRAMS) FORMED IN 12 DAYS IN VARIOUS CONCENTRATIONS OF ACID

CONCENTRATION	FUSARIUM OXYSPORUM				FUSARIUM TRICHOTHECIOIDES			
	N/1000	N/500	N/250	N/125	N/1000	N/500	N/250	N/125
Formic acid								
I............	Non-weighable	Non-weighable	Non-weighable	Non-weighable	Non-weighable	None	None	None
II...........	Non-weighable	Non-weighable	Non-weighable	Non-weighable	Non-weighable	"	"	"
Average....	....	....	....	....	....	....	....	....
Acetic acid								
I............	....	....	....	....	0.8	1.2	3.2	5.8
II...........	....	....	....	....	0.8	2	3.4	6.6
Average....	....	....	....	....	0.8	1.6	3.3	6.2
Proprionic acid								
I............	1.4	2.0	5.0	None	1.4	2.6	4.0	None
II...........	2.6	2.4	5.8	"	1.2	3.4	6.6	"
Average....	2.0	2.2	5.4	....	1.3	3.0	5.3	....
Butyric acid								
I............	4.8	7.6	10.4	None	2.4	5.0	None	None
II...........	5.4	9.0	13.8	"	3.6	5.6	"	"
Average....	5.1	8.3	12.1	....	3.0	5.3	....	....
Oxalic acid								
I............	0.4	0.4	0.4	1.2	0.8	Non-weighable	Non-weighable	Non-weighable
II...........	0.8	1.2	1.2	1.2	0.8	Non-weighable	Non-weighable	0.2
Average....	0.6	0.8	0.8	1.2	0.8	....	....	0.1

A set of experiments was run also in which solanin in various percentages was added to glucose media. Because of the high cost of solanin, only 1 cc. solution was used in each test. The results are given in table X.

These differences, that is, greater versatility in the use of carbon sources, greater resistance to inhibition, and intoxication, may well play an important rôle in determining the difference in behavior of these two organisms.

TABLE X

DRY WEIGHT (IN MILLIGRAMS) FORMED BY *Fusarium oxysporum* AND *F. trichothecioides* IN 6 DAYS

	Percentage solanin				
	1	2	0.25	0.226	0
Fusarium oxysporum....	10.6	8.1	13.2	10	13.8
Fusarium trichothecioides	2.5	2.4	7.8	....	9.3

DISCUSSION.—The versatility of these organisms in using various carbon sources in their metabolism is of great interest. This almost omnivorous ability to use carbon compounds, including the simplest fatty acid, the highly oxidized fatty acids, the long carbon chain acids, the alcohols, mono- and poly-hybic, glycerin and fats, the mono-, di-, and poly-saccharides, including the dextrines, starches, hemicelluloses, and true celluloses, assigns to them an important rôle in the carbon cycle, and at the same time must help render them the formidable and destructive enemies of the root crops that they are.

The methods suggested by APPEL (2), namely, rigid inspection of potato fields, immediate destruction of all plants that show the slightest symptoms, quarantining of non-certified seed stock, alone give promise of keeping these troubles in check. Disinfection of storage cellars and of potatoes when put into storage, together with storage at proper temperature, will help combat these diseases, especially the dry rot induced by *F. trichothecioides*.

Conclusions

1. *Fusarium tuberivorum* Wilcox and Link is the same as *Fusarium trichothecioides* Woll.

2. Both *Fusarium oxysporum* and *F. trichothecioides* can produce both tuber rot and wilt of the potato plant.

3. The wilt is induced by destruction of the root system and by clogging of the xylem elements in the stem, and is, in mild cases, marked by such symptoms as discoloration of leaves, curling and rolling of leaves, and production of aerial tubers.

4. Under field and storage conditions *Fusarium oxysporum* is more probably responsible for wilt than is *F. trichothecioides*, and the latter more responsible for tuber rotting.

5. The optimum and maximum temperatures of *Fusarium oxysporum* are higher than those of *F. trichothecioides*. *F. trichothecioides*, however, grows well at 8–10° C., while *F. oxysporum* does not. These facts may explain in part the fact that *F. oxysporum* produces more wilt than *F. trichothecioides*, and that the latter causes more tuber rot.

6. *Fusarium oxysporum* has a more rapid, superficial, and spreading habit of growth than has *F. trichothecioides*. This may be associated with a greater oxygen requirement for *F. oxysporum*, and may account for the frequenting of xylem elements by this fungus.

7. Both organisms possess a striking ability to use the most diverse carbon materials as carbon sources in their metabolism. *Fusarium oxysporum* has a greater range in its ability, and can utilize the materials more readily, although not so completely as does *F. trichothecioides*.

8. *Fusarium oxysporum* is less subject to inhibition in growth and intoxication than is *F. trichothecioides*.

9. Solanin is not toxic to either organism, although it seems to inhibit somewhat the growth of *Fusarium trichothecioides*.

The writer acknowledges his indebtedness to Dr. E. MEAD WILCOX and to Dr. WILLIAM CROCKER. They not only made this research possible, but they gave freely of advice and criticism, and lent encouragement by their interest in the progress of the investigation.

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AERIFEROUS TISSUE IN WILLOW GALLS

A. COSENS AND T. A. SINCLAIR

(WITH PLATES II–IV AND FIVE FIGURES)

COSENS (2) has demonstrated recently that in certain willow galls there is a production of aeriferous tissue in regions where it is not present normally. This investigation has been undertaken for the purpose of explaining the unexpected appearance of this tissue. Many different species of *Salix* and *Populus* were examined in order to determine the normal distribution of the tissue in their various organs. As an important adjunct of the work experiments have been conducted for the purpose of observing the influence of certain factors on its production, such, for example, as temperature, water, nutrient solutions, and light.

Stated in general, intercellular spaces are found in the parenchymatous tissues of nearly all plants. These usually remain small and inconspicuous, although connected with one another in such a way as to form a continuous gas-containing system of cavities. In many plants, however, especially hydrophytes, these spaces are so large and conspicuous and formed on such a regular plan as to impart the well known characteristic appearance to the areas in which they occur. Such a typical tissue is designated here aeriferous tissue.

Concerning the occurrence of this tissue in certain histological regions of *Salix* when under abnormal stimulation, two explanations present themselves: (1) that the tissue is an "environmental" modification, produced by the action of the plant protoplasm in direct response to certain conditions of the environment; (2) that the tissue is a distinct type, probably of wider distribution in the ancestral forms of the group, but now absent in certain regions of the modern species, since the power to produce it is dormant, but reinstated when these tissues are subjected to suitable stimulation. One example will suffice to illustrate the line along which attention has been directed. In many aquatic plants there are two types of leaves; for example, in *Proserpinaca palustris* L. the leaves on the submerged portions are divided, while those on the aerial portions are entire. Explanations, on experimental grounds, have been offered by various workers, notably McCallum, Burns, and Goebel, to account for this phenomenon of heterophylly.

McCallum (4) conducted a series of experiments with *Proserpinaca palustris* L., in which both submerged and aerial plants were grown under various degrees of illumination, and found that the type of leaf developed was entirely independent of the light relations. Further experiments were performed in which plants were kept in a perfectly saturated atmosphere containing normal amounts of carbon dioxide and oxygen, and it was observed that the water form of leaf persisted. It was then assumed that the essential factor common to moist air and water was inhibition of transpiration, and confirmatory experiments from this standpoint were carried out, in which transpiration was induced in submerged plants by high osmotic pressure. The conclusion arrived at was that the aerial type of leaf is developed, even under water, if sufficient moisture is withdrawn from the protoplasm.

From the preceding experiments the conclusion deduced was that the only factor, constant in all cases where the water form of leaf developed; was the checking of transpiration and constant increase in the amount of water in the protoplasm, while those chemical and physical conditions, resulting from partial withdrawal of water, are associated with the aerial type. The main point is that the modification in the form of the leaf was construed as environmental, that the form of the leaf is especially plastic under the influence of the transpiration factor.

Burns (1) studied the same plant and arrived at conclusions entirely different from those formed by McCallum. He experimented with cuttings and seedlings, and found that in the case of the former the disturbance of the vegetative activity caused a change that might be accompanied by a reversion, but concluded that environmental factors did not determine, in McCallum's sense, the type of leaf produced. A verification of this was obtained from the production of seedlings under varied conditions of light, temperature, moisture, etc. He also found in the case of plants grown from seed that the first leaves to appear were always divided, and that the entire leaves were of later growth. From further experiments he obtained the following data. All stems, regardless of external conditions, produced the water type in the autumn. Stems from which the vegetative points were removed in June threw out side branches with the water type of leaf, while normal plants under the same external conditions were producing the land type. In summer all plants, whether in water or air, developed the aerial form of leaf. Also, at the time of flowering only entire leaves were found. The change from the water to the land type took place earlier on strongly growing than on weak stems.

From his experimental results BURNS concluded that there are two forms of leaves, an adult and a juvenile, and that the production of these is not controlled by any one or more definite factors of the environment, such as light, moisture, temperature, etc. But under favorable vegetative conditions the adult form, the entire leaf, is produced, while under unfavorable the juvenile divided leaf is developed. Further, a reversion to the primitive or divided type may be associated with unsuitable vegetative conditions.

GOEBEL (3) experimented for several years with plants that exhibited the phenomenon of heterophylly, and his views fully confirm those of BURNS. He summarizes his conclusions concerning *Proserpinaca* as follows:

> Es ist klar, dass die Wasserumgebung nicht die Ursache der Blatteilung ist. Ebensowenig hängt sie ab vom Licht, der Temperatur, dem Gasgehalt des Wassers und dem Kontaktreiz als Solchem. Die einzige Folgerung, die sich aus meinen Versuchen ableiten lässt, scheint die zu sein, dass Proserpinaca palustris zwei Formen hat, eine "Folgeform" und eine "Jugendform." Unter guten vegetativen Bedingungen hat sie das Bestreben, die Folgeform mit den ganzen Blättern, Blüte und Frucht hervorzubringen, unter ungünstigen Vegetationsbedingungen hat die Pflanze das Bestreben, die Jugendform mit dem geteilten Blatt zu bilden.

In view of the satisfactory results obtained from investigations on such morphological features, it seems entirely reasonable to hope that the anatomical aberrations occurring in galls may be capable of explanation by pursuing similar lines of inquiry and by making use of the anatomical principles unfolded by SCOTT, JEFFREY, and others in recent years. Several of these aberrations have been studied by the senior author; in this paper that of aeriferous tissue only is dealt with in detail.

Distribution of aeriferous tissue in galls

Aeriferous tissue is abnormally present in galls produced by certain species of at least two distinct orders of insects. Thus it has been located in the dipterous galls *Rhabdophaga triticoides* Walsh and *R. strobiloides* Walsh, stem galls on *Salix cordata* Muhl., and *Phytophaga rigidae* O.S. on the stem of *Salix discolor* Muhl. It also occurs in the hymenopterous gall *Pontania pomum* Walsh, a leaf gall on *Salix cordata* Muhl.

FIGS. 1, 2.—Fig. 1, Gall of *Rhabdophaga triticoides* Walsh on *Salix cordata* Muhl.; fig. 2, Galls produced by *Rhabdophaga triticoides* Walsh.

The stem gall *Rhabdophaga triticoides* Walsh is usually terminal or nearly so (text fig. 1), but sometimes occurs at varying distances from the tip of the stem; two or three galls occasionally are spaced along a branch and separated by sections of normal stem, as shown in text fig. 2. The gall, especially when of the terminal type, bears a remote resemblance to a head of wheat. It is produced by a very marked shortening of the stem of the host, accompanied by a decided increase in its diameter. The contraction of the infected stem is so pronounced that the lateral buds, which would normally occupy 25–30 cm. of the stem, are crowded into a space of 4–5 cm. As the characteristic alignment of the lateral buds is approximately maintained on the abnormal part of the stem, there are 5 fairly well defined rows of buds on the gall. In each of these buds a larval chamber is located.

3 4

FIGS. 3, 4.—Fig. 3, Gall produced by *Rhabdophaga strobiloides* Walsh on *Salix cordata* Muhl.; fig. 4, Gall of *Phytophaga rigidae* O.S. on *Salix discolor* Muhl.

Practically the entire pith and the cortex of this gall are composed of aeriferous tissue. It is absent only immediately around the zonal areas of the larval chambers, and in these regions it can be seen to have been obliterated by compression. The tissue is of the most typically aeriferous type, consisting of polygonal spaces bounded by strands seldom more than one cell in width. The distribution and character of this tissue can be seen in figs. 1 and 2.

The gall *Rhabdophaga strobiloides* Walsh (text fig. 3) is invariably terminal, since the solitary larva occupies the tip of the growing point, and thus prevents any elongation of the stem past the infected area. The condensation of the stem axis that results is, in this case also, the chief gall-producing factor, the top-shaped mass of aborted leaves representing the normal leafage of nearly a meter of stem. The entire cortex of the receptacle of this gall is composed of aeriferous tissue. It commences to appear in the pith, immediately beneath the compact tissues of the gall zones, and extends to a considerable distance from the point of infection (fig. 4.)


FIG. 5.—Galls on *Salix cordata* Muhl. produced by *Pontania pomum* Walsh.

In contrast with the two preceding species, a marked condensation of the stem of the host does not enter into the production of the gall *Phytophaga rigidae* O.S. (text fig. 4). This is apparent from the normal spacing of the buds that persist on the exterior of the gall. The pith of the stem in this form is occupied by the larva of the producer, and only small areas of parenchyma remain outside of the protective sheath of the gall, but in these aeriferous tissue can be detected. The cortex, which is very thick, consists entirely of the same tissue (fig. 6). Aeriferous tissue is also developed far in excess of the normal in the cauline stelar gaps comprised within the gall. A section of such a stem gap is shown in fig. 3.

The normal occurrence of aeriferous tissue in the Salicales will be discussed in detail later, but it may be stated here that, while it occurs in the stem pith of the three galls just described, it is not present in the corresponding regions of normal *Salix*.

The well known spherical gall produced by the sawfly *Pontania pomum* Walsh on the leaves of *Salix cordata* Muhl. (text fig. 5) furnishes another example of aeriferous tissue in association with a gall-producing stimulus. In its earliest stages the gall consists of a singularly compact tissue (COSENS 2), but gradually the aeriferous characters become evident, until almost the entire mass of the adult gall consists of tissue which is thus differentiated. Fig. 5 is a typical section from such a mature gall.

Distribution of aeriferous tissue in normal plants

For the purpose of determining the distribution of this tissue in normal plants, the following species of Salicaceae were examined: *Salix fragilis* L., *S. cordata* Muhl., *S. discolor* Muhl., *S. humilis* Marsh., *S. longifolia* Muhl., *S. lucida* Muhl., *S. nigra* Marsh., *S. petiolaris* Sm., *S. purpurea* L., *S. rostrata* Richards, *S. serissima* Fernald, *Populus balsamifera* L., *P. deltoides* Marsh., *P. grandidentata* Michx., *P. nigra* L., *P. tremuloides* Michx.

CORTEX.—At first sight the cortex, both primary and secondary, of all these species appears to consist of a compact tissue, but on further examination a well defined aeriferous tissue was found in the primary cortex of every one. It can be seen in sections taken from the apex of the stem and for some distance back (fig. 10), but in older regions it is destroyed by compression. The amount formed varies somewhat in different species. Thus in *Salix nigra*, *S. cordata*, and *S. fragilis* the entire tissue forming the primary cortex is aeriferous; the meshes are relatively large, and the walls inclosing them are as a rule but a single cell in thickness. In *S. rostrata* and *S. petiolaris* it is especially well differentiated at the angles of the stem; at some levels it is confined to these regions.

Among the poplars the largest amount of this tissue is possibly to be found in *P. nigra* and *P. tremuloides*. In the former it is very pronounced, for the cortex is an entire lattice work of it, and the air spaces are large and bounded by strands that vary from one to three cells in width. The meshes in *P. tremuloides* are likewise regular and clearly defined, but they attain their greatest dimensions at the angles of the stem. *P. balsamifera* is peculiar in that they are most abundant and characteristic immediately around the leaf traces, especially in close approximation to the bundles. Indeed, at the nodal levels the entire cortex consists of aeriferous tissue. It is less apparent in *P. grandidentata* and *P. deltoides*, for, though present, it is not as abundant nor as regularly defined as in the other species. Incidentally it may be stated that wherever this tissue occurs in the cortex of the normal plant it is much more abundant and better developed in corresponding parts of stem galls. This also applies to *Salix*.

PITH.—The pith is distinctive and important. It has been noted that the pith in the galls cited is occupied by a very pronounced aeriferous tissue, but this is not the case with the normal pith of any species of willow except in the reproductive axis. In every species examined it is a compact tissue without any indication of other than small, ordinary, inconspicuous intercellular air spaces. The pith illustrated in figs. 8 or 10, for example (a normal pith), stands out in striking contrast to the pith of the gall of fig. 2. It is especially noteworthy that in the latter case the medullary and cortical tissues are identical, both in their conformation and in the size and shape of their cellular constituents, while in the normal, the cells of the pith are much larger and closely packed. The air spaces of the aeriferous tissue do not result as an indefinite elongation of the cells, as in certain oak-leaf galls, but are the result of cell divisions which follow a definite law with regard to their polarity. The compact normal pith and the open abnormal pith appear to be fundamentally different types on this basis and on the basis of the peculiarities in the distribution of the latter to be noted.

Fig. 7 is a photograph of a normal stem of *Populus balsamifera* taken from a new shoot, and it illustrates the difference manifested by *Populus* in comparison with *Salix*. In all poplars investigated the plan of organization of the pith is that of an aeriferous tissue, though lacking the regularity found in the cortex. Were it established, as some believe, that *Populus* is the more primitive genus, this phenomenon would be important in explaining the aeriferous tissue in the pith of the galls of *Salix*.

NODE.—We have been taught to regard the node as a conservative part of the stem, one in which discarded tissues may still persist, so that much interest attaches to an examination of this region. It is at the node more than elsewhere in the stem that the aeriferous tissue is most abundant and characteristic in both willows and poplars. Thus in *P. balsamifera* it is particularly abundant immediately around the leaf traces, especially in close approximation to the bundle. The branch gaps likewise are occupied by this tissue, in the case of *Salix* extending well into the pith (fig. 8). In stem galls, for example, *Phytophaga rigidae* O.S. on *Salix rostrata*, this feature is always very pronounced; but most significant of all, the pith at the bases of the branches is typical aeriferous tissue. Fig. 9 illustrates this feature clearly, and is interesting in comparison with fig. 10; both are from *Salix lucida*, the former from the base of a branch, the latter from an internode.

LEAF.—The petiole is sometimes a vestige carrier, so that it is of interest to note that aeriferous tissue has been located here in several species of *Salix* and *Populus*, for example, *Populus balsamifera*, *Salix humilis*, *S. fragilis*, and *S. cordata*. As a rule it is confined to the part of the petiole adjacent to the stem. Elsewhere the ground tissue is of the compact type. In the blade there is no indication of it, as the palisade layer and spongy parenchyma are of the ordinary type. Yet in the leaf gall *Pontania pomum* on *Salix cordata*, practically all of the ground tissue is aeriferous.

SEEDLINGS.—The seedlings of *Salix serissima* Fernald only were examined. These were obtained by sprouting the seeds in a moist chamber between layers of filter paper in an incubator, and then pricking the young seedlings into small fern pans. Different stages were examined, but in none was aeriferous tissue found in the pith. It is present in the cortex.

ROOT.—The roots of the Salicaceae are devoid of a pith. The primary cortex, however, was examined in the roots of seedling plants of *Salix serissima*, and in young roots of *Populus balsamifera* and *P. nigra*, and was found to consist in part of a well differentiated aeriferous tissue.

INFLORESCENCE.—No part of the plant is considered more important as a retainer of vestigial tissue than the reproductive axis. It is of especial interest to find, therefore, that aeriferous tissue is developed and often beautifully typical and abundant in the pith as well as in the cortex in the reproductive axes of all of the species of *Salix* and *Populus* that were investigated. This is especially noteworthy in the case of *Salix*, because here and at the bases of the branches alone is pith of this type to be found. Figs. 11, 12, 13, and 14 are photographs of the pith of sections cut from the catkins of *Salix purpurea*, *S. humilis*, and *S. cordata*. Figs. 11 and 12 are of *S. purpurea* at different stages of development, the former from a young catkin, the latter from a mature one. This tissue is sometimes more abundant and typical in the pith of the staminate catkin, but a striking similarity exists in the form and distribution of the tissue in the two catkins of the same species. An examination of both was made in the case of *Salix alba*, *S. lucida*, and *S. longifolia*. In *S. rostrata*, *S. cordata*, and *S. nigra*, the aeriferous tissue of the pith is more clearly defined than in the cortex, but is present in both. The reverse is true in *S. lucida*, while in *S. discolor* there is no greater development in the one region than the other. The pith in the catkin of *S. petiolaris* is peculiar because it consists of a large-meshed aeriferous tissue, and often there are but two or three large spaces bounded by slender lines of cells.

Influence of environment on occurrence of aeriferous tissue

For the purpose of ascertaining to what extent the production of aeriferous tissue in the Salicales is dependent on external factors, several experiments were conducted in the growing of plants under conditions in which certain components of the environment could be controlled. Cuttings from various species were grown in culture solutions, in water, some totally immersed, and in soil. The effect produced by the variation of the illumination and temperature was also noted in a number of species. The following list comprises the more important experiments.

LIGHT RELATIONS.—Cuttings from *Salix nigra* Marsh., *Populus nigra* L., *Populus balsamifera* L., and others were sprouted in pots of earth. In the conservatory some of these were placed in a situation to receive the maximum light, others in a more sheltered position, and finally several in a light-proof chamber. The only effect noted was in the case of the sprouts grown in the dark. In those experiments the stems produced were flattened, and the tissues, which were greatly compressed, contained many parenchyma cells of particularly large size, but no aeriferous tissue.

TEMPERATURE RELATIONS.—Cuttings were sprouted as in the preceding series, but the experiments were conducted at several different temperatures. With the exception of an increase in the rate of growth, tissue effects were not produced.

INHIBITION OF TRANSPIRATION.—Cuttings from various species of *Salix* and *Populus* were weighted down in tall cylinders containing water and allowed to sprout when completely submerged. Some of these cuttings were placed in bright sunlight and others in more shaded situations. Sections were taken from the newly produced shoots at different levels, as the growing point, the base of the sprout, near its origin from the main stem, and also from shoots that had just emerged from the water. In this series the most marked effect found was the flattening of the stems and the compression of the tissues proportional to the depth at which they were grown. In no case was there any increase in the amount of aeriferous tissue produced; indeed, in the sprouts produced on cuttings from *Salix nigra* Marsh., the tissue was entirely lacking.

CULTURE SOLUTIONS.—Cuttings were sprouted in various nutrient solutions, including all of the formulae recommended by MACDOUGAL (5), and the results were checked by shoots produced in ordinary water. In the sprouts produced on *Salix* cuttings in nutrient solutions aeriferous tissue was not developed in the pith. While it was invariably present in the pith of the *Populus* shoots, it was not increased in amount beyond the normal production.

The results of all the experiments seem to justify the conclusion that the stimulus to the development of aeriferous tissue in the Salicales is not involved in light, in temperature relations, in the nutrient conditions imposed by the conditions of our experiments, or in checking of transpiration.

Discussion

As already stated, two theories present themselves in explanation of the unexpected appearance of aeriferous tissue in certain regions of insect galls where it is normally absent. The tissue may be regarded as a direct effect of the action of certain environmental factors on the protoplasm of the host; or it may be considered a hereditary tissue that has been reinstated owing to the awakening of latent characteristics in the protoplasm of the infected plant, on account of unusual conditions associated with the presence of the gall producer.

Concerning the alteration by experiment of the environment of the host plant, all attempts were unsuccessful either to increase the amount of aeriferous tissue normally present or to cause its development in regions where it is not found under normal conditions. Although the inhibition of transpiration is commonly regarded as an important factor in the production of this tissue, it was not increased to the smallest extent in the *Salix* sprouts developed under conditions of reduced transpiration. Indeed, if these experiments had given positive results, it would still be necessary to show that the conditions presented by galls are those of restricted transpiration. This is clearly not the case in *Rhabdophaga triticoides* Walsh, the gall in which the development of the tissue is the most pronounced and its characteristics the most typical. As stated previously, the lateral buds that contain the larval producers of this gall are situated on a length of stem, the internodes of which are shortened to a very marked degree. The occupation of the buds by the larvae, however, has not checked the development of the leaves in the axils of which the buds are produced. The galls shown in text figs. 1 and 2 were collected after the leaves had fallen. This results in the leaf area of the abnormal part of the stem being increased far beyond the normal leafage, a condition that we can scarcely associate with reduced transpiration. Thus the direct evidence from experiments leads us to believe that environmental factors of light, temperature, moisture, etc., are not directly operative in the production of aeriferous tissue.

The anatomical structure of the normal stems of the Salicales strongly favors the same view by furnishing facts apparently explainable only on the supposition that we are here dealing with a true heritable tissue. In accordance with the general view concerning the retention of ancestral characteristics, certain regions of plants are regarded as more tenacious of these structures than others. For example, it is commonly recognized that they may persist in the reproductive axes, petioles, leaf or branch traces, and roots. The tissue under consideration must be regarded as primitive in accordance with this general principle. It occurs in the pith and cortex of the reproductive axes of all species of *Populus* and *Salix*. It is found in the pith at the bases of the branches of the stem of *Populus* and *Salix*, and only in that region in the case of the latter genus. Further evidence in support of the same view is furnished by its presence in the branch gaps, its occurrence in petioles and the vicinity of stem and leaf traces, and in the cortex of the root. This prominent development of the tissue in the vestigial carriers of both *Populus* and *Salix*, taken in conjunction with our experimental results, presents strong evidence in favor of the theory that the aeriferous tissue in the Salicales is of ancestral type.

The power of the gall stimulus to arouse latent properties in the protoplasm of the host, expressed by its ability to produce an aeriferous tissue not normally present in *Salix*, is also exemplified in the case of glandular tissue and trichomes in certain galls on other plants. It may be stated as a general principle that glands, when they are present normally in a tissue of the host, are always more plentiful or larger in the gall originating from that tissue. A striking example (COSENS 2) of this is furnished by the pithy spherical stem gall produced on *Solidago canadensis* L. by *Eurosta solidaginis* Fitch. But in some cases glands are produced in galls on parts of the host normally glandless. Thus, they are plentiful in the stem gall *Neolasioptera perfoliata* Felt on *Eupatorium perfoliatum* L., but are not present in the same location in the normal plant. In fact, they were found in normal plants in the transitional region between stem and root only, both in this species and in *E. urticaefolium* Reichard.

Trichomes exemplify the same principle. When the gall produces unusual trichomes, the abnormal forms can almost invariably be found on the reproductive axes of the host. Thus, while the normal hairs on the leaf of *Quercus macrocarpa* Michx. are of the stellate type, those of the reproductive axes are acicular, corresponding exactly to those composing the pubescence of the leaf gall *Eriophyes querci* Garman on this host. Also, the convoluted type of hair present on the *Acarina* dimple gall on the leaves of *Acer Negundo* L. are exactly reproduced by the form occurring on the reproductive axes, although the normal leaf hair is straight.

An almost dormant characteristic has also been aroused in *Rosa blanda* Ait. by the cynipid producer *Rhodites multispinosus* Gillette (COSENS 2). The gall produced is always exceedingly spiny, although the stem of the host from which it originates is usually unarmed. But it is worthy of note that the production of spines is a marked character of other species of the genus. Doubtless further investigations will show that examples of this kind can be almost indefinitely multiplied.

The reinstatement in a gall of vestigial characteristics of the plant has an important bearing on the question of gall formation. The producer has long been recognized as exercising a directive control over the activities of the protoplasm of the host, but these examples of the rehabilitation of dormant characters show that the forces operative in gall formation are of wider scope. Under these conditions unexpected structures and unusual combinations may well be produced, and in the interpretation of the morphology of any gall it becomes necessary to discriminate carefully between these two classes of organs and tissues, that is, between those that are simply environmental modifications of the normal and those that are vestigial or in use in other parts only of the plant. Incidentally it may be added that there remains no authentic instance of any organ or tissue in a gall that is new, ontogenetically or phylogenetically, to the host.

This investigation was carried on at the Botanical Laboratories of the University of Toronto, and the authors wish to acknowledge their indebtedness to Professor J. H. FAULL for invaluable criticism throughout the whole course of the work.

UNIVERSITY OF TORONTO

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EXPLANATION OF PLATES II–IV

PLATE II

FIG. 1.—*Rhabdophaga triticoides* Walsh on stem of *Salix cordata* Muhl.: transverse section showing general arrangement of larval chambers and distribution of aeriferous tissue throughout the cortex and pith of the gall; ×10.

FIG. 2.—*Rhabdophaga triticoides* Walsh on stem of *Salix cordata* Muhl.: transverse section in which is shown the typical character of the aeriferous tissue produced under the stimulus of the gall producer; ×50.

FIG. 3.—*Phytophaga rigidae* O.S. on *Salix discolor* Muhl.: a stem gap which shows aeriferous tissue produced in excess of normal amount; ×90.

FIG. 4.—*Rhabdophaga strobiloides* Walsh on stem of *Salix cordata* Muhl.: a longitudinal section showing aeriferous tissue produced in pith of gall axis; ×25.

PLATE III

FIG. 5.—*Pontania pomum* Walsh on *Salix cordata* Muhl.: section of a nearly full-grown gall in which aeriferous tissue is produced throughout its mass; ×18.

FIG. 6.—*Phytophaga rigidae* O.S. on *Salix discolor* Muhl.: gall chamber includes all the pith area of the stem; aeriferous tissue of the characteristic type occupies whole of cortical region of the gall; ×10.

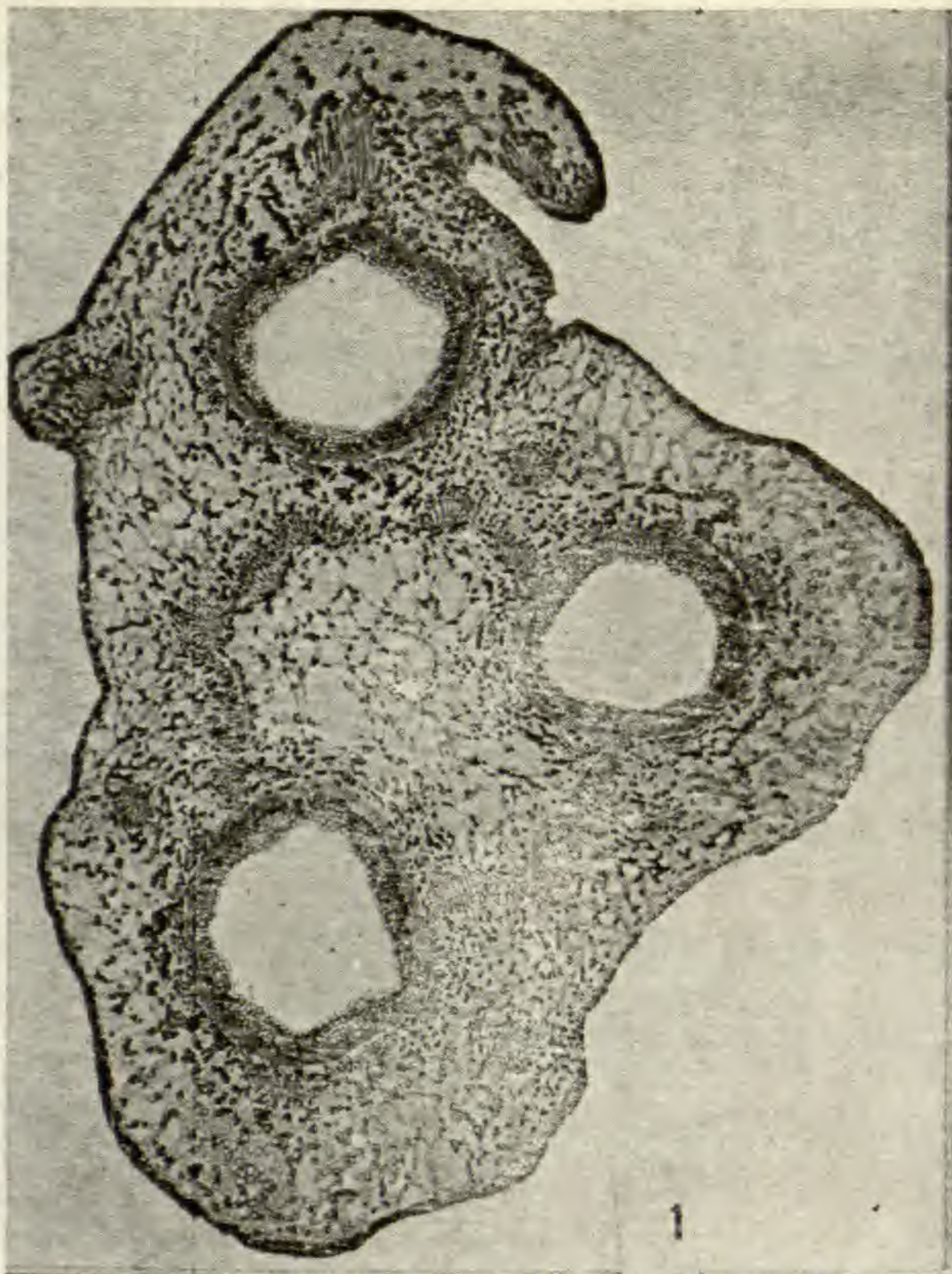
FIG. 7.—*Populus balsamifera* L.: transverse section of stem pith in which aeriferous tissue is normally present; ×80.

FIG. 8.—*Salix humilis* Marsh.: section of normal stem at level of a stem gap; it illustrates the compact nature of the *Salix* pith and the differentiation of aeriferous tissue in the stem gaps; ×65.

PLATE IV

FIG. 9.—*Salix lucida* Muhl.: section through base of a normal stem, showing aeriferous tissue produced in the pith; ×60.

FIG. 10.—*Salix lucida* Muhl.: transverse section of normal stem a short distance behind the growing point; aeriferous tissue constitutes the entire cortex, but is completely absent from the pith; this compact nature of the pith is typical for all species of *Salix* except in region of bases of branches as illustrated in preceding figure; ×12.



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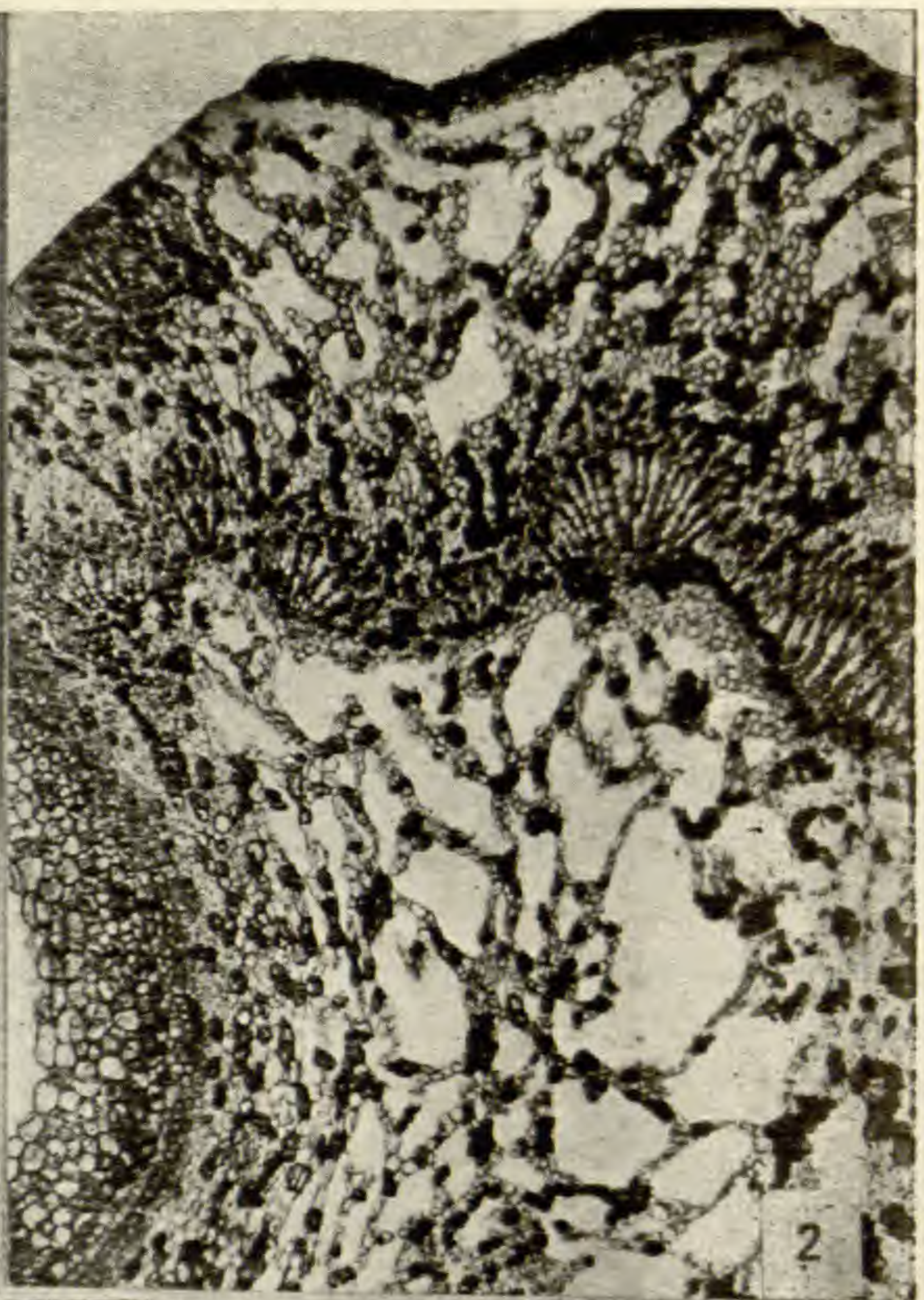

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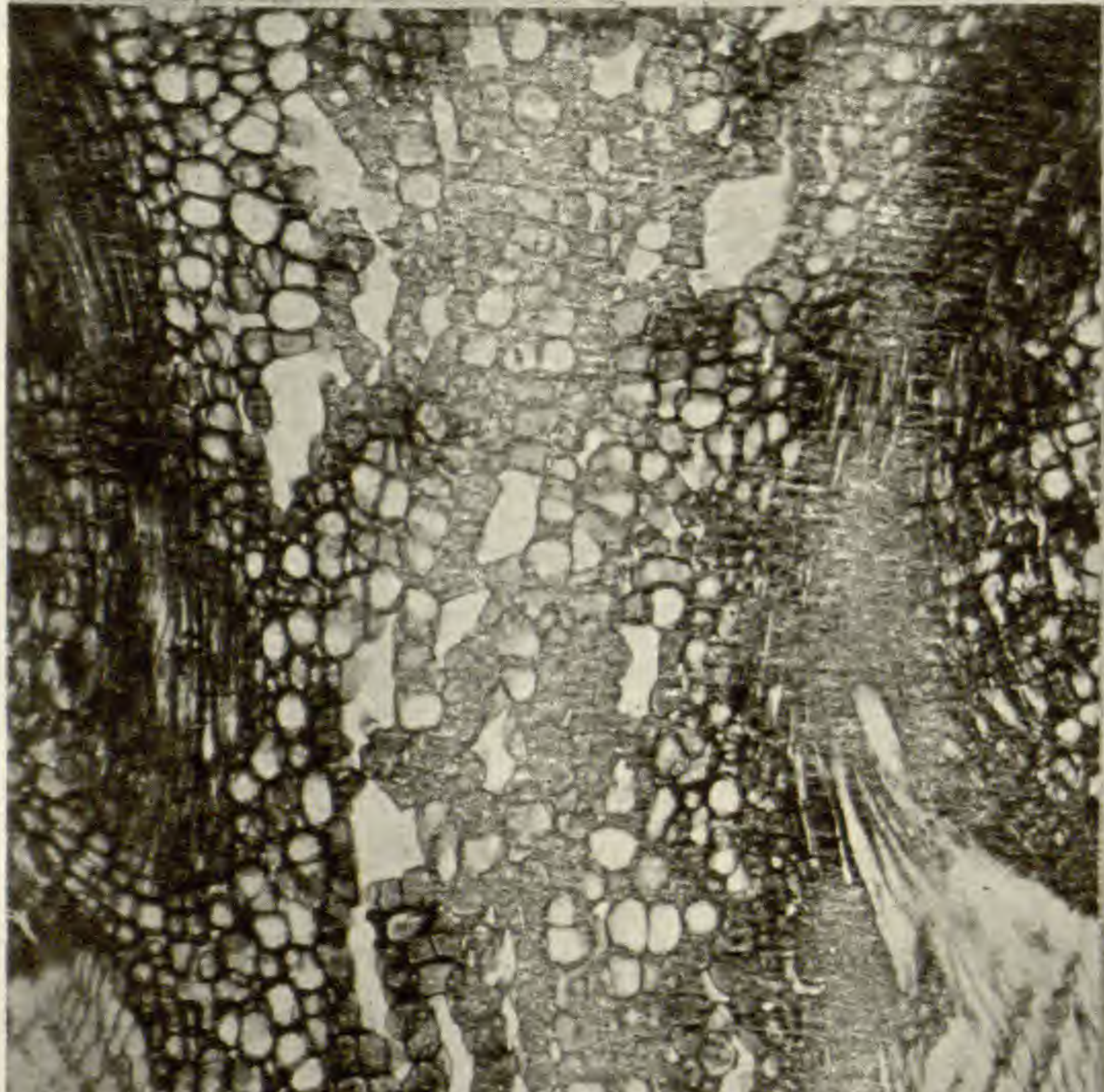

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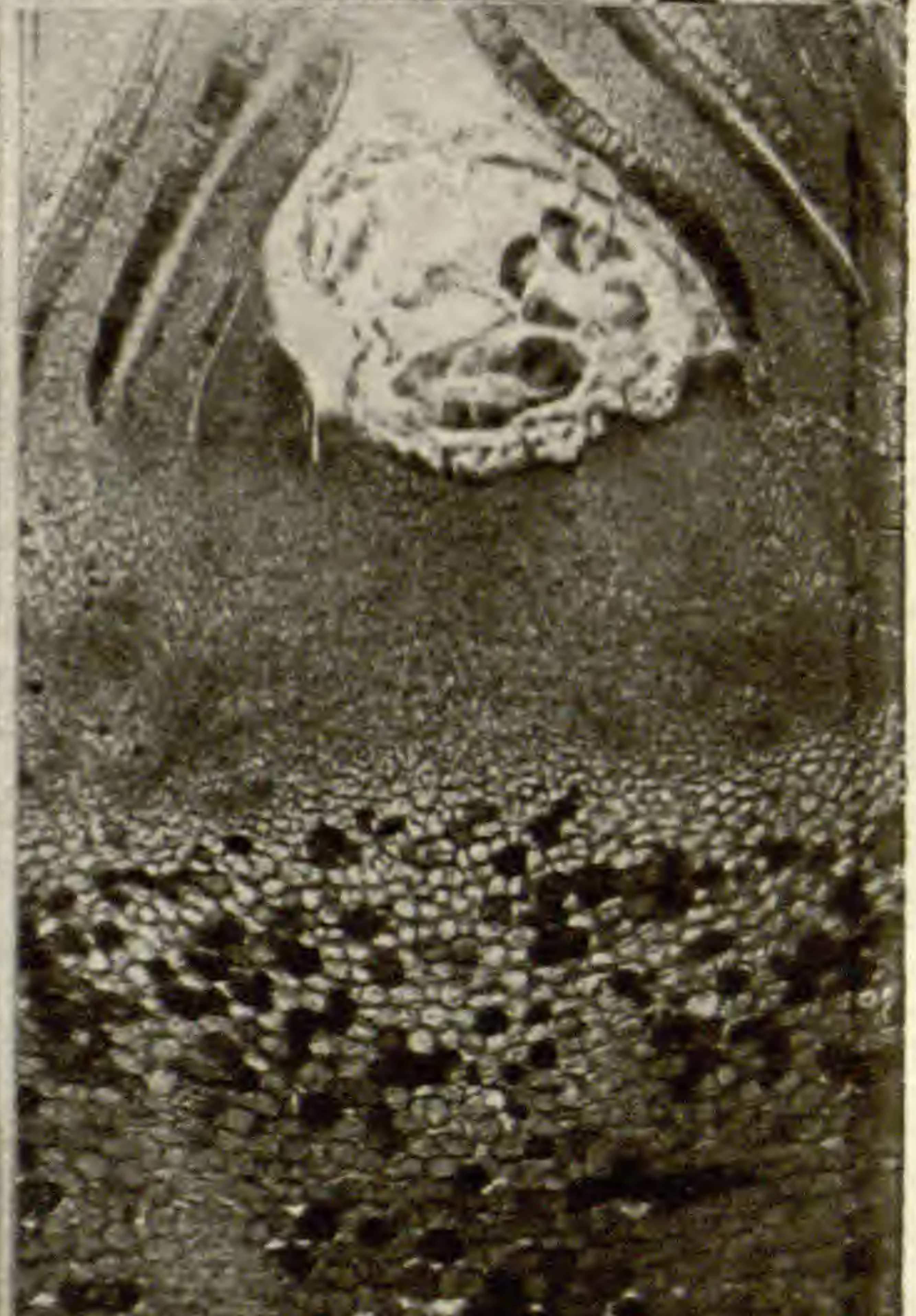

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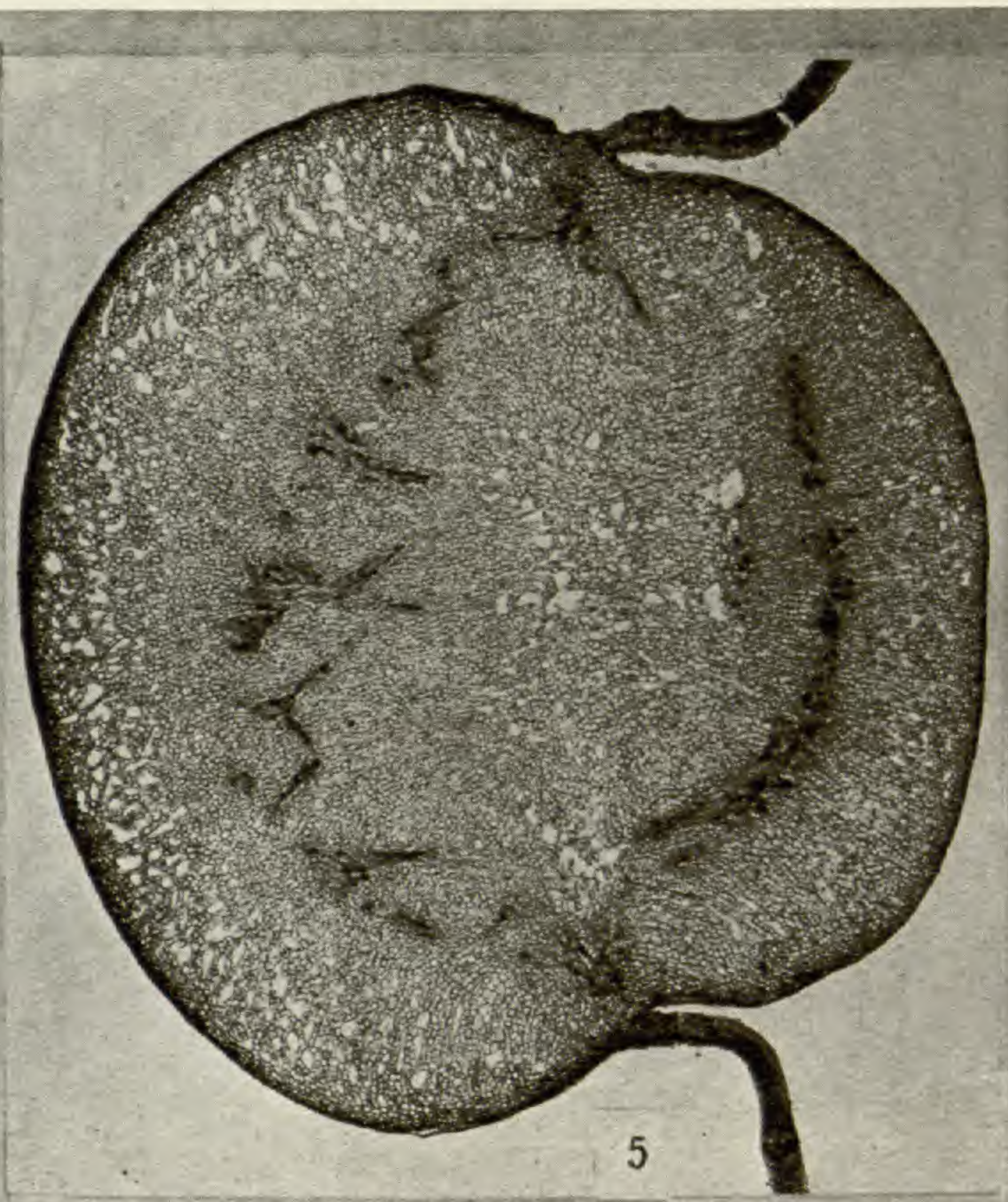
COSENS and SINCLAIR on AERIFEROUS TISSUE



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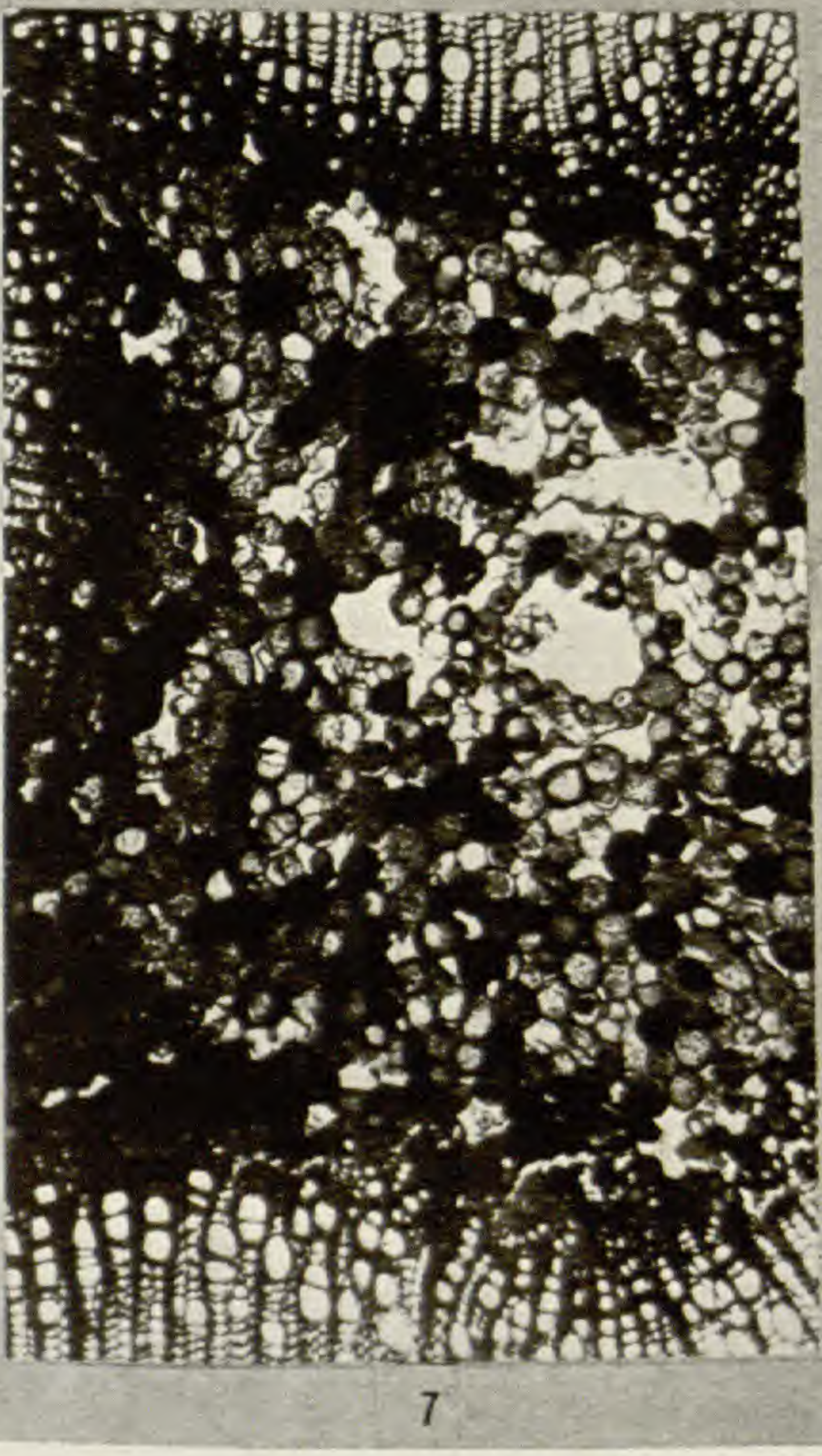

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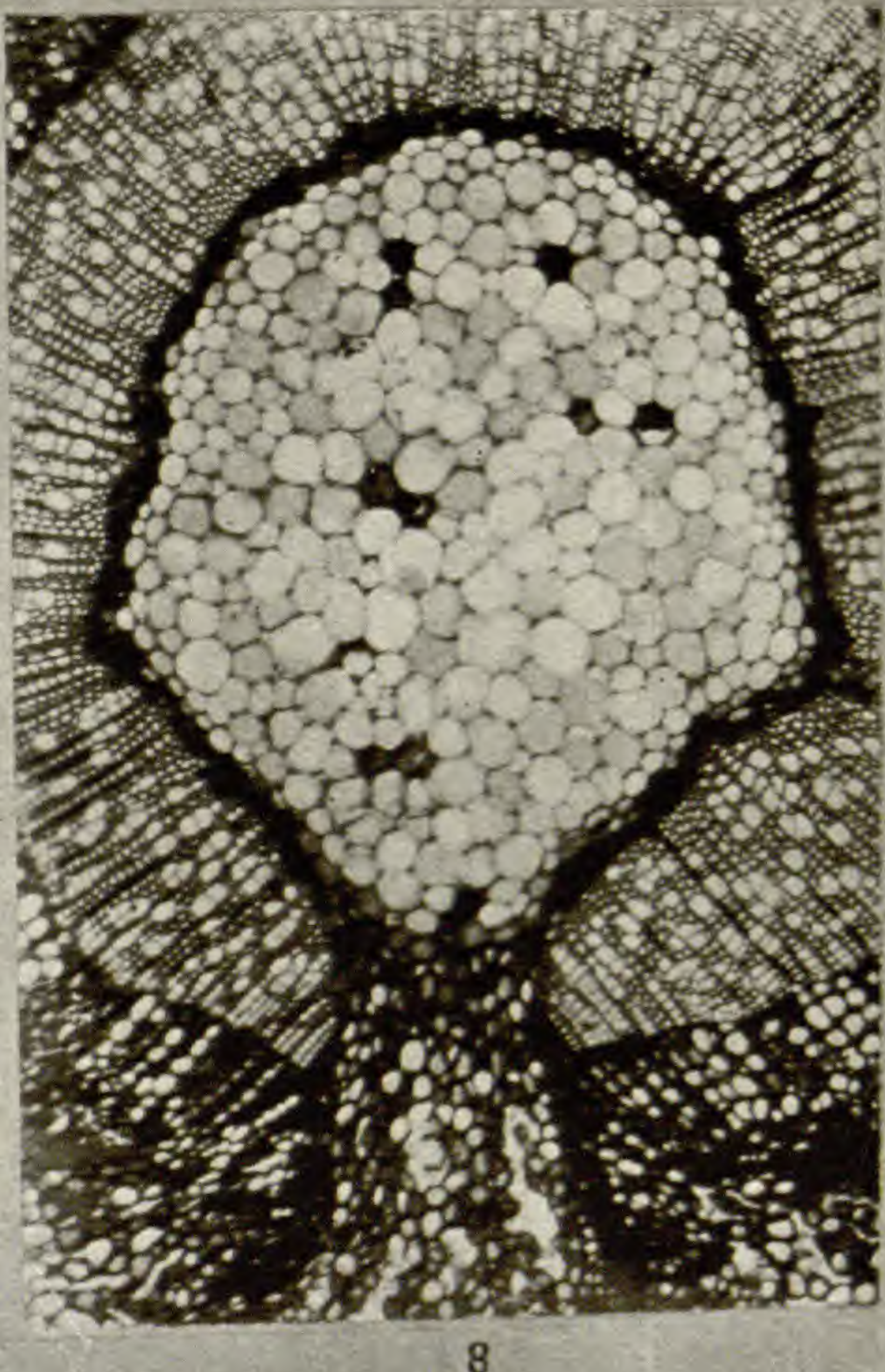

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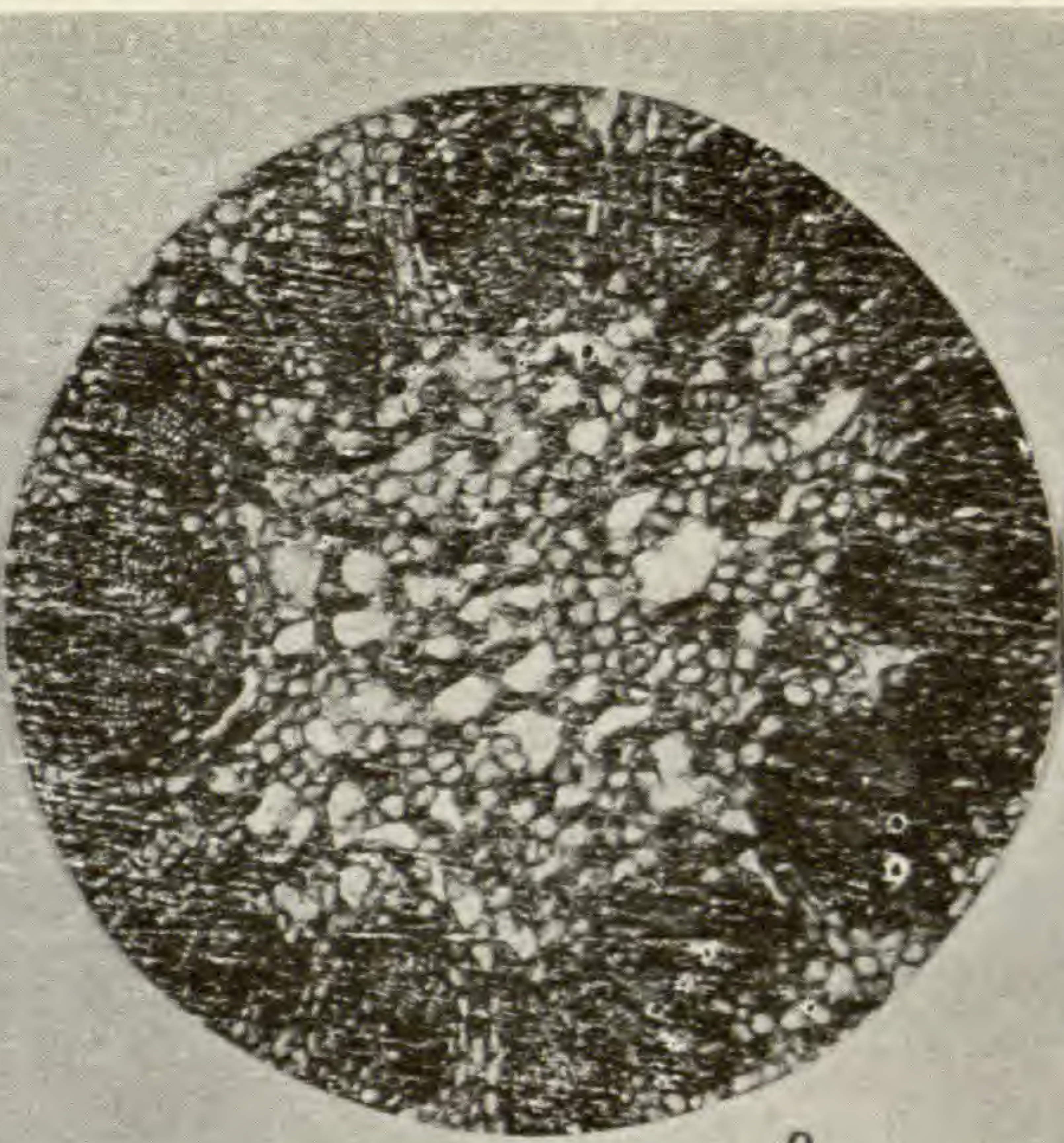
COSENS and SINCLAIR on AERIFEROUS TISSUE



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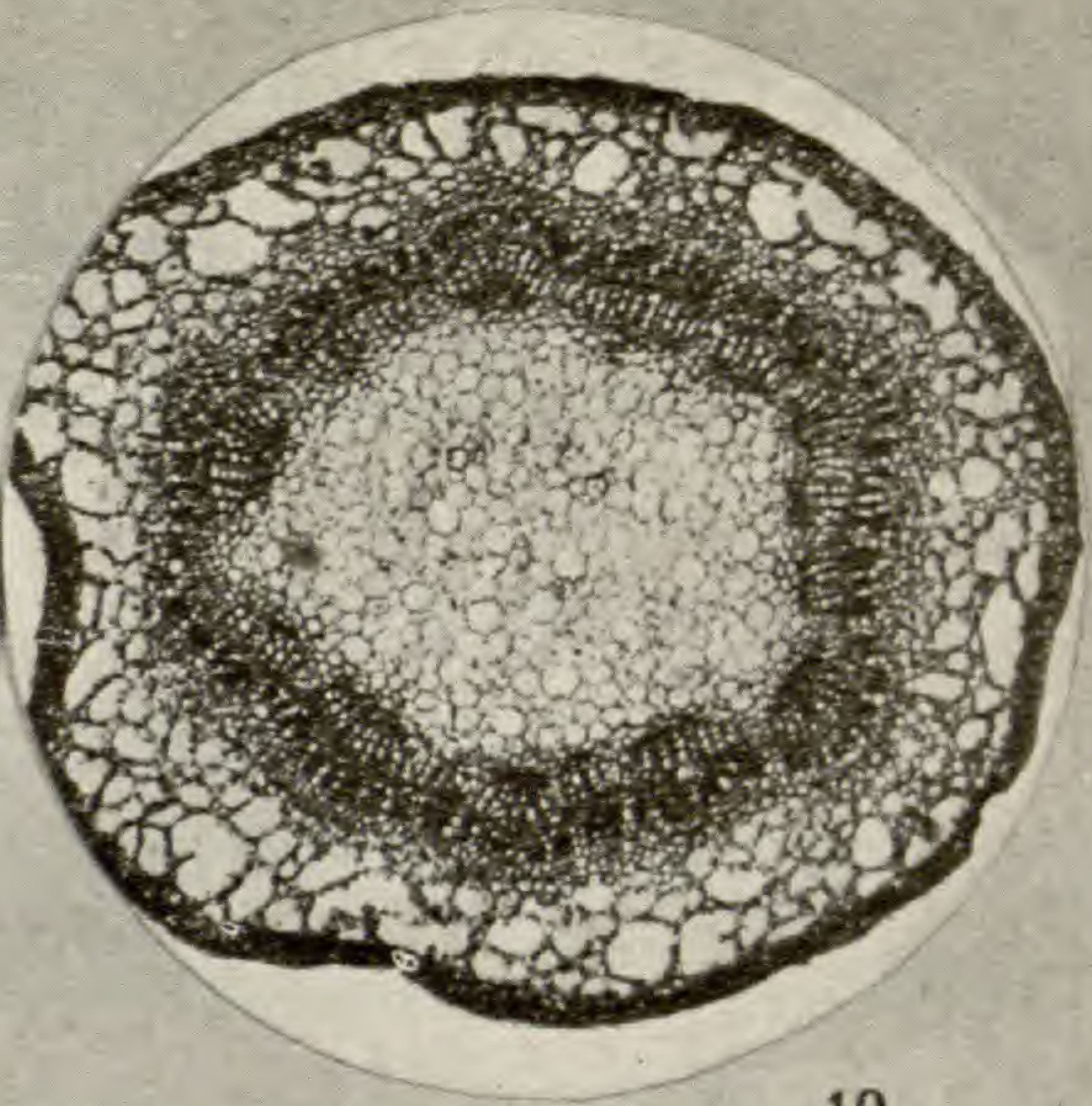

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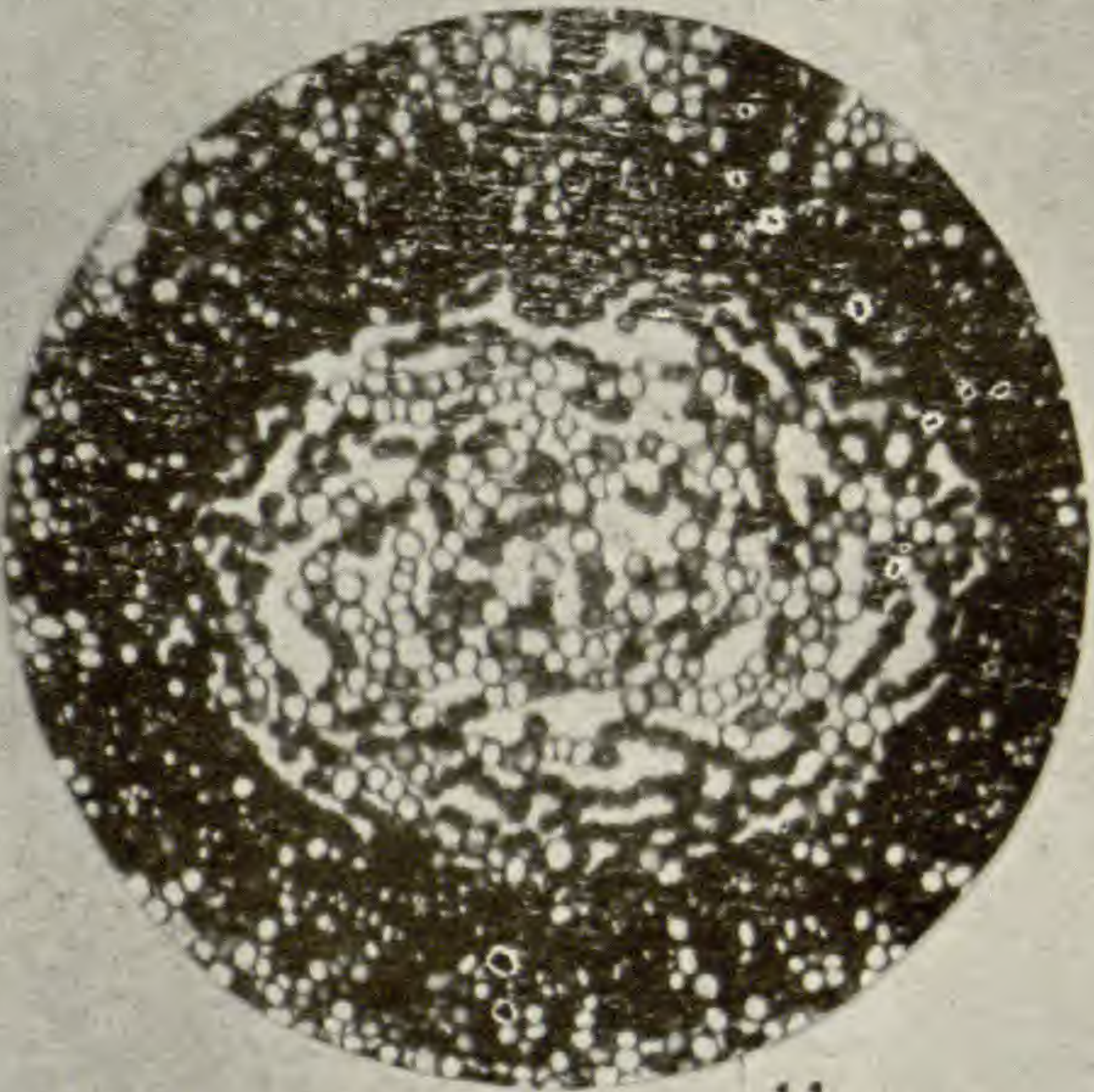

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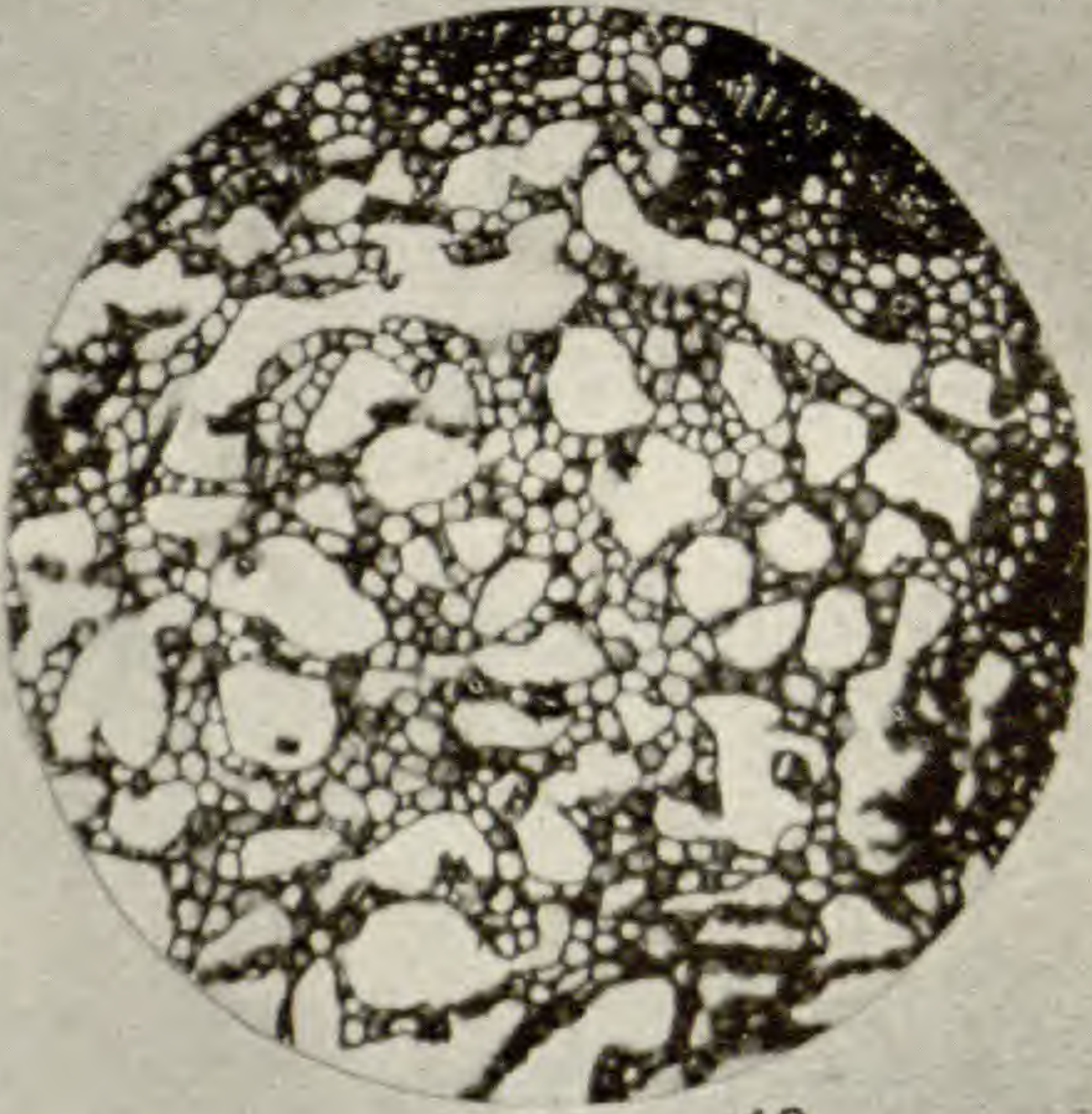

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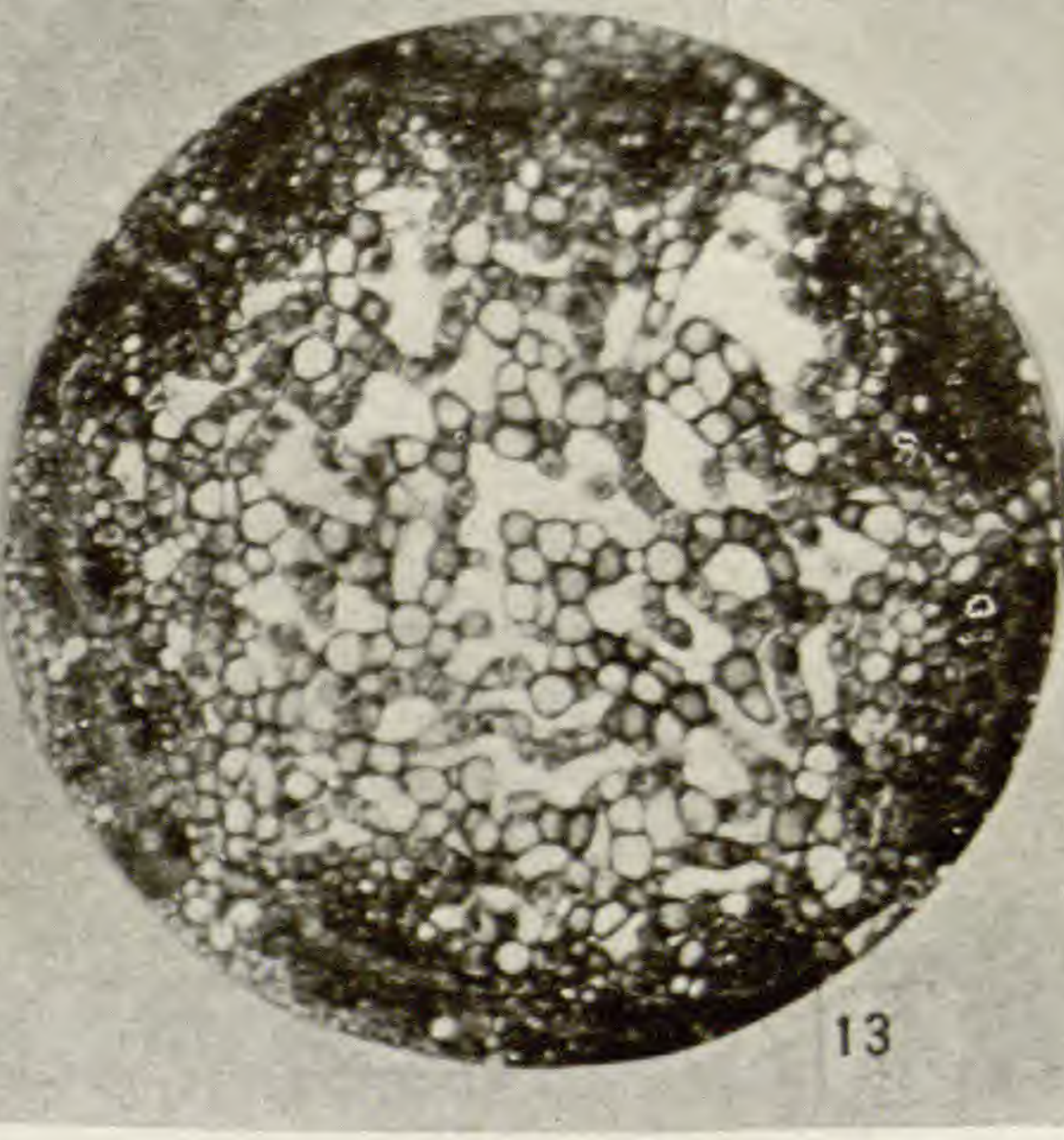

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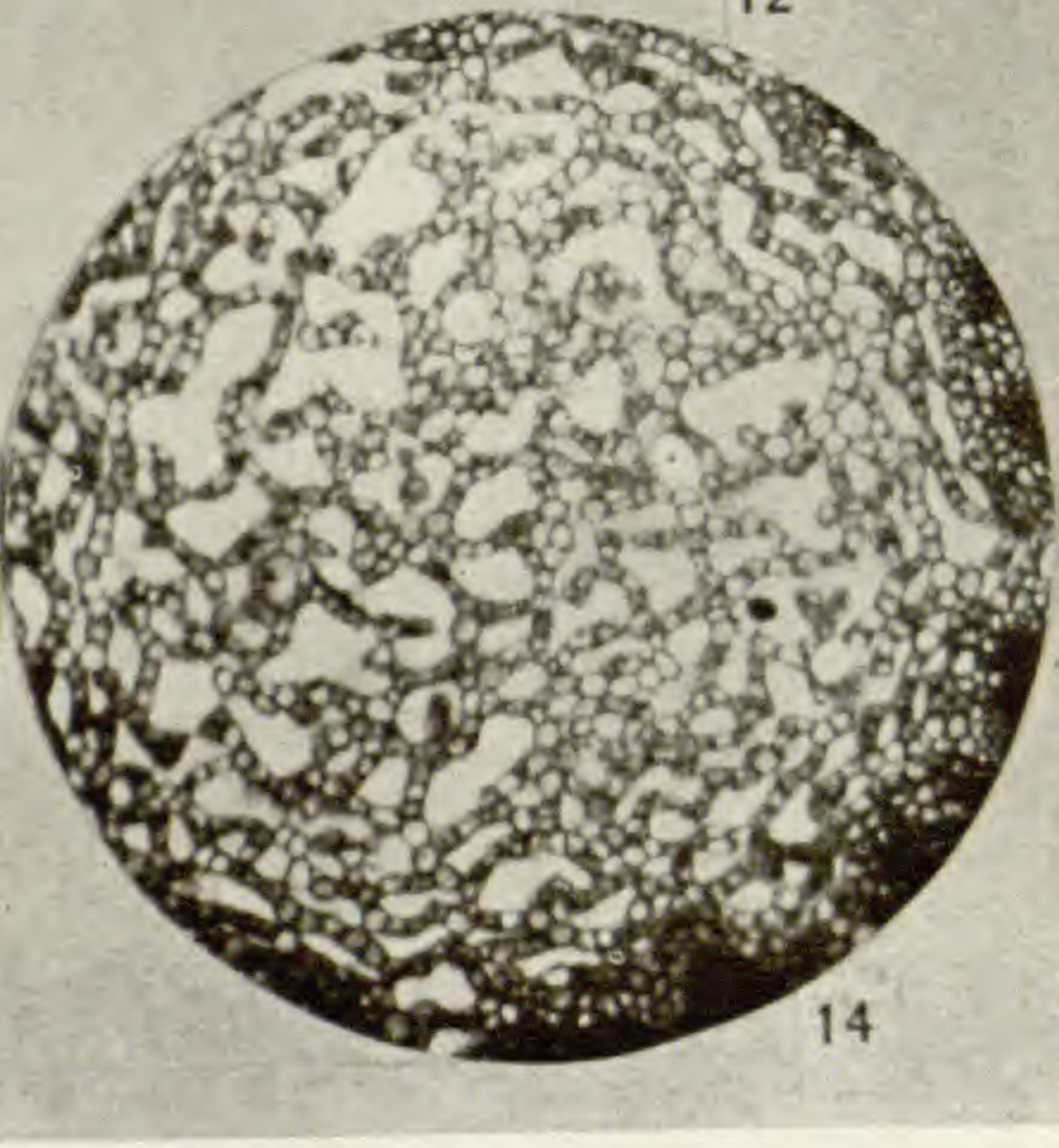

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COSENS and SINCLAIR on AERIFEROUS TISSUE

Fig. 11.—*Salix purpurea* L.: section across the male reproductive axis when the catkin is just emerging from the bud; aeriferous tissue is clearly differentiated in the pith; ×70.

Fig. 12.—Same as preceding except that the catkin was sectioned at a later stage; aeriferous tissue has opened out, making more apparent the air spaces and the separating strands of cells; ×70.

Fig. 13.—*Salix humilis* Marsh.: showing aeriferous tissue developed in the pith of the male reproductive axis; ×70.

Fig. 14.—*Salix cordata* Muhl.: aeriferous tissue in the pith of the male reproductive axis; a comparison with fig. 2 shows similarity of this tissue with that incited by gall stimulus in the stem pith of this species of *Salix;* ×70.

CHONDROMYCES THAXTERI, A NEW MYXOBACTERIUM

J. H. FAULL

(WITH PLATES V AND VI)

The Myxobacteriaceae constitute an extremely interesting assemblage of forms, because of their apparent relationship to the bacteria on the one hand, and to the slime molds, particularly the Acrasieae, on the other hand. The individual plants are bacteria-like rods in all cases, and after a vegetative period these swarm together to organize a definitely shaped pseudo-fructification, without, except in the one genus *Myxococcus*, undergoing any marked morphological changes themselves. These fructifications are not comparable to the more or less heaped up colonies characteristic of certain bacteria, but, as THAXTER (9) has pointed out, are strictly comparable to the "fructifications" of the Acrasieae, the only other group in which there exists "a similar concerted action of aggregates of individuals toward a definite end, namely, the production of a more or less highly differentiated resting state." Whether they are to be regarded as a family of bacteria or as a division of the Myxomycetes, close to, if not included within the Acrasieae, remains undecided. BAUR (1) and QUEHL (4) and more recently PINOY (3) incline strongly to the former view; VAHLE (10) and others incline to the latter view.

Of the fewer than 30 known bona fide species, distributed among 3 genera, 11 have been referred to the complex and variable genus *Chondromyces*. The one described in this paper adds another, *Chondromyces Thaxteri*, n. sp., in some respects the most remarkable of all. This form appeared in small quantity on deer dung from Algonquin Park Forest Reserve, Ontario, and was successfully cultured for more than two years, or until the identity of the species was established, when it was allowed to die out.

The fructifications, or more properly pseudo-fructifications, are ordinarily yellowish, but may vary from flesh-colored to reddish orange, in this as in other respects showing great variability, and ranging 250–750 μ in height, averaging about 350 μ. They are usually simply stalked, but the stalks may fork or even branch more than once, as is shown in fig. 5. The stalks are broad-based, narrowing slightly toward the apex, and yellowish in color. Within their gelatinous matrix are considerable numbers of rods, especially in the lower part. It may be noted also that many members of the colony contributing to the fructification are left on the medium in the immediate neighborhood.

At the tip of the stalk a single head of rods may be found, but more frequently several are formed, resulting from a lobing or branching of the mass that has attained this level, an example of which is represented in fig. 3. These lobes or heads may be sessile or short-stalked. This habit calls to mind *C. pediculatus* Thaxt., in which species the lobes at once ripen as the ultimate cysts, and *C. catenulatus* Thaxt., in which, however, the lobes first elongate and then contract at intervals, due to secondary movements of the rods, giving rise to chains of cysts. But development goes further here. A striking phenomenon next takes place, namely, a heaping up of the rods in radially arranged masses over the entire surface of each head, in the form of elongated cones (figs. 7, 11) or, more rarely, in the form of cylinders (figs. 4, 12), likewise of comparatively great length. In the first instance the apices of the cones are often attenuated to such an extent as to be tipped with but single rods. The surface of the now bristling head is directly invested closely by a yellowish transparent membrane, a secretion from the rods.

The cylindrical form has its counterpart in *C. crocatus* B. and C., in which, however, the cylinders become sharply abstricted at the base, and mature as cysts, when ripe falling away from the cystophore and from one another at the slightest touch; and similarly the conical form, which is the more frequent of the two, recalls *C. apiculatus* Thaxt., possibly its most closely related species. In *C. apiculatus* the cones develop into cysts as do the cylinders in *C. crocatus*, but this is first initiated by a secondary migration of the rods to the centers of the cones, the covering membrane simply shrivelling at the bases and apices.

In *C. Thaxteri* the purpose of the cone and cylinder formations is not evident, for immediately succeeding the completion of the envelope there is a migration of the rods back to the center of the head, within which they form a smooth, more or less spherical mass, the membranes of the protuberances persisting as somewhat shrivelled and twisted or striated appendages, containing no rods except such occasional stragglers as may happen to have adhered to their inner surfaces.

The bristly heads, without further morphological changes, then mature as cysts, which drop off almost as lightly as do the spore-like cysts of *C. crocatus*. Figs. 3, 4 or 7, 9, and 1 represent in sequence the stages that have been described. The course of development just outlined is entirely independent of variations in moisture, food supply, light, and temperature, so far as I have ever observed; always the formation of cones or cylinders on the surfaces of the heads, and their subsequent abandonment, the rods retreating over the route along which they came. The ripening of the head is completed by a movement of rods away from the short stalk, and a shrivelling or contraction of the membrane at that point, which thus explains in part the mode of dehiscence (fig. 1).

The number of cysts, as already indicated, varies from one to several, usually 3–4, but sometimes there may be as many as 20–30. Not infrequently fusions take place in the early stages between neighboring developing heads, as shown in fig. 2. When mature, the cysts are spherical or more commonly depressed spheres or thick disks, and average about 140 μ in extreme width, though varying from 65 to 165 μ. These measurements include the appendages, which vary from 30 to 15 μ in length, and from 10 to 22 μ in breadth at their bases.

On germination of the cysts the rods swarm out through the basal scar of the membrane, leaving the bristly empty husk behind. Fig. 14 indicates the way in which this process takes place, and also shows that the appendages on the membrane have been septated off in no way by an inner secondary membrane.

Attention has already been called to the many variations exhibited by *Chondromyces Thaxteri*, a phenomenon shared to some extent by other species; variations in color, striking variations in every dimension of the fructification, variations in the branching of the stem, and in the number and size of the cysts and their appendages. Compare, for example, figs. 1, 2, 5, 8, 9, and 10. Many species might easily be based on a series of mounts made from a single culture. There is no doubt, of course, that these are simple fluctuating variations of very wide range. It is also almost certain, although not demonstrated, that many of these are due to environment; the local differences of food supply and moisture on the substrata on which they grow cannot be inconsiderable; but this does not explain all.

The life history was followed carefully throughout in its main features, and was found to conform closely to the account given by THAXTER for *C. crocatus*. Confirmation is given also of the fact that the contents of the rods are characterized by deeply staining masses, especially just before and during the resting stage, one to three or four being readily demonstrable. Eosin differentiates them especially well in the cystophores, where the imbedded rods, stained deep pink, stand out in striking contrast to the yellowish translucent matrix. This stain also brings out the uneven distribution of the rods in the cystophore; often there is a thin outer zone almost free from them, and, too, they are much more abundant toward the base than elsewhere, gradually diminishing in number toward the apex. The contents of the cysts stain deeply; the envelope remains unstained, chitinous yellow in appearance, showing for the cysts at all events that the color is not restricted to the rods, as stated by BAUR.

Many attempts were made to secure pure cultures, and a variety of media was employed. The only one that yielded tolerable results was the natural substratum of the organism. Moreover, even on this it was impossible to secure good results if the cysts alone were planted. There was always an admixture in flourishing cultures. What relation the impurities bore to the *Chondromyces* I did not determine. It may be a case of succession of floras, in which the ground must first be prepared for the *Myxobacterium*. So far as I have been able to learn, the same feature is true of other species that have been cultivated. PINOY (3) has recently investigated this feature of *Chondromyces crocatus*, and he announces that a *sine qua non* condition for obtaining a complete development of the fructification is the presence of a special bacterium, a *Micrococcus* related to *M. latens*. He demonstrated this by first isolating the coccus and then planting it alongside rods of *Chondromyces* on the surface of the same sterilized medium. It was only where the resultant colonies grew together and mingled that perfect fructifications of *Chondromyces* were formed, although the relation does not appear to be lichenicolous in character. He affirms that if the *Micrococcus* be present, normal cultures can be obtained on many different kinds of media. I know of no further observations on this subject. ZEDERBAUER (11) cannot be quoted, because he was not working with Myxobacteriaceae as he supposed. In my own cultures a filamentous fungus with septate hyphae was constantly present in small amount, and not infrequently the threads of this fungus are imbedded in the cystophore, although never in the cysts. But ample observations make certain that whatever rôle it plays, if any, it is not a necessary constituent of the fructification at any stage.

The species of *Chondromyces* that have been so far described constitute a rather close evolutionary series; and the outstanding character in which the evolutionary forces are manifested is fundamentally not one of form, size, or color, but of movement. Thus we may begin with a form like *C. muscorum* Thaxt., in which single, simple, unstalked cysts are formed. In *C. serpens* Thaxt. and *C. lichenicolus* Thaxt. there is a movement in a horizontal direction, resulting in sessile, elongated, and often confluent, coil-like cysts. The primary movement is vertical in *C. gracilipes* Thaxt. and a simple stalked cyst results. In some species there may be a forking of the stalk, but in most cases there is a radial movement from the primary stalked mass, resulting in the formation of heads of small cysts, for example, in *C. crocatus*. In these cysts there may be secondary migrations, a single one toward the center of the cyst in *C. apiculatus* Thaxt., or several, the centers of migration being separated at intervals in a radial line. The last is illustrated by *C. catenulatus* Thaxt., with its heads of cysts in chains.

In the ontogeny of *C. Thaxteri* every stage in the vertical and radial line of evolution relative to this phenomenon of movement is traversed, and then there is a beginning of a migration of the rods back over the same road before maturity is reached; and this order of development is invariably followed regardless of the conditions of the environment. As the secondary movement in *C. Thaxteri* carries the rods entirely out of the structures that apparently correspond to the cysts of *C. crocatus*, *C. apiculatus*, etc., it seems reasonable to conclude that *C. Thaxteri* stands at the extreme end of the main evolutionary branch of the genus as we know it at present.

Chondromyces Thaxteri, sp. nov.—Pseudoplasmodiis luteo- vel carneo-coloratis; baculis 3–6 μ×0.5 μ longis; fructificationibus luteis, 250–750 μ, vulgo circa 350 μ altis; stipitibus plerumque simplicibus, atque interdum ramosis; cystes setaceas, sub-globosas, breviter pedicellatas vel sessiles, 1–7 vel plures numero gerentibus; quae cystes integrae 65–165 μ, vulgo circa 140 μ latae sunt; setae autem sunt conicae, 15–30 μ longae et ad bases 10–12 μ latae.—In fimo cervino nascens in Algonquin Park, Ontario, Canada.

UNIVERSITY OF TORONTO

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EXPLANATION OF PLATES V AND VI

FIGS. 1, 2, 5, 8, 9.—Mature "fructifications" of *Chondromyces Thaxteri;* ×240.

FIG. 3.—An immature stage; ×240.

FIGS. 4, 7.—An immature stage, but later than the one in fig. 3; they represent the cone or finger-like processes formed on the "heads" of the fructification; ×240.

FIG. 6.—An early stage in the heaping up of the rods; ×240.

FIG. 10.—Cysts on the left hand branch are mature, those on the right are rather younger than the ones represented in fig. 3; ×195.

FIGS. 11, 12.—Processes from figs. 7 and 4 respectively, drawn on a larger scale; ×720.

FIG. 13.—Part of a mature cyst (fig. 8) on a larger scale, showing empty husks of processes after rods have migrated back from them; ×720.

FIG. 14.—A germinating cyst; ×720.

FAULL on CHONDROMYCES THAXTERI

FAULL on CHONDROMYCES THAXTERI

THE MODE OF ACTION OF PLANT PEROXIDASES

G. B. REED

(WITH TWO FIGURES)

In a recent paper[1] it was shown that while colloidal platinum markedly accelerates the rate of oxidation of various substances by hydrogen peroxide, it does not ordinarily bring about oxidation in the absence of hydrogen peroxide. Experiments were described, however, which show that when the colloidal metal is charged with oxygen (by making it an anode) it rapidly brings about a certain amount of oxidation. By repeating the charging process at sufficiently frequent intervals colloidal platinum might be made to bring about the oxidation of various substances at a rate approximating that affected by hydrogen peroxide and colloidal platinum. From this it was concluded that the action of the colloidal metal in accelerating oxidation by hydrogen peroxide (that is, its peroxidase action) is due to the taking of oxygen from the peroxide by the metal to form a compound which is a more efficient oxidizing agent than the original peroxide.

This information, gained from a study of a simple peroxidase reaction where the constitution of the catalyzer was known, has made possible an analogous investigation of the more significant and complicated problem of the nature of the peroxidases produced in living tissue. Since, as has frequently been pointed out, the peroxidase action of colloidal platinum closely resembles that of the plant peroxidases, it seemed probable that the mechanism of the reactions must be similar. Accordingly, the experiments which proved fruitful in a study of the platinum reaction have been repeated, as nearly as the material permitted, with certain plant peroxidases.

The very active ferment of horseradish root was first investigated. About 150 gm. of the finely chopped tissue was mixed with twice its volume of distilled water and allowed to stand for 24

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[1] REED, G. B., Mechanism of oxidase action. BOT. GAZ. 62:53–64. 1916.

hours. On filtering, a clear, pale yellow extract was obtained, which exhibited very active peroxidase properties.

The peroxidase activity of this extract was determined quantitatively by the method of BACH and CHODAT.[2] To 100 cc. of a solution containing 0.2M potassium iodide and 0.05M acetic acid 20 cc. of the horseradish peroxidase extract was added; the mixture was kept well stirred and at a constant temperature of 15° C., and at frequent intervals portions of 5 cc. were removed and titrated with 0.01M sodium thiosulphate solution (with starch indicator) for the iodine liberated in the oxidation. The values obtained in this way are plotted in the curve *OA*, fig. 1. The rate of oxidation of hydrogen peroxide in the absence of a ferment is shown by the curve *OB* of the same figure. By subtracting the ordinates of the curve *OB* from the ordinates of the curve *OA* we obtain the curve *OC*, which expresses the effect of the catalyzer. For subsequent comparisons the curve *OC* is also plotted as *OC*, fig. 2.

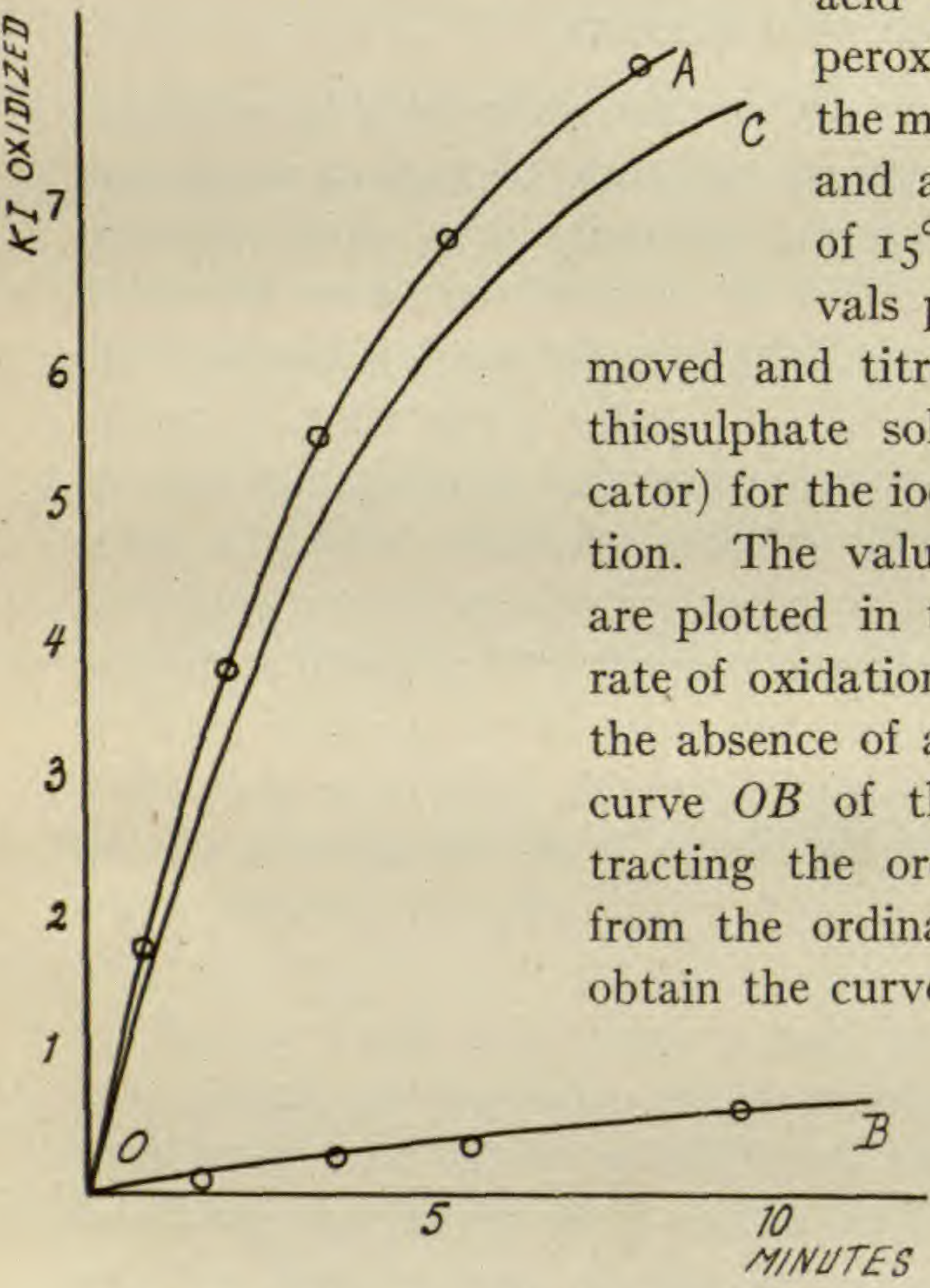

FIG. 1.—Curves of oxidation of potassium iodide by hydrogen peroxide: *OA*, in presence of horseradish peroxidase; *OB*, in absence of a catalyzer; *OC*, the curve resulting when the ordinates of *OB* are subtracted from those of *OA*; ordinates represent number of cc. of 0.01M $Na_2S_2O_3$ required to combine with the iodine in 5 cc. of reaction mixture; abscissae represent time in minutes.

The fresh peroxidase extract in the absence of a peroxide showed no oxidative activity; on adding portions to solutions of potassium iodide and starch or to gum guaiac free from peroxide no oxidation took place. In this respect it was analogous to an ordinary colloidal platinum solution. The platinum may be made

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[2] BACH, A., and CHODAT, R., Ber. Deutsch. Chem. Gesells. **35**:2466–2470, 3943–3946. 1902.

active by charging it with oxygen, either electrically (by making it an anode) or by a chemical method (exposing it to potassium permanganate). Obviously it was not possible to charge the plant peroxidase with oxygen electrically; hence the chemical method was adopted. To about 100 cc. of the same peroxidase solution as that used in the previous experiment concentrated potassium permanganate[3] solution was added drop by drop until the permanganate was no longer reduced, as shown by the color. A small excess of fresh extract was then added to reduce any free potassium permanganate present. The mixture was then filtered free from a small amount of brown precipitate consisting of manganic hydroxide and probably also some organic manganese compounds. In this way a clear solution, somewhat deeper yellow in color than the original extract, was obtained; but this solution possessed a very marked oxidative activity. Solutions of potassium iodide, gum guaiac, or pyrogallol mixed with portions of it, were rapidly oxidized. This new compound was very unstable; when heated rapidly to 60° C. its ability to oxidize gum

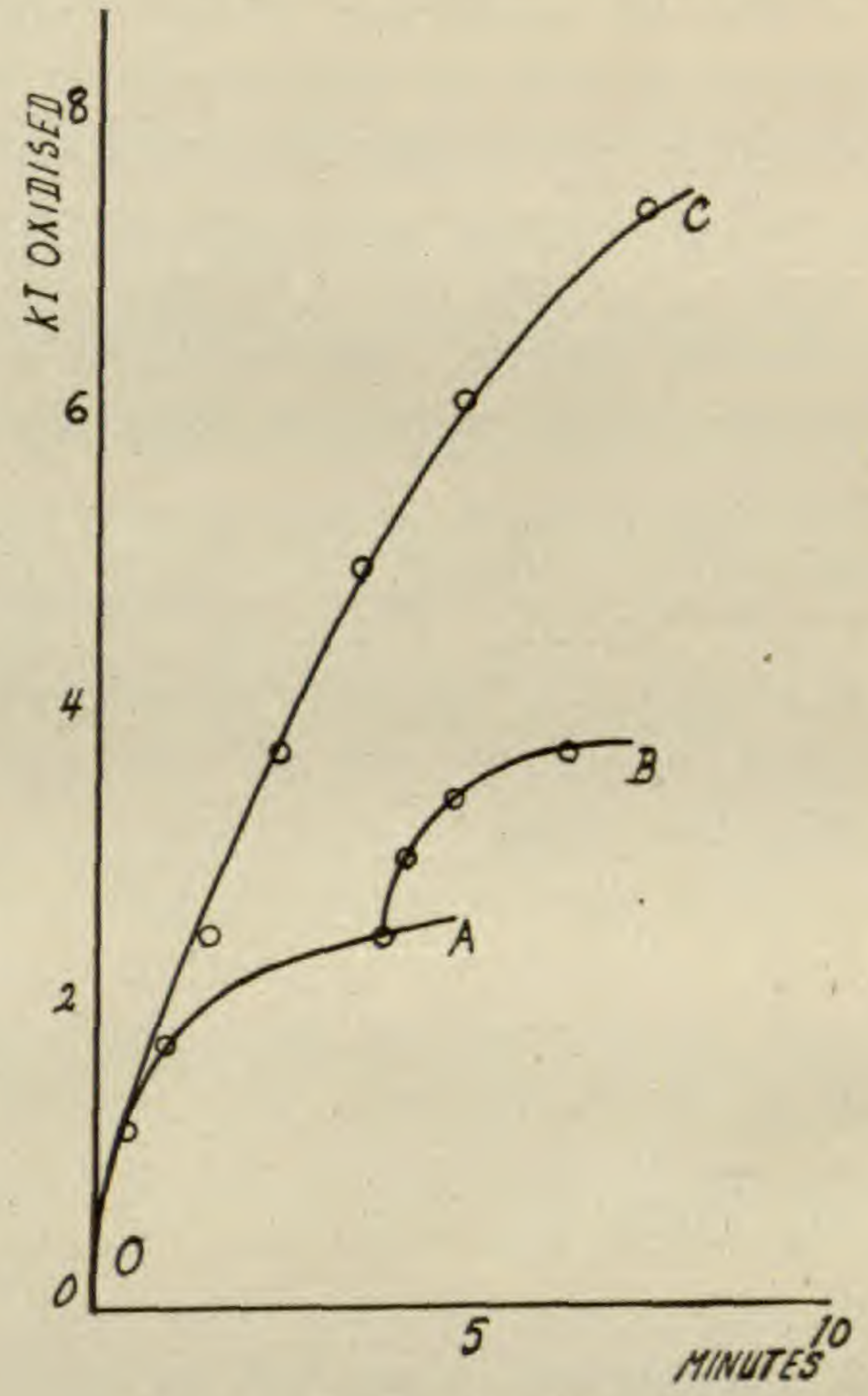

FIG. 2.—Curves showing oxidation of potassium iodide; lower curves *OA* and *AB* represent successive oxidations in the absence of hydrogen peroxide by horseradish peroxidase which had been treated with potassium permanganate at the beginning of each oxidation; curve *OC* represents oxidation by hydrogen peroxide in presence of untreated horseradish peroxidase; ordinates represent number of cc. of 0.01M $Na_2S_2O_3$ required to combine with the iodine in 5 cc. of reaction mixture; abscissae represent time in minutes.

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[3] A concentrated solution was used so that the amount added was not sufficient to dilute appreciably the peroxidase extract.

guaiac or potassium iodide directly was destroyed, but its peroxidase activity was still maintained, as shown by a vigorous action on the addition of hydrogen peroxide.

The full significance of the change effected in the extract by treatment with the oxidizing agent was only apparent, however, after determining quantitatively its oxidizing ability and comparing it with the activity of the fresh extract in the presence of a constant supply of oxygen from hydrogen peroxide. This was done in the following manner. To 100 cc. of a solution consisting of equal volumes of 2M potassium iodide and 2M acetic acid 20 cc. of the *treated* horseradish extract was added and the amount of oxidation measured by titrating samples of 5 cc., at frequent intervals, with 0.01M $Na_2S_2O_3$. The curve *OA*, fig. 2, expresses the results in such form that they may be compared with the peroxide reaction which has already been described and which is plotted in the curve *OC* of this figure.

From this comparison it will be apparent that the amount of oxidation by the new preparation is small; but it was found possible after equilibrium had been nearly reached to renew its activity by a second treatment with potassium permanganate. For this purpose the ferment was removed from the potassium iodide solution (after it had been acting 5 minutes) by adding about 2 volumes of 95 per cent alcohol. The small amount of white precipitate which appeared (about half an hour after adding the alcohol) contained the greater part of the peroxidase. This was centrifuged out, washed in 80 per cent alcohol, dissolved in a few cc. of water, and again treated with potassium permanganate in precisely the same manner as before. On adding this preparation to a potassium iodide solution as previously used, oxidation followed at a rate indicated by the curve *AB* (fig. 2). The smaller amount and somewhat slower rate of oxidation are probably due to loss of ferment in the precipitation and subsequent treatment. An attempt to repeat the process a third time was not successful, only a faint cloudiness appearing on the addition of alcohol to the reaction mixture.

By an inspection of the curves (fig. 2) it is apparent that if it were mechanically possible to repeat the process indefinitely the oxidation could probably be indefinitely prolonged. Moreover, by subjecting the peroxidase to the oxidizing action of the permanganate at sufficiently frequent intervals, it is apparent that the reaction could be made to proceed as rapidly as the curve *OC* (fig. 2), which represents the hydrogen peroxide reaction catalyzed by untreated peroxidase.

Hence we may conclude that just as platinum is recharged with oxygen by hydrogen peroxide, as soon as some of the oxygen has been removed from the platinum by a reducing agent, so the horseradish peroxidase is recharged by hydrogen peroxide under similar conditions.

An investigation of potato peroxidase gave exactly similar results. It may be of interest to note that by treating the peroxidase from the inner tissues of the potato with permanganate it could be made to behave like the oxidase (that is, peroxidase in combination with the oxygenases) from the outer part of the tuber.

It may be concluded, therefore, that in oxidation processes catalyzed by peroxidases two reactions are involved. The peroxidase combines with oxygen from the oxygenases (or from hydrogen peroxide, or possibly from some other source, since it is capable of taking it from potassium permanganate) to form an intermediate compound which is a more energetic oxidizing agent than the original source of the oxygen. The final stage in the oxidation is then affected by this intermediate compound.

A new light is thus thrown on the mechanism of oxidation in living tissues which has so long proved baffling to investigators. The difficulties which beset this field of research are clearly illustrated by the fact that a great amount of careful investigation was necessary before the conceptions of oxygenase and peroxidase could be established. The next step was to elucidate the connection between these entities. This now seems to be accomplished, but it would probably not have been done so soon or so satisfactorily without first making a careful study of the simpler conditions to be found in oxidations which are catalyzed by platinum.

In this connection it is of interest to recall that VAN SLYKE and CULLEN[4] have shown recently that in the fermentation of urea by soy bean urease there is a combination between urea and ferment

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[4] VAN SLYKE, D. D., and CULLEN, G. E., Jour. Biol. Chem. **39**:141–180. 1914.

which eventually splits to form carbon dioxide and ammonia. From the course of the reaction these authors have devised a formula which applies to several enzyme reactions which had previously been measured, particularly the hydrolytic action of invertase and lactase. It seems possible that it may prove to be generally true that the catalyzer combines in a definite manner with the reacting substances.

LABORATORY OF PLANT PHYSIOLOGY
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BRIEFER ARTICLES

THE HALL HERBARIUM

Students of botanical taxonomy in the Central States and America are to be congratulated upon the donation to the Field Museum of Natural History of the herbarium of the late ELIHU HALL. The family of Mr. HALL, after long deliberation, decided that in the herbarium of the Field Museum the botanical work of their father would be advantageously preserved in a highly referable form; his individuality maintained; and his material most carefully organized.

The herbarium of Mr. HALL comprises about 35,000 sheets, particularly rich in western and southwestern United States plants. It contains the original HALL plants of Texas, the American Plains, and Oregon; the HALL and HARBOUR collections of the Rocky Mountains and American Plains; and a unique series of western species grown at Athens, Illinois, from seeds of plants collected from Texas northward to Oregon. The latter series is carefully labeled, so that in every case the original plant may be directly compared with the specimen of the same as grown in the new environment.

Mr. HALL was an active and careful conductor of exchanges from 1858 to 1870, a fact that results in large series of plant specimens contributed to his herbarium by BOLANDER, California; BRANDEGEE, Colorado; BUCKLEY, Texas; CANBY, various localities; CHAPMAN, Florida; CLINTON, New York; COUTHOUY, Ecuador; CURTISS, Virginia and Florida; FAXON, Florida; FENDLER, New Mexico; FORSHEY, Texas; GARBER, Florida; GATTINGER, Tennessee; HALE, Louisiana; HOWELL, Oregon and Washington; JAMES, California; JONES, Utah; LOOMIS and CROOM, North Carolina; MACOUN, Canada and British Columbia; MOHR, Alabama; PARRY and PALMER, Mexico; RAVENEL, South Carolina; RIDDELL, Texas; SHORT, Kentucky; TORREY, various localities; VASEY, Illinois and California; VOLLUM, Texas; WOLF, Colorado; WRIGHT, New Mexico; McOWEN, South Africa; MUELLER, Australia; and various other American and foreign collectors and herbarists.—C. F. MILLSPAUGH, *Field Museum of Natural History, Chicago.*

CURRENT LITERATURE

BOOK REVIEWS

A textbook of physiological chemistry

On account of the rapidly growing importance of physiological chemistry in modern plant physiology, a recent textbook by MATHEWS[1] will be found of particular interest to plant physiologists. Much of this large volume is of equal interest to plant and animal physiologists. Those features which concern the plant physiologist more especially are as follows.

The point of view seems more that of the biochemist than previous texts on this subject, which have been treated almost exclusively from the standpoint of the animal physiologist. This is doubtless due to the more extensive treatment of those phases of the subject not exclusively of interest to the animal physiologist, namely, the chemistry of the carbohydrates, fats, and proteins, and the physical chemistry of the cell. These subjects constitute part I (pp. 265). Some plant physiologists, perhaps selfishly, had hoped for even more extensive treatment of the more general phases of the subject.

Part II deals with "the mammalian body considered as a machine," and while it is of more direct interest to the animal physiologist, there are chapters which interest the plant physiologist as well, namely, the chapters entitled "Animal heat" and "Metabolism under various conditions." In the former chapter there is given a summary of our present knowledge of respiration and a historical résumé of its development. The brief historical discussion which the author has given in connection with the treatment of various phases of the whole subject of physiological chemistry seems especially commendable. The statement made early in the text that the energy of the body comes "immediately from the union of living matter or its constituents with oxygen" indicates that he leans toward the PFLÜGER-VERWORN conception of respiration. This conception and the opposing view of HOFMEISTER are treated in the latter chapter under respiration.

Part III is devoted to practical work and methods. An admirable laboratory course dealing with the general phases of the subject and a course of special methods in quantitative analysis of plant and animal tissue form the basis. In the treatment of this practical work the author gratefully acknowledges the able assistance of his colleague F. C. KOCH, under whose direction, for the most part, these courses have been developed. A separate index to part III is an excellent feature.—LEE I. KNIGHT.

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[1] MATHEWS, ALBERT P., Physiological chemistry, a textbook and manual for students. 8vo. pp. 1040. New York: Wood. 1915.

Artificial parthenogenesis and fertilization

This belated notice of LOEB's book[2] was suggested by the fact that it does not seem to have become known in botanical circles to an extent worthy of its significance. The material is almost wholly zoological, but the book deals with such fundamental problems that it cannot fail to be of interest to all biologists. Chap. iv, on "Hydrolytic processes in the germination of oil-containing seeds," and chap. xxix, on "Artificial parthenogenesis in plants," are of special botanical interest. It must not be inferred from the title that LOEB attempts a systematic presentation of the phenomena in animals and plants; on the contrary, he presents for the most part his own results, his own methods of work, and his own points of view; and as these have concerned so preponderatingly the subject of artificial parthenogenesis, fertilization comes in for relatively scant attention. Nor must it be supposed that the book represents any definitive standpoint with reference to the problems presented. LOEB has already discarded some of the working hypotheses outlined in his book, and has gone beyond the standpoint of the book in other particulars.

The book is of interest in two particular respects: (1) as a presentation of the author's own classical work in this field which he has done so much to illuminate, and (2) as a splendid example of biological analysis which takes a particular genetic process out of the realm of morphology and attempts a thorough physiological analysis, relating it as far as possible to physico-chemical processes on the one hand and to seemingly remote biological processes on the other hand. The author is so fresh and original in his point of view that one cannot fail to profit by the presentation as a study in scientific method. There are few biologic books in which this connection between hypothesis and discovery is more convincingly illustrated.—F. R. LILLIE.

Phoradendron

Probably no genus of plants has been given more elaborate and detailed treatment than the exclusively American genus *Phoradendron* has received in the recent monograph by TRELEASE.[3] It is the record of a prolonged and painstaking study of a difficult and poorly known group. In this monograph 277 species are recognized, and their geographical distribution is very interesting. The two primary groups are named "Boreales" and "Aequatoriales," and the names indicate their distribution. Of the 66 species of the former, 28 occur in the United States, 48 in Mexico, 2 in Central America, and none in the West Indies and South America; while of the 211 species of the latter, none occur in the United States, 29 in Mexico, 20 in Central America, 38 in the West Indies, and 134 in South America. The new species are remarkably

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[2] LOEB, J., Artificial parthenogenesis and fertilization. The University of Chicago Press. 8vo. pp. x+312. *figs. 87.* 1913.

[3] TRELEASE, WILLIAM, The genus *Phoradendron*. Large 8vo. pp. 224. *pls. 245.* Urbana: University of Illinois. 1916. $2.50.

numerous, reaching 102, of which 29 are Boreales and 73 Aequatoriales. This well emphasizes the fact that the genus was very poorly understood.

The book includes all the features of a complete monograph in the way of discussion, keys, descriptions, citations of stations and exsiccatae, etc. The discussion includes such subjects as a historical account of the taxonomy of the group, an extended analysis of the characters used, the facts of parasitism, the origin of the genus, and the range of the species. The numerous plates, which are unusually clear photographic reproductions of types and authentic specimens, approach in value for reference a set of actual specimens.—J. M. C.

Soils

RUSSELL[4] has written another most interesting little book upon soils. His position as director of the famous Rothamsted Experiment Station, which has probably contributed more to our knowledge of soils than any other institution, and his established ability as a writer and as an authority on soil subjects, lead one to expect much from a book written by him, and in the present instance this expectation is fully justified. While the book is directly practical, dealing with matters that tillers of soils need most to know, it leaves the reader with a clear grasp of the main principles established by science and practice in this field. RUSSELL shows a happy breadth of view in treating the subject in this as in his earlier works. Some of our American writers on soils can profit by his example in this respect.

The revision of RUSSELL's monograph on *Soil conditions and plant growth*[5] should have been noted at an earlier date. The first edition has already been reviewed in this journal.[6] While the second edition involved no essential changes, the third edition has a new chapter on the relationship between the micro-organic population of the soil and the growth of plants, also added sections in various other chapters of the book, along with other modifications that bring the book up to date. The book stands alone as a clear, terse, exact statement of the soil conditions in relation to plant growth.—WILLIAM CROCKER.

MINOR NOTICES

Microscopy of vegetable foods.—A second edition of WINTON'S volume under this title has appeared.[7] The general scope and purpose of this valuable

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[4] RUSSELL, E. J., A student's book on soils and manures. pp. ix+206. *figs. 3.* Cambridge Press. 1915.

[5] ———, Soil conditions and plant growth. VIII. pp. viii+190. *figs. 9.* 3d ed. Longmans, Green, & Co. 1915.

[6] BOT. GAZ. 55:167–168. 1913.

[7] WINTON, ANDREW L., The microscopy of vegetable foods, with special reference to the detection of adulteration and the diagnosis of mixtures. With the collaboration of Dr. JOSEF MOELLER and KATE BARBER WINTON. 2d ed. Imp. 8vo. pp. xiv+701. *figs. 635.* New York: John Wiley & Sons. 1916. $6.50.

work were stated in this journal[8] upon the appearance of the first edition. The second edition has incorporated the results of ten years of activity in the examination of human and cattle foods. Among the features of the edition are additions to the sections on wheat and flour; a complete revision of such parts of the chapter on oil seeds as treat of mustards, rapes, cruciferous weed seeds, and linseed; a description of the histology of alfalfa; and a revision of the sections on pomes and drupes. The arrangement of material, analytic keys, lists of adulterants, and the suggestions as to diagnosis are of great practical service in the campaign against sophistication by unscrupulous manufacturers and dealers.—J. M. C.

Plant anatomy.—STEVENS[9] has published a third edition of his well known *Plant Anatomy*, which speaks well for the growing interest in the subject, and also for the quality of the book. The other editions were reviewed in this journal,[10] so that the scope of the work has been described. In the present edition the most important addition is a section on the phylogeny of the vascular bundle, which could not be omitted from the plant anatomy of today. Naturally, there are also additions that include various improvements in technique. The author is a thoroughly good teacher, which means a well organized book and clear presentation.—J. M. C.

The first American Botany.—SAMUEL N. RHOADS has published a facsimile reprint of YOUNG'S *Catalogue d'arbres arbustes et plantes herbacées d'Amérique*, published in Paris in 1783, which is claimed to be the earliest published book written by an American botanist and devoted exclusively to American plants. The editor has given a prefatory account of the author, WILLIAM YOUNG, Jr., of Philadelphia, adding a very interesting personality to the known list of pioneer American botanists, and one whose chronological position in our American literature should be recognized. The book is privately printed in Philadelphia.—J. M. C.

North American flora.—The fifth part of Vol. 9 continues the presentation of the Agaricaceae by MURRILL,[11] 10 genera being presented, which include 311 species, 134 of which are described as new. The largest genus is *Prunulus*, with 106 species, 53 of which are new; the next is *Gymnopus*, with 93 species, 45 of which are new. New species are also described in *Geopetalum* (5), *Mieromphale* (2), *Omphalopsis* (14), and *Omphalina* (15).—J. M. C.

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[8] BOT. GAZ. 41:300. 1906.

[9] STEVENS, W. C., Plant anatomy, from the standpoint of the development and functions of the tissues and handbook of microtechnic. 3d ed. 8vo. pp. xviii+399. *figs. 155*. Philadelphia: Blakiston's. 1916. $2.50.

[10] BOT. GAZ. 46:306. 1908; 50:470. 1910.

[11] MURRILL, W. A., North American Flora 9: part 5. pp. 297–374. Agaricales: Agaricaceae (pars), Agariceae (pars). New York Botanical Garden. 1916.

NOTES FOR STUDENTS

Periodicity in tropical trees.—KLEBS[12] has added some important evidence on the much disputed question of the cause of periodicity in tropical trees. It has been observed that some tropical trees in the very nearly constant climate of their native habitat show no vegetative periodicity, while most of them show an alteration of growth and rest in some form or other. VOLKENS and others attribute this behavior or difference in behavior to hereditary characters; while KLEBS thinks of it as the result of external (nutrient) conditions acting upon the specific structure (hereditary characters) of the plants. KLEBS would say that the course of development of any plant or plant primordium is determined by two things, specific structure of the unit and the condition under which it develops. Every such unit has many potentialities so far as course of development is concerned; the one realized depends upon the condition under which it develops. The reviewer feels that the dual nature of the determiners of the course of development of the individual should sink deeply into the minds of all botanists. It will clear away much foggy thinking frequently manifested by such statements as "this character is due to heredity" and "that one to environment." *Every character is due to both.* Here the speculative philosopher's attempt to get at the *one essential factor* fails, for there are two.

The work was done in the greenhouse at Heidelberg on the following forms in the main: *Terminalia Catappa, Theobroma Cacao, Albizzia stipulata,* and *Pithecolobium Saman.* KLEBS states that since growth depends upon a great number of factors, rest can be procured by any one of these sinking to a certain minimum, and this minimum varies greatly for the different species. KLEBS worked especially with light and nutrient salts. The light intensity and its daily durations at Heidelberg gradually fall to a low minimum late in December. With good salt supply there were 3 types of response to this seasonal light minimum: (1) buds were formed and unfolded continuously throughout the year, but the leaves were smaller and their daily growth less during the time of minimum light (*Terminalia, Albizzia, Pithecolobium*); (2) leaves were formed throughout the season, but these did not unfold normally from November to January (*Theobroma*); (3) plants rested during the months of minimum light (*Eriodendron anfractuosum* and probably *Sterculia*).

The growth inhibiting or rest producing effect of diminished light may be shown in summer. This is most marked in complete darkness, which readily throws even *Albizzia* and *Pithecolobium* into rest. *Albizzia* continues to vegetate in darkness if it has much stored food, so that the effect of diminishing light seems to be related to reduced food supply and not to such specific action as KLEBS found for the beech. Limited light and limited salt supply may act together in producing rest. *Albizzia, Pithecolobium,* and *Terminalia* were

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[12] KLEBS, G., Über Wachstum und Ruhe tropischer Baumarten. Jahrb. Wiss. Bot. **56**:734–792. 1915.

thrown into rest periods at the winter period of minimum light if they were pot bound, but not with good salt supply. The part played by specific structure was evident here, for light amounts that produced continuous unfolding in buds of *Terminalia* gave buds that did not unfold in *Theobroma*, and complete rest in *Eriodendron*.

Intense photosynthesis resulting in high carbohydrate supply produces rest if salts are insufficient. One growth period may exhaust the salt supply, thus producing rest which lasts until the salt supply rises because of nonconsumption and induces a second growth period. KLEBS suggests that this is the condition that throws into dormancy the buds formed in the spring on trees growing in our climate. The salts have been reduced by the formation of stems and leaves and the carbohydrates are abundant, owing to the activity of the newly formed leaves. He suggests that high photosynthesis along with low salt supply may produce dormancy in a similar way in trees of the tropics Several workers have shown that older individuals of a tropical species are more likely to show a rest period than young ones. This KLEBS attributes not to age so much as to limited nutrients conditioned by a multiplication of buds dependent upon a single absorptive and conductive system.

KLEBS criticizes JOST and SIMON for classifying salts with anesthetics as mere stimuli to growth, rather than thinking of their nutrient significance. He speaks of salt addition as a quantitative increase of an already present and *absolutely necessary growth factor*. In this connection he emphasizes the error of assuming that the soil is a constant factor. In such a complex system of organisms and organic and inorganic materials in ever shifting equilibria one must expect periodic changes in supply of nutrient salts as well as in other factors. The frequently observed fact that different individuals of the same species in the tropics show marked differences in vegetative periodicity may be due to the soil factor.

KLEBS speaks of throwing *Pithecolobium* into rest at will (by salt reduction) or active growth (by salt addition) with the same sort of ease as *Vaucheria* can be caused to reproduce by zoospores on the one hand, or by oospores on the other; or water to assume the form of a solid under one condition and that of a liquid under another condition. His evidence leaves little doubt that the tropical trees in general can be made to behave quite like *Pithecolobium*. It seems that KLEBS has established his general contention of the dual determination of periodicity in these forms, but there are some minor conceptions that are less happy.

He classifies all nutrient salts together as if they all have the same effect upon the course of development, while agriculturists have fully demonstrated that nitrates and phosphates in some respects have opposite effects. He implies that salts have their effects mainly as nutrients (building materials), while the extensive work on antagonism probably deals with general physical or colloidal effects, and there is evidence that metallic ions are of importance in catalysis. Moreover, it is not yet shown whether high nitrate supply induces vegetation and succulence through materials (proteins, etc.) built from it or through its lyotropic effects, and whether the partially contrasting effects of phosphates depend upon the first or second condition. Periodicity in salt absorption which has been observed in trees and grains is also minimized It seems evident that to get far back of the general proposition which KLEBS has apparently proved, there is need of a careful study of internal conditions of the plant, anatomical, chemical, and microchemical, as well as the application (by injection or otherwise) of various salts and carbohydrates and products manufactured from them to be sure of the effective agents.—WILLIAM CROCKER.

Morphology of Gnetum.—THOMPSON[13] has done a much needed service in making a thoroughgoing investigation of *Gnetum*. We have had too meager information in reference to this important and puzzling genus, owing mainly to the difficulty of obtaining material representing any extended and close series of stages. THOMPSON obtained his material during a visit to the Malay Archipelago, and chiefly from the Botanic Garden at Buitenzorg and the adjacent country. As a consequence, he has published an extended account of all the structures related to reproduction. Several species were investigated, and especially two species that represent the extremes of variation. The details are so numerous that they cannot be repeated in a review, but some of the more important facts may be mentioned. Certain strobili were found in which the flowers are arranged in a spiral, and are thus very suggestive of catkins of Amentiferae. In the development of the megasporangium the 3 envelopes arise in acropetal succession, and the micropylar tube, which THOMPSON regards as a style, develops conducting tissue. No vegetative cells appear in the development of the male gametophyte, and the microspores frequently germinate in the micropylar tube at a distance from the nucellus. At fertilization stage the female gametophyte consists of approximately 256 or 512 free nuclei; and after the entrance of the pollen tube rapid divisions occur, multinucleate compartments being formed and all the nuclei in each compartment fusing.

THOMPSON concludes that the strobili of *Gnetum* are closely related to the catkins of the Amentifereae; that the flowers are reduced from a bisporangiate condition; that the inner "envelope" of the ovulate flower is an ovary homologous with that of the angiosperms and bearing a true style (the micropylar tube); that the female gametophyte is gymnospermous in its early stages and angiospermous in the later ones; that the fusion of nuclei preceding endosperm formation is a forerunner of that in angiosperms; that the angiosperms have been derived from ancestors very much like modern Gnetales, and that, in fact, the genus *Gnetum* should probably be classified with angiosperms.—J. M. C.

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[13] THOMPSON, WALTER P., The morphology and affinities of *Gnetum*. Amer. Jour. Bot. 3:135–184. *pls. 2–7.* 1916.

Orthogenetic saltation.—BENEDICT[14] has undertaken a study of the variations of the Boston fern (*Nephrolepis exaltata bostoniensis*). His title is selected to emphasize two points: (1) the variations are discontinuous (saltation), and (2) they occur in a definite series along a few limited lines (orthogenetic). It seems that from this fern there have arisen more than 100 forms in less than 15 years. The present paper is concerned chiefly with a record of the facts. Uniform and highly developed cultural conditions are thought to favor the preservation of variations which under wild conditions would be eliminated.

The variations are classified as progressive and regressive, implying in the one case increasing departure from the parent form and in the other case a return toward the parent form. Progressive variations have appeared along three main lines: (1) increasing division of the leaf, observed through 5 vegetative generations; (2) increasing ruffling of the pinnae, observed through 3 generations; and (3) dwarfing, observed through 3 generations. Regressive variations rarely if ever show a complete return to the parent form. The coefficient of variation for progressive variations is very low, probably between 1:1,000,000 and 1:1,000; while regressive variation is much more common. The variations are all discontinuous and the differences are said to be as great as those existing between many wild species of ferns. The main difference between these variations and those shown by wild forms is that the former do not survive natural conditions, the variation usually being accompanied by diminished vigor.—J. M. C.

Anomalous endosperm and the problem of bud sports.—EMERSON[15] has recorded some new and interesting cases of hybrid maize kernels in which half of the endosperm shows a different combination of Mendelian characters from the other half, although it is obvious, from the fact that both parts show xenia, that the endosperm as a whole is due to double fertilization. He discusses two hypotheses, either of which might account for such kernels, namely, EAST and HAYES' hypothesis of somatic segregation and his own hypothesis of somatic mutation. A third, suggested years ago by COULTER and CHAMBERLAIN, seems to the reviewer more plausible than either. In certain plants it has been observed that the division of the primary endosperm nucleus begins before the constituent nuclei have lost their identity. If such a condition were to be demonstrated in maize, we would have a mechanism for the production of mosaic endosperm which could be called neither segregation nor mutation, in the sense in which EMERSON obviously uses the latter term

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[14] BENEDICT, R. C., The origin of new varieties of *Nephrolepis* by orthogenetic saltation. I. Progressive variations. Bull. Torr. Bot. Club 43:207–234. *pls. 10–15*. 1916.

[15] EMERSON, R. A., Anomalous endosperm development in maize and the problem of bud sports. Zeitschr. Ind. Abstamm. u. Vererb. 14:241–259. 1915.

when he speaks of the origin of a recessive Mendelian variety as a bud sport. The hypothesis of incomplete triple fusion is in a way a compromise between the inapplicable and discarded older hypotheses involving entire suppression of the triple fusion and the later ones involving no gross cytological aberrations whatever. Although heartily in accord with EMERSON'S well-considered views in regard to somatic mutation in general, the reviewer must confess to a distrust of drawing a close parallel between bud sports and anomalous endosperm development.—H. H. BARTLETT.

Tubers of Nephrolepis.—SAHNI[16] has investigated the vascular anatomy of the tubers borne on the underground stolons of *Nephrolepis*, and has uncovered a very interesting situation. The vascular strand of the stolon penetrates the base of the tuber as a protostele for a short distance, and then expands into a funnel, acquiring in succession internal phloem, pericycle, endodermis, and "ground tissue." Later the funnel-like stele breaks up, at the same time expanding enormously, into a hollow network of ribbon-like strands (each concentric in structure) inclosing gaps of irregular shape and size. These strands converge again into a single protostelic strand, which usually ends in the apical "mamelon." Root strands arise promiscuously from this reticulate stele. SAHNI calls attention to the fact that the conspicuous gaps that appear in this latticed cylinder cannot be explained as leaf gaps, since there is no trace of leaves on the tuber. He suggests that it is a case of a solid stele dilated sufficiently to transform it into a hollow network. TANSLEY has suggested that it is the dilation of a protostele that converts it into a siphonostele.—J. M. C.

Soil Science.—Many will welcome the founding of the new journal *Soil Science*. It is published (first number January 1916) at Rutgers College, with JACOB G. LIPMAN as editor-in-chief, and NICHOLAS KOPELOFF and CARL R. WOODWARD as assistant editors, along with 23 consulting editors, representing experts in this line from various parts of the United States and from 9 foreign countries. The editor-in-chief outlines the scope of the journal in the following statement: "*Soil Science* is to be devoted to problems in soil physics, soil chemistry, and soil biology. Papers dealing with problems in plant physiology, agronomy, bacteriology, or geology will be accepted only when they may contribute directly to our knowledge of soil fertility." He feels that greater cooperation will be gained among American workers on soil problems by a common channel of publication, in contrast with the previous distribution of articles through a number of American and several foreign journals. Promptness of publication is also of great importance. No doubt this will bring a welcome if only a slight relief to overcrowded journals in a number of lines.—WILLIAM CROCKER.

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[16] SAHNI, BIRBAL, The vascular anatomy of the tubers of *Nephrolepis*. New Phytol. 15:72–80. *figs. 3.* 1916.

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THE

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NEW DIMORPHIC MUTANTS OF THE OENOTHERAS

HUGO DEVRIES

(WITH FIVE FIGURES)

Among the previously described mutants of *Oenothera Lamarckiana* Ser. there is a form which, although fertile with its own pollen, yields a dimorphic progeny. Some of the individuals exactly repeat the stature and characters of their parent, but others return to the type of *O. Lamarckiana*. Besides these, new mutants, especially *O. oblonga*, are relatively numerous. The two main types are produced in varying proportions, according to the individual cultures. The typical specimens may be as few as 10 per cent, or as numerous as 80 per cent. In most instances, however, they show a proportion of about 35–40 per cent. Considering the much smaller individual strength of the typical ones, as compared with the atavistic specimens, these figures may be regarded as indicating a splitting, ordinarily, into nearly equal parts.

This inconstant mutant is *O. scintillans*.[1] Exactly the same phenomenon of splitting has been observed recently in a number of new types. In the first place, in *O. stenomeres* mut. *lasiopetala*, described by BARTLETT.[2] In the second place, it has occurred in my own cultures, among the new mutants of *O. Lamarckiana*, as well as among those of another American species, described under

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[1] The mutation theory. Chicago. 1909. Vol. I, p. 377; and Gruppenweise Artbildung, p. 257. 1913.

[2] BARTLETT, H. H., Mutations of *O. stenomeres*. Amer. Jour. Bot. 2:100–109; see also 2:146. 1915.

the preliminary name of *O. biennis Chicago*.[3] I shall deal with this one under the name *O. saligna*, and designate the new inconstant mutants of *O. Lamarckiana* as *O. cana*, *O. pallescens*, *O. Lactuca*, and *O. liquida*. As far as investigated, they all follow the rule that in every generation they split up into two ordinarily almost equal groups of typical specimens and of atavistic individuals which, in all cases, exactly duplicate the characters of *O. Lamarckiana*.[4] Moreover, they show a relatively high degree of mutability.

With one of them, *O. cana*, I have made a number of crosses with allied forms, in order to ascertain that it behaves in the same manner as *O. scintillans*, and that the same conception of heterogamy must be applied here also. In this mutant the pollen carries only the hereditary qualities of *O. Lamarckiana*, and the specific marks of the mutant are handed down to their progeny through the ovules only.[5] This conception of heterogamy may be considered to hold good for the other inconstant types also.

The same behavior is found in *O. lata*, but since this form never produces any fertile pollen in my cultures and has to be fertilized by *O. Lamarckiana* in order to produce seeds, the evidence which it affords is less stringent than that given by the self-fertile dimorphic races.

Oenothera Lamarckiana mut. *cana*.—Among a number of dubious mutants from *O. lata* which were cultivated as biennials in 1906–1907, a plant was noticed in the third generation of that family with narrower leaves of a gray color, evidently constituting a new type. It was very vigorous, reached a height of about 2 m., and was self-fertilized. It will be designated as *O. cana* from *lata* no. 1, since the first family of *O. cana* was derived from it.

Next year the same mutant type was recognized among the young rosettes, issuing from different samples of seeds of *O. lata* (fig. 1). All in all there were 5 specimens of *O. cana*. In order to determine the frequency of this mutant I have made two cultures

[3] Gruppenweise Artbildung, p. 52. *fig. 18* and *pl. 6*. 1913.

[4] In the wild condition such a splitting would evidently cause a race to die out after a few generations, especially since the atavists are very fertile and much stronger than the mutant form. As a matter of fact, inconstant wild species of this type are not known. See The mutation theory, Vol. I, p. 380.

[5] Gruppenweise Artbildung, p. 273. 1913.

on a sufficiently large scale, using the seeds produced by my pure strain of *O. lata* fertilized by *O. Lamarckiana*. The seeds of 1909 gave 564 seedlings, with 18 per cent *lata* and 2 per cent *cana*. Those of 1908 gave 1550 seedlings, 8 per cent of which were *lata* and 9 per cent were *cana*. Other mutants appeared in these cultures in different proportions, as usual.

FIG. 1.—*Oenothera Lamarckiana* mut. *cana:* a young plant showing the narrow leaves by which it is easily distinguished from rosettes of the *Lamarckiana* type in the same sowings; June 11, 1915.

Among the seeds of pure *O. Lamarckiana*, *O. cana* is much the rarer. In 1913 I fertilized, on 5 strong biennial specimens, almost all the flowers during two months and got sufficient seed to have 20,000 seedlings in 1914. Of these only 6 were *cana*, giving a percentage of 0.03 per cent. In the same boxes 7 *rubrinervis* and 5 *scintillans* appeared as mutants, showing that the mutation coefficients for these three forms do not essentially differ from one another.

Stray mutations into *cana* have appeared in later years in different cultures, as, for instance, in 1913 in those of *O. laevifolia* and *O. scintillans*. Three mutations from *O. pallescens* will have to be recorded in the pedigrees relating to this form. It seems probable that *cana* mutants also have appeared in previous years, but have not been distinguished from other narrow-leaved types, of which there have always been quite a number in the larger cultures. Many other mutations also have escaped observation during a series of years until a single specimen developed into a strikingly new type.

I have cultivated *O. cana* mostly as annuals, but in some instances as biennials. In both cases the stature is the same as that of *O. Lamarckiana*, but in the annuals the stems are slender and the foliage rather loose, whereas the biennials have thick and strong stems with dense foliage. The leaves are narrow, with a shorter blade and a longer petiole, and of a very striking gray color. The flower buds are long and thin, contrasting sharply with those of *O. Lamarckiana* and even more so with those of *O. pallescens* and *O. Lactuca*. The spike is less dense than in the parent species and the fruits are more cylindrical and narrower, containing fewer seeds (fig. 2). In the flowering condition, as well as in the stage of young rosettes, the plants are now easily recognized, but at other periods of their development it is often difficult to identify and count them, some specimens showing their marks very clearly, but others resembling more or less their *Lamarckiana*-like sisters.

The easiest marks are afforded by the flower buds. Measured the day before opening and with the tube and ovary, their size varies, as a rule, from 75–80 mm., against 80–95 mm. in *O. Lamarckiana* cultivated under the same conditions; means 77.5 against 90 mm. The breadth, measured at the base of the conical part above the tube, is only 7 mm. The 4 tips at the top of the bud are more or less bent on one side, and this curious mark is so striking that it is often the first which draws the attention to a stray mutant of the *cana* type (fig. 2). The 4 lobes of the stigma are

FIG. 2.—*Oenothera Lamarckiana* mut. *cana:* flowering spikes of the two types into which each generation splits; on the left the *Lamarckiana* type, on the right the parental type, showing the thin buds and the bent tips of the calyx; the difference in height of the 2 spikes is the same as the mean difference in height of the 2 groups on the bed; third generation of mut. *cana* no. 3, photographed July 22, 1914.

more slender than in *O. Lamarckiana;* the anthers are thin, provided with a good supply of pollen on stout specimens, but often deficient in this production on the weaker ones, especially in annual cultures.

O. Lamarckiana mut. *cana.*—Among the *cana* mutants from *O. Lamarckiana* only one specimen has been self-fertilized. It arose in 1913 in the fourth guarded generation from a plant introduced into my garden in 1905 from the original field near Hilversum. It was only recognized at the end of July, when it opened its first flowers. It yielded few seeds, which gave rise to 19 seedlings only, all of which flowered in 1914. Of these, 13 exactly duplicated the type of *O. Lamarckiana*, 5 were *cana*, and one was a mutant *nanella*. These figures point to a percentage of 26 per cent *cana*.

O. cana from *lata* no. 1.—From the first mutant of 1906–1907, previously described, I have derived a pedigree family in order to try its constancy and got the following result:

1906–1907....Mutant Mutant
1912.........Second generation 16 per cent cana Lamarckiana
1913.........Third generation 24–34 per cent cana Lamarckiana Lamarckiana

The size of these cultures is given in table I.

TABLE I

Year	Generation	Parent	Offspring		Percentage of cana	Mutants
			Total	Flowering		
1912.........	Second	mutant	31	30	16	....
1913.........	Third	cana	49	48	24	1 nanella
1913.........	Third	cana	115	65	34	4 nanella
1913.........	Third	Lamarckiana	60	25	0	....

The offspring of two *cana* individuals of the second generation have been studied separately, as well as those of one specimen of the *Lamarckiana* type. The plants have been under observation through their whole lifetime, so far as space allowed, the numbers of the flowering individuals being given in the column next to that of the totals. The *cana* were all of the same type; the *Lamarckiana* exactly repeated the marks of the original species. Three of the dwarfs have flowered. They all had the marks of ordinary *O. La-*

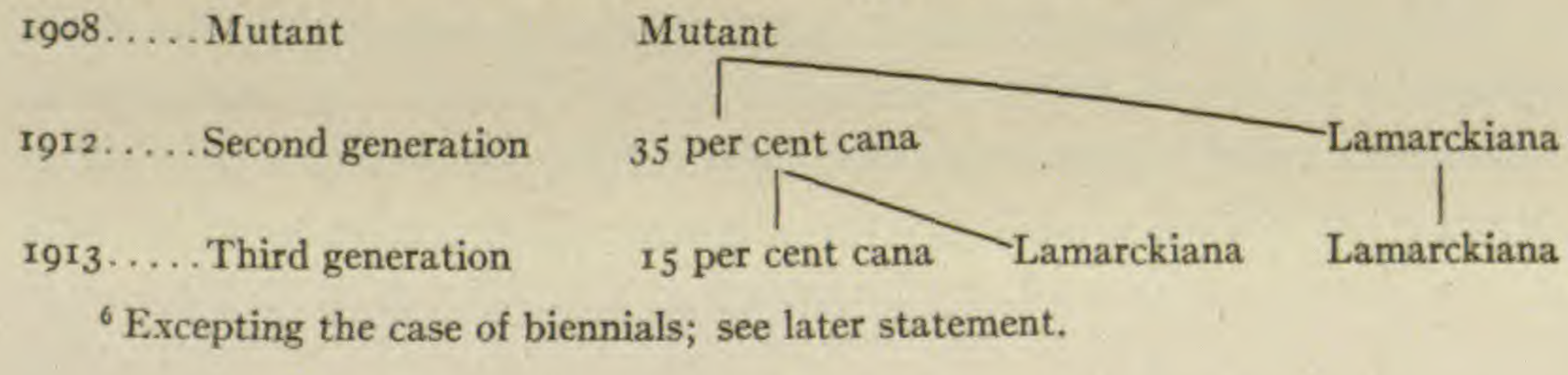
marckiana mut. *nanella* and none of those of *O. cana*. This has been the case in some of the other pedigree cultures of this type, but it should be remarked that in other cases the characters of *O. cana* may combine with the dwarf stature. Such dwarfs have the narrow gray foliage and are easily distinguished from typical *O. nanella* specimens.

The self-fertilized seeds of the *cana* individuals split in both generations into *cana* and *Lamarckiana*, just as *O. scintillans* splits into *scintillans* and *Lamarckiana*. The proportions 16–24–34 per cent with a mean of 25 per cent seem to indicate a splitting into nearly equal parts, with a loss on the side of the weaker form. The same deviation from equality will be seen in almost all the figures of this article, and the same explanation must be considered as applying to all of them. It is almost always the new type which is in the minority.[6]

The seeds of the *Lamarckiana*-like individuals do not give rise to a splitting of this kind, keeping true to their parent form. The same fact recurs in all the pedigrees to be mentioned later on, and for all the new dimorphic types. It may be taken to be the rule, therefore, although the trials have been only one or two in each instance. It agrees fully with the behavior of the analogous splitting products of *O. scintillans*.

O. cana from *lata* nos. 2 and 3.—In a culture of about 600 seedlings of *O. lata*, 4 mutants of the *cana* type arose in 1908. The *lata* strains had been derived from some annual mutants which had been produced by my *O. Lamarckiana* in 1905, and described in my *Gruppenweise Artbildung* (p. 247). From these mutants a second generation was cultivated in 1907, and fertilized by the pollen of *Lamarckiana*-like individuals of the same culture. Their seeds yielded the two *cana* mutants to be described here, and two others, from the seed of which only one generation has been studied.

The pedigree of mutant no. 2 is given below:



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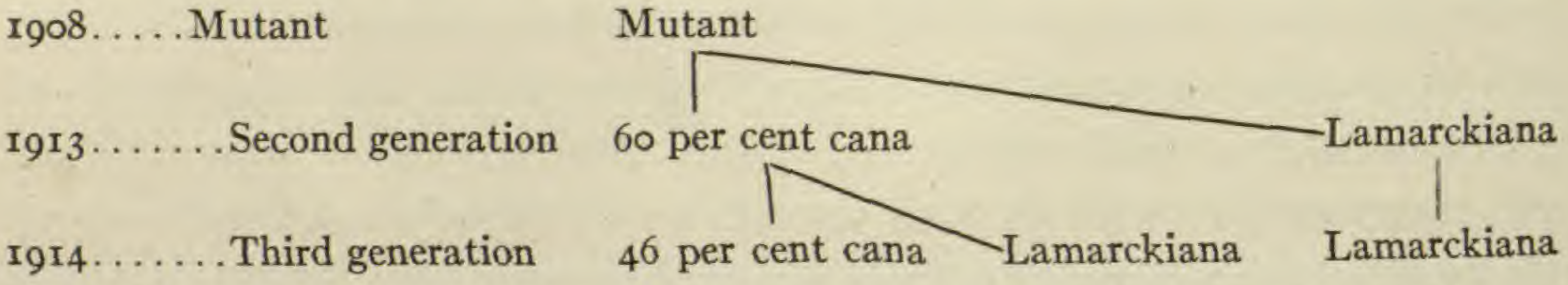
[6] Excepting the case of biennials; see later statement.

That of mutant no. 3 was as follows:



It is easily seen that they agree almost exactly with the pedigree of mutant no. 1, and simply give further proofs of the conclusions drawn from this. The size of the two cultures is given in table II.

TABLE II

YEAR	GENERATION	PARENT	OFFSPRING		PERCENTAGE OF CANA	MUTANTS
			Total	Flowering		
1908.....	A mutant no. 2	....	....	....	....	....
1912.....	Second	mutant	17	16	35	2 nanella
1913.....	Third	cana	55	45	15	2 nanella 2 new
1913.....	Third	Lamarckiana	60	25	0	....
1908.....	B mutant no. 3	....	....	....	....	....
1913.....	Second	mutant	30	30	60	1 lata, 3 nanella
1914.....	Third	cana	57	56	46	3 nanella
1914.....	Third	Lamarckiana	60	25	0	....

In each case the self-fertilized seeds of only one *cana* were studied, besides those of the mutants, and also those of only one atavist of the *Lamarckiana* type. The progeny of the latter proved to be uniform and like the parent, about one-half of the plants being studied in the flowering condition and the remainder in the stage of large rosettes of radical leaves in July. The *lata* mutant has flowered, as have the majority of the dwarfs. Two mutants of a new type arose, which will have to be described in another paper. They resembled *O. rubrinervis*, but lacked the characteristic brittleness of the stems of this form.

The second generation of mutant no. 3 has been the most vigorous one of all my annual cultures of *O. cana*. It was grown, moreover, under exceptionally favorable conditions. For this reason it has been chosen for making a series of crosses, which will be dealt with at the end of this article. The fact that, in this case, the percentage figures come so much nearer to equality of the two types than in the other cases is probably owing to this striking vigor of the race. The means are 25 per cent *cana* for no. 1 and no. 2, but 53 per cent *cana* for no. 3. The proportion of mutants among the seedlings of the *cana* individuals is 18 in 350, or about 5 per cent.

O. cana from *lata* nos. 4 and 5.—As previously mentioned, the progeny of two further mutants of the same origin have been studied. The offspring of one of them embraced only 15 individuals, of which 13 have flowered. There were 3 *cana*, 2 mutants (one *oblonga* and one of the same new type as in no. 2), the 10 remaining plants being externally like *O. Lamarckiana*. The second original mutant yielded only 11 offspring, among which 7 were *cana*, one *Lamarckiana*, and 3 *oblonga*. Although these cultures do not justify the calculation of percentage figures, they evidently support the conclusions drawn from the three former ones, and argue for the conception that this form of splitting is typical for *O. cana*.

INFLUENCE OF CULTURE ON PERCENTAGE FIGURES.—I have shown[7] that the percentage figures for the splitting of *O. scintillans* in the succeeding generations may differ for different families. Sometimes it is only 15 per cent, more often it varies between 34 and 39 per cent, and in rare cases it reaches 69–93 per cent. Subsequent experiences have suggested the idea that these differences are due mainly to outward conditions or to the method of cultivation, and that favorable influences must increase the percentage of individuals with the type of *scintillans* and diminish the percentage of *Lamarckiana*-like specimens.

The self-fertilized seeds of the *cana* individuals previously mentioned have given the following percentages of specimens with the *cana* type: 15 and 16 per cent, 24–34 and 35 per cent, and 46 and 60 per cent, the two latter being found in a culture which excelled the others in vigor. Evidently these figures run parallel to those of *scintillans* and the variability must have the same cause in both cases.

In order to ascertain the nature of this cause I have tried to answer two questions, namely: (1) are the percentage figures

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[7] The mutation theory. Chicago. 1909. pp. 388–391.

different on different parts of the main spike of a plant and on different branches; and (2) are they different for annuals and biennials, provided that the individual strength is in both cases as great as possible? The following experiments will show that the first question is to be answered in the negative, but the second in the affirmative; or in other words, the percentage figures depend upon individual vigor of the plants, and this between the widest possible limits.

The second generation of mutant no. 3, cultivated in 1913, was the most vigorous of all my annual cultures, as already mentioned. I chose for my experiment, therefore, the strongest individual of this group, having the largest supply of pollen in its anthers, and fertilized its flowers on the main spike and on a lateral branch in small bags, each with its own pollen. At the time of harvest I separated the fruits in groups of 10 each; there were 4 of these groups on the main spike and 2 on the branch. In the spring of the following year (1914) I sowed the seeds of these 6 lots separately. I counted the seedlings in the stage shown in fig. 1 without transplanting them. The *cana* were easily distinguished from the *Lamarckiana* by their narrower leaves and gray color. There were a number of dwarfs, which combined with this character those of *cana* and will be called *cana-nanella*. I have planted them out after finishing the countings and found them true dwarfs of the *cana* type. About a dozen of them flowered as annuals, and some flowered the following year as biennials. The result of the countings is given in table III.

TABLE III

	Number of seedlings	Percentage of cana	Percentage of cana-nanella	Percentage together
A, main spike				
base	57	40	5	45
second group	114	30	4	34
third group	121	31	4	35
top	129	35	5	40
B, lateral branch				
base	95	39	4	43
top	94	31	11	42

The means for the whole plant are 34, 6, and 40 per cent. It is easily seen that the deviations from the means fall within the limits of ordinary chance, although all the seeds from the 10 capsules of each group have been sown. Thus it is clear that the first and the last fruits of a spike and those of a side branch may give the same percentage figures of specimens of the parental type. Moreover, the mean value is not essentially different from the means of the pedigrees, as just given, which was 33 per cent. We may conclude, therefore, that the mean percentage for all my annual cultures is about 30–40.

In order to compare the influence of biennial culture upon this figure, I chose three healthy and very vigorous rosettes of 1913 and kept them through the winter under glass. They had been reared from seeds of a biennial mutant belonging to the group of *cana* mutants from *lata*, from which pedigrees no. 2 and no. 3 were derived; but this special culture stayed in the rosette condition during 1913. In 1914 three plants of the *cana* type became very vigorous, reaching about double the height of the annual plants and growing up to more than 2.5 m. Their stems also had twice the thickness of the others, the foliage and flower spikes were very dense, and the flowers much stouter. Every evening 4 or 5 flowers opened on the same spike, against 1 or 2 in ordinary cases. The number of fruits on a spike was 60–80, whereas 40 fruits, as just given, is a high value for an annual plant. All of these fruits were self-pollinated in little bags, and yielded 1–1.3 cc. of seeds from 10 fruits, whereas the annuals give only 0.5–0.9 cc. of seeds in 10 fruits. We may summarize these details by saying that my biennial specimens of 1914 were about twice as vigorous as the very best of all my annual cultures.

TABLE IV

Plant	Fruits	Seeds in cc.	Seedlings	Percentage of cana
No. 1.......	63	6.6	590	96
No. 2.......	79	10.0	1099	93
No. 3.......	64	6.3	277	97

All the seeds were sown in boxes in 1915 and the seedlings counted out, without being transplanted, in the stage corresponding to fig. 1, when the differentiating marks were very sharp. The three plants gave the results shown in table IV.

The remaining 4–7 and 3 per cent were mostly of the *Lamarckiana* type, with some mutants belonging to *albida*, *oblonga*, and *nanella*. I had saved the fruits and their seeds in 7 or 8 groups, beginning at the base of the spike, and sown the seeds separately. But, just as in the previous case, there were no appreciable differences in the percentage figures between the higher and the lower groups.

The main result is that the percentage of specimens of the *cana* type, which runs 15–60 per cent on annual individuals, may increase to 93–97 per cent on very vigorous biennial plants. It is thus clearly seen to be dependent upon the method of cultivation. Obviously this rule may be applied to the percentages of *O. scintillans*, as previously discussed, and to those of *O. pallescens* and the other new dimorphic mutants to be described in this article.

Oenothera Lamarckiana mut. *pallescens* (fig. 3).—Among all the mutants which arose in my garden from *O. Lamarckiana*, this form most closely resembles the parent type. In early stages the rosettes are the same, and in springtime, when still in the boxes, I have not as yet succeeded in distinguishing them. It is not until about 6 weeks after planting out on the beds that the differentiating marks begin to show (fig. 4). In the middle of June the leaves are clearly shorter, and the blade is set off from the narrowly-winged petiole by a sharp indentation. This character causes the rosettes to be more open because the petioles hardly touch one another.

This spatulate form of the leaves remains, for a long time, the best mark of the race; but when the stem grows up, the whole plant is much more slender than the parent form (fig. 3). The stem is thin and low; in July, when the first flowers open, it often reaches only 75 cm., when the corresponding specimens of *Lamarckiana* are already 1 m. and more in height. After a time, however, this difference disappears, since the spike is more elongated. It is less dense than in *Lamarckiana;* the bracts are much shorter and strikingly broader; the flower buds are large and conical, the flowers somewhat smaller, although still larger than those of *O. biennis;* the pollen is abundant and the fruits are short and thick, containing a good supply of seed. The foliage is of the same green

FIG. 3.—*Oenothera Lamarckiana* mut. *pallescens:* the 2 types into which each generation splits; to the left the *Lamarckiana* type; to the right the parental type; August 1914.

color as in the parent form, but much more crinkled and uneven, not as gray as in *O. cana*, nor as hairy as in this form.

The impossibility of distinguishing the young plants before planting out evidently makes this mutant less fit for the determination of splitting percentages, because the sorting and counting has to be done on the beds. In my experiments I have always counted the individuals of the two types at the beginning of the flowering period, since at this time the limits between the two groups are the most sharp.

Moreover, this similarity between the mutant and the parent species must diminish the chances of discovering mutant specimens of the new type. This is probably the reason why it was not observed before 1911. Since that year new mutants of the *pallescens* type have more than once arisen from *O. Lamarckiana* and from some of its derivatives, especially in 1914. All of these mutants exactly resembled the first one in their whole structure and in all their marks.

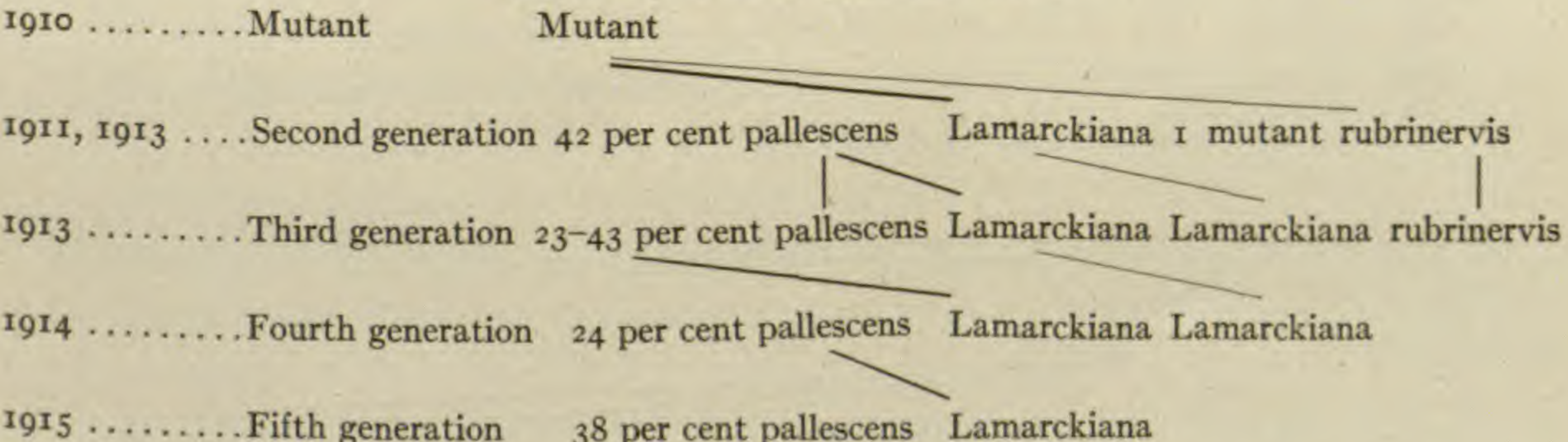
FIG. 4.—*Oenothera Lamarckiana* mut. *pallescens:* 3 typical leaves of the rosette of radical leaves; June 16, 1914.

I have made pedigree cultures of the offspring of my first three mutants. These arose from seed of the same parent plant of 1909, which belonged to the second generation of a guarded strain of *O. Lamarckiana*, derived from a rosette collected in 1905 in the original field near Hilversum. One part of this seed was sown in 1910 and yielded, among about 500 specimens, 1 *pallescens*, together with 1 *rubrinervis*, 3 *oblonga*, 2 *lata*, 1 *scintillans*, 1 *nanella*, the specimen of *O. Lamarckiana* mut. *semigigas* described by STOMPS,[8] and a narrow leaved specimen, exactly resembling the type described and figured by GAGER[9] for a derivative of *O. biennis*. The specimen of *pallescens* was discovered by chance, since almost none but mutants and a number of doubtful specimens had been planted out; it occurred among the latter and was distinguished as a new type only at the time of flowering. Thereupon, another part of the same sample of seeds was sown in 1911 and yielded two more specimens of *pallescens*, among about 250 flowering individuals.

The self-fertilized seeds of these three mutants gave rise to a mixed progeny, the smaller half of which resembled the parent, whereas the remainder presented the type of *O. Lamarckiana*, duplicating this in all of its special marks and during all the stages of their development. In the following description I will, therefore, indicate them simply as *Lamarckiana*, without discussing the question whether some internal characters might perhaps be different. But externally there is no difference; moreover, the progeny of this derivative *Lamarckiana* behaves exactly like that of normal ones. This splitting into these two types has repeated itself in the following generations and in all of the cases investigated.

Moreover, the *pallescens* seems to be mutable to a higher degree than *O. Lamarckiana* itself; for, although my cultures have been necessarily small, the number of mutants is very striking, reaching 20 among about 500 specimens, or 4 per cent. From the first three mutants I have derived three pedigree families, which I will now briefly describe.

PEDIGREE OF MUT. *pallescens* NO. 1



1910 ....Mutant Mutant

1911, 1913 ....Second generation 42 per cent pallescens Lamarckiana 1 mutant rubrinervis

1913 ....Third generation 23–43 per cent pallescens Lamarckiana Lamarckiana rubrinervis

1914 ....Fourth generation 24 per cent pallescens Lamarckiana Lamarckiana

1915 ....Fifth generation 38 per cent pallescens Lamarckiana

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[8] STOMPS, THEO. J., Die Entstehung von *Oenothera gigas* DeVries. Ber. Deutsch. Bot. Gesells. **30**:406. 1912.

[9] GAGER, STUART C., Cryptomeric inheritance in *Onagra*. Contrib. Brooklyn Bot. Garden no. 3, Bull. Torr. Bot. Club **38**: 461-471. *figs. 2*. 1911.

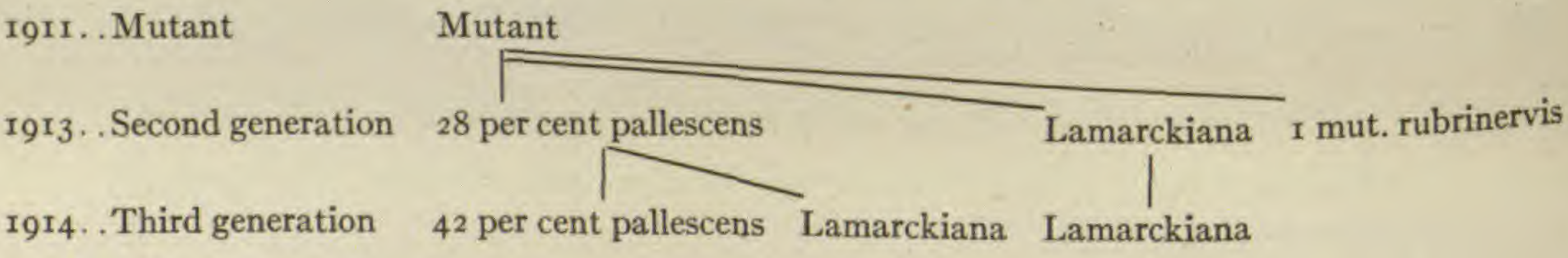
The size of these cultures is given in table V.

TABLE V

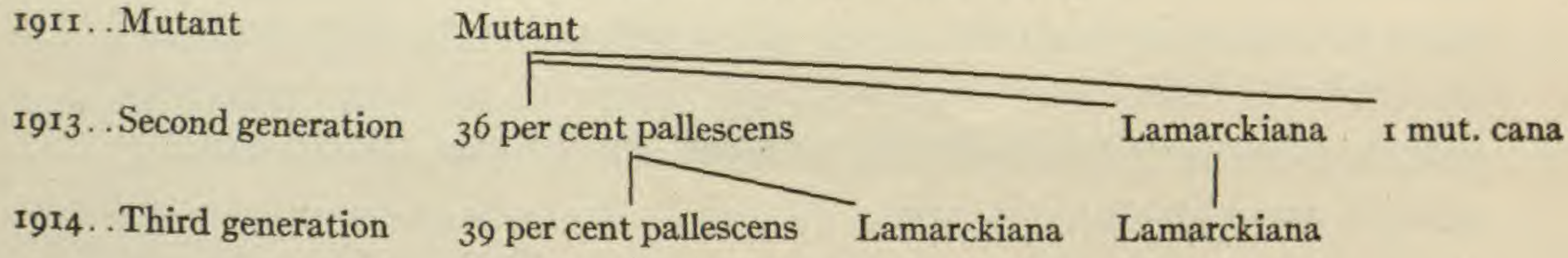
Year	Generation	Parent	Total offspring	Flowering individuals	Percentage of pallescens	Mutants
1911.....	Second	mutant	129	6	....	1 rubrinervis
1913.....	Second	mutant	40	40	42	1 liquida
1913.....	Third	pallescens	69	69	23	....
"	"	pallescens	65	65	43	1 nanella, 1 cana
"	"	Lamarckiana	90	50	0	1 scintillans, 1 lata, 1 albida
"	"	Lamarckiana	56	50	0	....
"	"	rubrinervis	70	25	0	....
1914.....	Fourth	pallescens	55	53	24	1 nanella
"	"	Lamarckiana	58	25	0	....
"	"	Lamarckiana	80	25	0	....
1915.....	Fifth	pallescens	60	25	38	....

The offspring of the 4 specimens of *Lamarckiana* had this uniform type, in the flowering specimens as well as in the other ones. These were examined in June and July when in large rosettes of radical leaves. The offspring of the mutant *rubrinervis* was also uniform and exactly resembled the race of this name in all its marks, and especially in the brittleness of its stems. The offspring of the original mutant, cultivated in 1911, embraced 129 plants, only 6 of which have flowered, the others having been destroyed before the significance of the culture had been realized. Among these 6, 2 were *Lamarckiana*, 3 *pallescens*, and 1 mut. *rubrinervis*. Their offspring were studied in 1913, as given in table V. Among the mutants the *rubrinervis*, *liquida*, *scintillans*, *lata*, and *cana*, as well as one *nanella*, flowered and proved their identity with the races of these names at that period.

PEDIGREE OF MUT. *pallescens* NO. 2



PEDIGREE OF MUT. *pallescens* NO. 3



The size of these cultures is given in table VI.

TABLE VI

Year	Generation	Parent	Total offspring	Flowering individuals	Percentage of pallescens	Mutants
A						
1911.....	mutant no. 1	....	....	....	....	....
1913.....	Second	mutant	25	25	28	1 rubrinervis
1914.....	Third	pallescens	57	57	42	5 nanella
1914.....	Third	Lamarckiana	60	25	0	....
B						
1911.....	mutant no. 2	....	....	....	....	....
1913.....	Second	mutant	25	25	36	1 cana
1914.....	Third	pallescens	25	25	39	1 lata, 3 nanella, 1 cana
1914.....	Third	Lamarckiana	65	25	0	....

Among the mutants the *rubrinervis*, both *cana*, one *lata*, and some *nanella* have flowered. The individuals of *pallescens* and *Lamarckiana*, which did not flower, were examined in June and July as large rosettes. Most of the flowering specimens were observed during the months of August and September.

The percentage figures of these tables vary from 23 to 43, the means for the 3 families being 33, 35, and 37 per cent, and the total mean being 35 per cent. On account of the evident weakness of the individuals of the *pallescens* type, as compared with their *Lamarckiana*-like sisters, these figures may be assumed to show that the splitting into two main types took place in about equal parts. The splitting is constantly repeated from the *pallescens* specimens, but the progeny of the *Lamarckiana* type retain this type uniformly.

I have made only one cross in these families, and that in order to ascertain the properties of the pollen of the *pallescens* individuals. I placed this pollen on the stigma of some flowers of

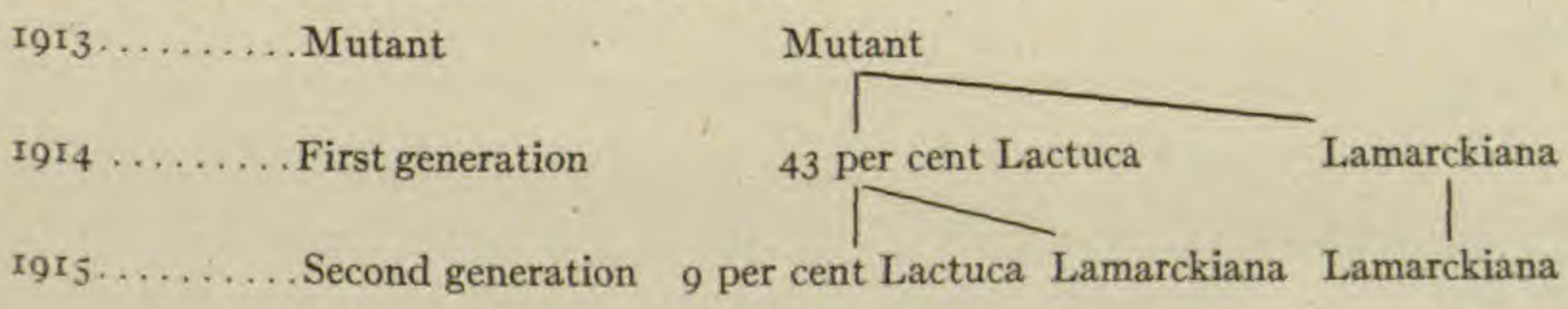
Lamarckiana in 1913, and got from the seeds a uniform generation of 60 flowering individuals, all of which proved to be *Lamarckiana.* I conclude from this fact that the pollen of the *pallescens* plants does not transmit the characters of the race, exactly as in *O. scintillans* and *O. cana.*

O. Lamarckiana lata mut. *Lactuca* (fig. 5, *C*).—In the summer of 1913 I found, in a race of *O. lata* which had been fertilized in the previous generations (1905 and 1907) by *O. Lamarckiana*, a weak plant which seemed to be new to me, but showed evident signs of affinity with the inconstant types of *O. cana* and *O. pallescens* as previously described. It was fertilized, therefore, purely by its own pollen. It yielded 0.8 cc. of seeds, which were sown in 1914 and gave rise to 65 plants, one of which was a mutant of the ordinary type of *O. nanella*, and subjected to the same bacterial disease which so often deforms the dwarfs of my race. Among the others, two types were represented in about equal numbers. One type was exactly like normal *Lamarckiana;* it counted 36 individuals, almost all of which have flowered, without showing any recognizable difference from the original wild species. The remaining 28 constituted a new and uniform type, repeating the characters of the parent plant of 1913, so far as these had been noticed and recorded. At the time of planting out, in the beginning of May, they very much resembled the compact rosettes of *O. nanella*, but without any signs of the disease. About the middle of June, when the rosettes of the type of *Lamarckiana* were growing very fast, those of the new

FIG. 5.—Typical radical leaves of *A*, *Oenothera Lamarckiana* mut. *liquida; B*, mut. *cana; C*, mut. *Lactuca;* June 1914.

type remained small, their leaves reaching only about half the length and half the breadth of those of their sisters (7×3 cm. against 14×5 cm.). Their blades were sharply set off from the winged petioles (fig. 5, *C*), and thereby they much resembled those of *O. pallescens* (fig. 4). This resemblance continued during the development of the stem and the flower spikes; but even as the rosettes were smaller and more densely leaved, the stems were lower and weaker and less branched. The leaves were narrower and folded along the middle vein, instead of being broad and flattened, as in *O. pallescens*. The flower buds were as thick and as large as those of this species, and the flowers also reached the same size, the petals having a length of 4 cm. During the flowering period the differences from *O. pallescens* grew gradually less, and at the end, in September, the new type seemed to be only a weak form of this latter, reaching a less height and being almost unbranched. Artificial self-fertilization has been difficult, since in many flowers the pollen was in an imperfect condition. Four specimens yielded a sufficient harvest (0.5–1 cc.). The sister plants of the *Lamarckiana* type showed an abundance of seed, exactly as the *Lamarckiana* of pure origin does.

The next year (1915) I sowed the seeds of one specimen of the parental type and of two of the type of *Lamarckiana*. The first gave only 44 seedlings, of which 4 were *Lactuca*, one *nanella*, and the others *Lamarckiana*. The two other sowings gave 248 and 283 offspring of their own type, without any *Lactuca* specimens, but with some dwarfs. The number of them was 11, or 4 per cent in the first group, but only one in the latter group. Combining these results we get the following pedigree:



Although only two generations from the seeds of the original mutant have been cultivated, it is evident that this new form behaves exactly like the inconstant races of *O. scintillans*, *O. cana*, and *O. pallescens*. Under favorable conditions it splits into about equal numbers of the mutant type and of the type of *O. Lamarckiana*. Moreover, a mutant dwarf has been produced.

O. Lamarckiana mut. *liquida* (fig. 5, *A*).—In 1912 and 1913 a new type of mutant was discovered, which came next to *O. scintillans*, had flat and smooth leaves like that form, but the foliage was much broader and lighter green. The individual mutants resembled one another in all respects, save the size of the flowers; they were very slender and had leaves about two-thirds the size of those of *O. Lamarckiana*. The pollen was sometimes abundant, but in other flowers rather scanty.

All in all, I had 6 mutants of this type. One came from seed of *O. Lamarckiana*, but unfortunately it failed to produce good seeds, although the fruits were well developed. Four others arose from *O. lata*×*Lamarckiana*, two in 1912, which also did not yield fertile seeds, and two in 1913. The last one was found in the culture of *O. pallescens* mut. no. 1, and mentioned previously. The seeds of the 3 fertile mutants were sown in 1914 and yielded small cultures, which split up into two types, one repeating the mutant parents in all respects, and the other differing in no visible way from ordinary *O. Lamarckiana*. Besides these there were some mutants which happened to belong to allied types. Table VII gives the size and constitution of these cultures.

TABLE VII

SECOND GENERATION OF *O. liquida*

Mutant 1913 from	Lamarckiana	liquida	Mutant	Total	Percentage of liquida
pallescens	11	6	....	17	35
lata	61	26	1	88	30
lata	13	8	2	23	35
Total	85	40	3	128	31

In each of these cultures 15 individuals have been allowed to flower, about one-half of these being the *liquida* type and the other half the *Lamarckiana* type. The 3 mutants were *scintillans*, *pallescens*, and *cana*. All 3 have grown vigorously and flowered in August and September; they differed in no respect from the races of the same names.

The percentage of typical individuals in the second generation is about the same as for *O. cana* (25 and 53 per cent), for *O. pallescens* (35 per cent), and for *O. Lactuca* (43 per cent). From this it may be concluded that the 4 races have the same hereditary constitution which, moreover, is the same as in *O. scintillans*.

The next year (1915) I cultivated a third generation of the second mutant of the table (mutant no. 1 from *lata*). The harvest had been small, as in the previous generation, and only 33 seeds germinated. Of these 9 were *liquida*, 1 was *pallescens*, 1 *oblonga*, and the others *Lamarckiana*. All of them have flowered. The percentage for *liquida* was 27, or about the same as in the first generation. Moreover, I have sown for each of the 3 cultures of 1914 the seeds of one or two typical individuals, and also for each of them the seeds of two of the atavistic or *Lamarckiana* type. These 6 last sowings contained 150–300 seedlings each, together 1311, of which 8 were mutants (3 *oblonga*, 4 *lata*, 1 *cana*); the remainder were all of the *Lamarckiana* type, no *liquida* occurring among them. The seedlings of the 4 *liquida* specimens gave the results indicated in table VIII.

TABLE VIII

THIRD GENERATION OF *O.* MUT. *liquida*

Race issued from	Total of seedlings	Percentage of liquida
pallescens	84	25
pallescens	47	28
lata no. 1	80	41
lata no. 2	26	35
Total	237	32

The countings were made in June and July in the boxes in which the seeds had been sown; the plants were all young rosettes with leaves 15 cm. long in the *Lamarckiana* type, and 6–10 cm. long in the *liquida* specimens. The differences were clear and sharp. The table shows that the splitting was almost exactly the same in the third as in the second generation.

Dimorphic races do not seem to be rare among the mutants of *O. Lamarckiana*, and have been observed to spring also from its hybrids with other species; but the characters are not always as sharp as in the instances described, or the production of seeds is too insufficient for further cultures. Only one case may still be mentioned here. It was a mutant from *O. lata*, discovered in 1914, the self-fertilized seeds of which gave a dimorphic second generation, consisting of 19 plants of the parental type, 47 of the *Lamarckiana* type, besides 2 mut. *oblonga* and 2 mut. *lata*. Almost all of these flowered in 1915. Those of the parental type were strikingly like one another, constituting a wholly new form, with very long, narrow, dark green leaves, the stems low and scarcely branching, the spikes rich with bright flowers like those of *Lamarckiana*, and with a good supply of pollen. The fruits, however, were cylindrical and very thin, containing only a few good seeds. The plants excelled in beauty the species and most of its other mutants, but on account of its slight fertility I do not propose to continue the culture. It may be called *O. superflua*.

O. biennis Chicago mut. *saligna*.—In the second generation of my race of *O. biennis Chicago*[10] I found in 1913, among 870 normal individuals, two specimens of a weaker, narrow-leaved type, which differed sufficiently from the former mutants of this species, namely, from *O. biennis Chicago* mut. *salicifolia* and mut. *salicastrum*,[11] to be considered a new form. One of these new mutants died before flowering, the other yielded, after self-fertilization, a small but sufficient harvest of seeds. One-half of these seeds were sown, but only 17 specimens germinated and grew up into flowering plants. Of these 9 repeated the type of the parent, but 8 returned to the size, vigor, and characters of *O. biennis Chicago*, the grandparent. Although the numbers are very small, they point to a splitting into equal parts, as in the splitting mutants of *O. Lamarckiana* just described.

The difference was already evident in March, when the seedlings were only two months old. In June the rosettes were large, but smaller than those of the species, the leaves smooth and narrow. The stems grew up to about one-half the height of their atavistic sisters, and began to flower in September, having a length of 60–120

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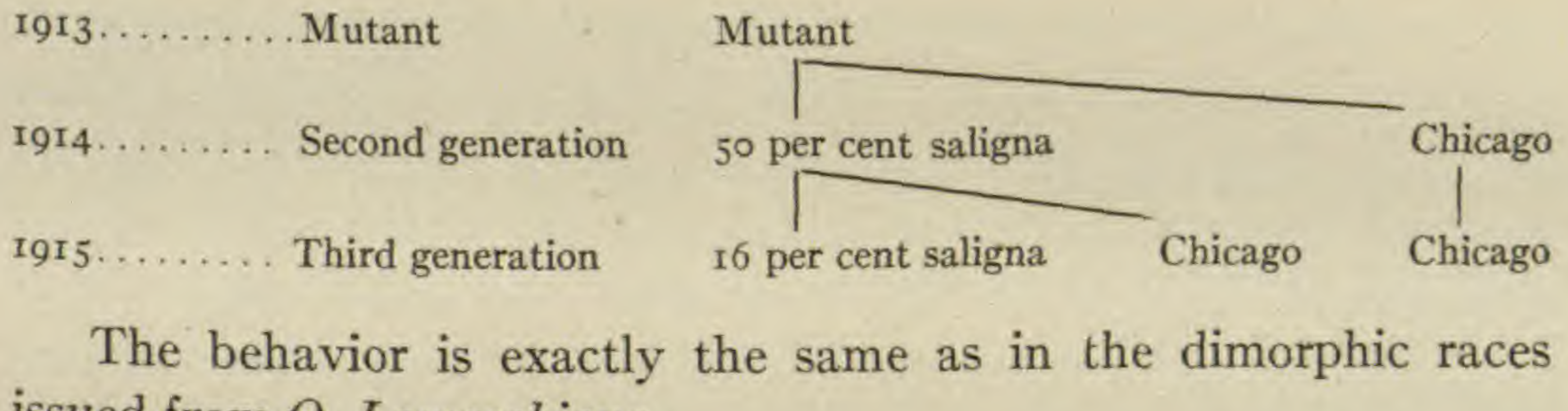
[10] Gruppenweise Artbildung, pp. 34, 52, etc. 1913.

[11] Gruppenweise Artbildung, p. 304. *figs. 110, 111.*

cm. The spikes were densely flowered, the flowers a little smaller, the fruits thin and long. The production of pollen was insufficient in many flowers, but this may have been the effect of the individuals being transplanted from their boxes to the bed in June, which is relatively late in the season. The seed developed badly and contained only a small percentage of normal grains.

I have sown the seeds of 8 of the 9 specimens with the parental type, and of two of the type of *O. biennis Chicago*. The first split into two types, the second only repeated the form of the parent. The splitting percentages were 11, 12, 13, 15, 15, 17, 18, and 25, with a mean of 16 per cent, but the germination had been very poor, giving only 444 seedlings for the 8 boxes. The progeny of the two specimens of the *Chicago* type was uniform with 252 and 60 seedlings. I counted them in May and June, and left one group of each type to flower. The group from the *saligna* type contained two flowering *saligna*, identical with those of the second generation; that of the atavists 60 flowering plants of the uniform type of *O. biennis Chicago*. From these facts we may conclude that in this race all of the specimens of the parental type give a dimorphic progeny, while the offspring of the plants with an atavistic type remains uniformly so. Resuming the cultures, we get the following pedigree:



The behavior is exactly the same as in the dimorphic races issued from *O. Lamarckiana*.

Crosses of *Oenothera Lamarckiana* mut. *cana*.—As indicated on p. 258 I chose in 1913 the second generation of a *cana* mutant which arose from *O. lata* (no. 3) for a series of crosses. This culture was the most vigorous one of all my annual *cana* families, and its percentage figures seemed to be more normal than in the other cases. The crosses were made in both directions with the pure strains of my species and races described in *Gruppenweise Artbildung*, and the seeds were sown in 1914. This first generation was counted in July, during the beginning of the flowering period, but about one-half of some of the cultures were kept in the boxes so as to reach only the stage of rosettes at that time. No second generations have been cultivated. I will first describe the results, and afterwards give the necessary details concerning the several cultures.

The main purpose of these crosses was to decide the question whether the special characters of *O. cana* are handed down by the ovules only, as in *O. scintillans* and *O. lata*, or by the pollen also. Table IX gives the result of the crosses with wild species other than *O. Lamarckiana*. Here a splitting occurs into *laeta* and *velutina* or into *densa* and *laxa*, exactly analogous to that produced by *O. Lamarckiana* itself. The only exception is the pollen of *O. biennis*, which does not split; it gives with *O. cana* the same type as with the parent species. The result was very striking on the beds. No specimens of the *cana* type occurred in the cases where this mutant had been the father, whereas such individuals abounded in the results of the reciprocal crosses.

TABLE IX

CROSSES OF *O. cana* WITH OTHER SPECIES

Cross	Percentage of cana	Percentage of laeta	Percentage of velutina	Percentage of mutants
A				
O. cana×O. biennis Chicago	25	11	63	1
O. cana×O. Cockerelli	17	17	63	3
O. cana×O. Hookeri.......	28	14	58	....
O. Hookeri×O. cana.......	0	4	96	....
O. Cockerelli×O. cana.....	0	54	46	....
O. syrticola×O. cana......	0	51	49	....
O. biennis×O. cana.......	0	58	42	....
B		Percentage of densa	Percentage of laxa	
O. biennis Chicago×O. cana	0	24	75	1
O. atrovirens×O. cana.....	0	36	64	....
C				
O. cana×O. biennis.......	49	51		....

In this table, *O. syrticola* Bartlett is the *O. muricata* L. of my *Gruppenweise Artbildung*, and *O. atrovirens* Bartlett has been described in that book as *O. cruciata* Nutt. The other names are still the same as in my book.

With the same purpose crosses were made with *O. Lamarckiana* and some of its derivatives.

TABLE X

CROSSES OF *O. cana* WITH *O. Lamarckiana* AND ITS MUTANTS

Crosses	Percentage of cana	Percentage of Lamarckiana	Percentage of other forms
O. cana×O. Lamarckiana......	42	58	....
O. cana×O. nanella...........	21	71	8 nanella
O. cana×O. rubrinervis.......	40	25	35 subrobusta
O. Lamarckiana×O. cana	0	98	2 nanella
O. lata×O. cana	1	62	35 lata, 3 mutants
O. nanella×O. cana..........	1	5	94 nanella
O. oblonga×O. cana	0	82	15 oblonga, 3 mutants
O. rubrinervis×O. cana	0	52	48 subrobusta

The main result is the same. *In all the crosses of both tables the characters of O. cana are handed down through the ovules to a large part of the progeny, but not through the pollen.* The behavior is exactly the same as in *O. scintillans* and *O. lata*. The two specimens of *O. cana* from the crosses of *O. lata* and *O. nanella* must evidently be considered as mutants, that is, as having arisen from the fertilization of mutated sexual cells, since we have seen that such mutations occur from time to time, especially among the seeds of *O. lata*.

Apart from the appearance of plants of the *cana* type, the results of the crosses are, in every case, such as would be expected if *O. Lamarckiana* had been used instead of *O. cana*. In this respect they simply confirm the conclusions given in my book.

Let us now consider in its details the analogy of *O. cana* with the allied forms of *O. scintillans* and *O. lata*. Two cases offer themselves for this consideration. The first one is afforded by the crosses with *O. biennis*. Apart from stray mutants, these produce two types, one of which combines the visible marks of both parents, whereas the other wholly lacks the characters of the mother, but is simply like the hybrid of *O. Lamarckiana* and *O. biennis*, as described in my book. In the first group the combination is such as to make the characters of the mother the most striking in the hybrids, whereas those of the father, *O. biennis*, have only a less influence on the general type.

In table XI the figures of table IX are combined with the percentages derived from my *Gruppenweise Artbildung*, by taking the means of the two crosses for each case given on pp. 251 and 261.

TABLE XI

O. cana×*O. biennis* COMPARED WITH *O. lata* AND *O. scintillans*

Forms	Percentage of type of mother	Percentage of type of O. Lamarckiana ×O. biennis	Mutants
O. cana×O. biennis...............	49	51	....
O. scintillans×O. biennis.........	60	36	4
O. lata×O. biennis................	57	43	....
Mean.......................	55	43	....

In this table we see *that the characters of O. cana, even as those of O. scintillans and O. lata, are repeated in about one-half of the progeny, but not in the other half.* We may consider this as the simplest case. In the other crosses the proportions of *cana* are 17, 25, 28, with a mean of 23 per cent in table IX, and 21, 40, 42, mean 34 per cent in table X, and these figures may be assumed to point to a splitting into nearly equal parts with a loss on the side of the weaker form. Exactly the same behavior occurred among the progeny of the self-fertilized individuals of *O. cana*, as we have seen previously.

Let us now compare *O. cana* with the two allied forms in those crosses where the progeny splits into the twin hybrids *O. laeta* and *O. velutina*, as shown in table XII.

The types of *O. laeta* and *O. velutina* have been compared in each case with the twins derived from *O. Lamarckiana* by the same father.[12] The comparison embraced the whole lifetime from the germination in February until the production of the fruits in September. No differences have been observed.

From table XII we see that the splitting percentages are practically the same, whether the pollen is taken from *O. biennis Chicago*, *O. Cockerelli*, or *O. Hookeri*. For this reason I have given

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[12] DEVRIES, HUGO, On twin hybrids. BOT. GAZ. 44:401–407. 1907.

the mean for each group in the last columns of the table, and the reliability of this mean evidently depends strongly on this fact.

TABLE XII

SPLITTING INTO *laeta* AND *velutina*

FORMS	PERCENTAGE OF TYPE OF MOTHER	PERCENTAGE OF LAETA	PERCENTAGE OF VELUTINA	MEANS FOR THE GROUPS		
				Type of mother	laeta	velutina
O. cana×O. b. Chicago	25	11	63	23	14	61
O. cana×O. Cockerelli	17	17	63			
O. cana×O. Hookeri	28	14	58			
O. scintill.×O. b. Chicago	33	32	32	30	36	30
O. scintill.×O. Cockerelli	21	49	29			
O. scintill.×O. Hookeri	35	28	29			
O. lata×O. b. Chicago	21	24	55	27	25	46
O. lata×O. Cockerelli	21	31	48			
O. lata×O. Hookeri	39	18	43			
O. Lamarckiana×O. b. Chicago	....	19	81	......	18	82
O. Lamarckiana×O. Cockerelli	....	11	89			
O. Lamarckiana×O. Hookeri	....	23	77			
O. nanella×O. b. Chicago	....	41	59	......	41	59
O. nanella×O. Cockerelli	....	38	62			
O. nanella×O. Hookeri	....	45	55			

Theoretically a splitting into 4 groups of equal size should be expected, namely into *cana-laeta*, *cana-velutina*, *laeta*, and *velutina*.[13] Evidently one of the first two groups is suppressed. This conclusion holds good for *O. cana* just as for *O. scintillans*, but in the case of *O. lata* the fourth group is sometimes visible, a few specimens of the *lata*-like hybrids assuming at the same time the marks of *O. laeta* (about 1 per cent, *Gruppenweise Artbildung*, p. 255), whereas the remainder are clearly *lata-velutina*. It is probable that the same group is suppressed in both the other cases, and for the same reasons, which are as yet unknown. The fact that there are so often more *velutina* than should be expected probably has the same cause as the exuberant occurrence of this form in the crosses of *O. Lamarckiana* (82 per cent). The analogous crosses of *O. nanella*, given in the lower part of the table, show that these

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[13] DE VRIES, HUGO, On triple hybrids. BOT. GAZ. **47**:1–8. 1909.

diminutions of the *laeta* type may, at least in part, be considered as influenced by neighboring characters. The combination *O. cana nanella* has occurred in many instances during these experiments, either from the crosses of *O. cana* with the dwarfs of my race, or as occasional mutations in other cases. A repetition of my experiments, in which *O. cana nanella* would be chosen for fertilization by the other species or mutants used, would probably give the material to decide these questions.

Putting aside all of these more special considerations, we may conclude that *O. cana behaves in its crosses, as well as after self-fertilization, exactly after the manner of O. scintillans and O. lata.* From this fact and from the cross *Lamarckiana*×*pallescens*, previously mentioned, it seems probable that the other new dimorphic mutants, namely *O. pallescens*, *O. liquida*, and *O. Lactuca*, will follow the same rule if tried in the same way. This rule is evidently independent of the external types of their characters, but must depend upon internal properties of their hereditary qualities.[14]

I will now give briefly the necessary details concerning the crosses mentioned in tables IX and X. As already mentioned, all these crosses were made in 1913 with plants of the same origin. For every cross a single specimen was chosen and some flowers on the lower part of its main spike were castrated. The seeds were sown in February 1914, the seedlings transplanted into boxes, and from these, as a rule, about 25 specimens of each culture were placed in a bed in April and May, giving them a good soil and light exposure and plenty of space to insure a vigorous development until the time of flowering and of fruiting.

O. cana×*O. biennis Chicago.*—A group of 71 specimens, all of which produced high stems and 25 of which have flowered. One was a mutant, combining the gray narrow leaves of *O. cana* with the marks of the stature, foliage, and flowers of *O. lata*. The plants of the type of *O. cana* were exactly like pure *O. cana* at the beginning of the flowering period, in July, when they had reached a height of 70 cm. The remaining plants were like (*O. Lamarckiana*×*O. biennis Chicago*) *laeta* and *velutina*. The reciprocal cross yielded 59 specimens, of which 5 remained in the condition

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[14] On these questions see Gruppenweise Artbildung, pp. 268–295. 1913.

of rosettes. Of the remaining 54, about one-half or 25 have flowered, the others reaching this stage approximately at the time when they were pulled up and counted. One plant was a mutant, being a metaclinous *velutina*, just as described in my book on pp. 308–311. The others were *densa* and *laxa*, as should be expected, and agreeing with these types throughout their whole life.

O. cana×*O. Cockerelli.*—A culture of 63 specimens embracing 4 *cana*, 5 *laeta*, and 15 *velutina*, which have flowered, and a large number of rosettes of radical leaves. Two plants were mutants of the type of *O. lata* and one of them has flowered. Neither in the rosette stage nor at the time of flowering have the plants of the *cana* type showed any difference from ordinary *O. cana*, the characters of the father, also of its twin hybrid type, being invisible in them. Such was the case in almost all the beds containing the hybrids whose mother was *cana*, and this made the distinguishing and counting of this type quite easy and sharply defined, and therefore fully reliable. Short narrow leaves of a gray color, a slender spike with long, thin flower buds with nodding tips were everywhere the same distinguishing marks. The *laeta* and *velutina* had the ordinary type of these twins, as produced by *O. Cockerelli*.

The reciprocal cross yielded 19 annual and 13 biennial *laeta*, besides 4 annual and 23 biennial *velutina* of the same type. The annual plants have flowered; the biennials became stout rosettes in July and August.

O. cana×*O. Hookeri.*—Represented by 25 flowering plants, 3 younger ones, and 40 rosettes, and among the flowering individuals 5 *cana*, 5 *laeta*, and 4 *velutina*. The *cana* were like those of the pure type; the *laeta* and *velutina* did not differ from those of the cross *O. Lamarckiana*×*O. Hookeri*, some of the *velutina* being of a yellowish green in such a degree as not to be able to produce a stem. The reciprocal cross yielded only two *laeta*, one of which has flowered, among a culture of 60 specimens. The remainder were *velutina*, 24 flowering plants and 34 rosettes of radical leaves. The types were the same as those in the reciprocal cross.

O. syrticola×*O. cana.*—Represented by 60 specimens of the type of (*O. syrticola*×*Lamarckiana*) *laeta* and *velutina*. Of these 19 *laeta* and 6 *velutina* have flowered, reaching a height of 2 m. in

August. The remaining plants were pulled out as large rosettes in July.

O. atrovirens×*O. cana.*—Represented by 55 plants, among which 8 *densa* and 17 *laxa* have flowered. They were in all respects like the twins of the corresponding cross of *O. Lamarckiana.* Just as in this cross, some specimens had cordate petals and others had linear ones, repeating the *cruciata* type. But on the first plants stray flowers with narrower petals were found, from time to time, indicating a high degree of fluctuability rather than a splitting into two constant and uniform types.

The reciprocal cross yielded only 23 seedlings, 20 of which were yellow and died very early, and the 3 remaining ones were very weak, reaching only a height of 40–60 cm. when they flowered. They had the type of the *gracilis* of the corresponding cross of *O. Lamarckiana.* They were not mentioned in table IX.

O. cana×*O. biennis* L.—Among 70 plants of this culture, one-half had the type of *O. Lamarckiana*×*biennis*, and of these 15 have flowered. The other half were evidently *cana.* Of these, 28 had the stature of typical *O. cana*, but with some marks which indicated an influence of the father. The foliage was less gray, a darker green, with broader bracts, and more dense spikes with smaller flowers than in the other crosses. The stigmas were surrounded by the anthers, insuring natural self-fertilization, as in *O. biennis.* Besides these intermediate types there were 7 dwarfs, which had the gray, narrow, and pointed foliage of *O. cana*, and which in the table have been calculated together with the high specimens of the *cana* type.

The reciprocal cross yielded only *laeta* and *velutina*, together 57 plants, of which about one-half of each type have flowered.

O. cana×*O. Lamarckiana.*—The two types of this culture were exactly the same as in the self-fertilized offspring of the mutant. There were only 19 specimens, of which 6 were *cana*, 11 *Lamarckiana*, and 2 *nanella.* The dwarfs combined the marks of *cana* with those of *nanella* and have been calculated in the table with the *cana* specimens of tall stature.

The same cross had been made in 1907, the seeds being sown in 1913. In this case there were 50 offspring, among which 26 were *cana* and 23 were *Lamarckiana*, while one dwarf occurred. The percentage figures, 52 *cana* and 46 *Lamarckiana*, confirm those of table X.

The reciprocal cross yielded only normal *Lamarckiana*, 60 specimens with one dwarf. Of these 23 have flowered.

O. lata×*O. cana.*—In this case the differences were already very clear at the beginning of June. There were 34 *lata*, 61 *Lamarckiana*, and 3 mutants (one each of *cana*, *nanella*, and *oblonga*). In August 7 *lata* and 9 *Lamarckiana* flowered. They repeated the type of the hybrids of *O. lata* with the parent species.

O. cana×*O. nanella.*—Only 24 seeds germinated. These produced 5 *cana*, 17 *Lamarckiana*, and 2 dwarfs which had the characteristics of *O. cana*. All these plants have flowered.

The reciprocal cross gave 111 plants, of which one was a *cana* of normal stature, 105 were *cana* with the dwarfish stature of *O. nanella*, and 5 had the type of *O. Lamarckiana*. Two of this last type, the normal specimen of *cana*, and 22 dwarfs flowered in August.

O. oblonga×*O. cana.*—Already in June the differences were clear and unmistakable. Among 72 plants 59 were of the *Lamarckiana* type, 11 *oblonga*, 1 *albida*, and 1 *rubrinervis*. Of these, 2 *oblonga* and 12 *Lamarckiana* flowered; most of the others lived through the summer in the condition of large rosettes of radical leaves.

O. cana×*O. rubrinervis.*—Only 20 seeds germinated, and yielded 8 *cana* of the normal type, 5 *Lamarckiana*, and 7 *subrobusta*, all of which have flowered.

The reciprocal cross yielded 59 plants, but in only two types, which were the same as those in the first instance. Of these, 17 *Lamarckiana* and 8 *subrobusta* have flowered.

Summary

1. Besides *O. scintillans*, which splits under ordinary circumstances in every generation into nearly equal groups of plants of the same type and others of the type of *O. Lamarckiana*, I have cultivated pedigree families of 4 other mutants of *O. Lamarckiana* which behave in the same manner. They have been designated

as *O. cana. O. pallescens*, *O. Lactuca*, and *O. liquida*. Their *Lamarckiana*-like offspring are constant in their progeny. Besides the two main types, they produce, as a rule, a relatively high percentage of other mutants.

2. The specimens of the parental type are on the average produced in about 40 per cent, the other 60 per cent being *Lamarckiana* with some mutants; but these figures vary with the cultures and with the plants according to their individual strength. They may even increase, on very strong biennials, to 93–97 per cent for the parental type.

3. Dimorphic mutants of this type occur also in allied species of the *biennis* group, as has been discovered by BARTLETT in the case of *O. stenomeres* mut. *lasiopetala* and described in this article for *O. biennis Chicago* mut. *saligna*.

4. In the crosses with older species or with *O. Lamarckiana* and its derivatives, *O. cana* follows exactly the type of the analogous crosses of *O. scintillans* and *O. lata*.

5. In the dimorphic mutants, the special characters are handed down to the next generation through the ovules only. The pollen lacks these characters, and is, so far as investigated, not different from that of pure *O. Lamarckiana*.

6. The dimorphic mutants constitute a group in which the hereditary phenomena are evidently independent of the externally visible characters of the special members of the group, but must be assumed to have the same intrinsic causes in the different cases.

BOTANIC GARDEN
AMSTERDAM

GINKGO AND THE MICROSPORANGIAL MECHANISMS OF THE SEED PLANTS*

EDWARD C. JEFFREY AND R. E. TORREY

(WITH PLATES VII–IX)

The genus *Ginkgo* has long excited interest as the sole survivor of a group which was formerly numerously represented by species and widely distributed geographically. It is the purpose of the present article to show that its microsporangial structures have an important significance from the standpoint of the interpretation of microsporangia of seed plants in general. It will further be indicated in the present investigation that the affinities of the Ginkgoales are not with the Cycadales, as is ordinarily assumed, but with the abietineous subtribe of conifers, which one of us, in numerous articles devoted to the anatomical structure of living and fossil conifers, has attempted to show are the most primitive living representatives of the coniferous stock. Two important memoirs dealing with the genus under discussion have appeared in recent years. SEWARD and GOWAN[1] have given an interesting account of the living *Ginkgo*, together with statements in regard to its extinct predecessors and its botanical affinities. More recently SPRECHER[2] has published an admirable description of the anatomical structures of the various vegetative and reproductive organs in the genus.

The microsporophylls of *Ginkgo* are paired structures opening by means of a stomium along their inner faces. The opening mechanism is a jacket of cells, generally nucleated, with fibrous thickenings extending from the cavity of the mature sporangium to the epidermis. These thickenings are a structural feature of the mechanical layer, and are quite absent in the epidermal layer itself.

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

[1] SEWARD, A. C., and GOWAN, J., The maiden hair tree (*Ginkgo biloba* L.). Ann. Botany 14:109–154. *pls. 8–10.* 1900.

[2] SPRECHER, ANDREAS, Le *Ginkgo biloba* L. 8vo. pp. 208. *figs. 205.* Geneva. 1907.

The general distribution and topography of the mechanical tissues of the sporangium may be learned from the somewhat diagrammatic illustration appearing as fig. 17. This figure will be discussed more fully at a later stage. The anatomical details of organization of the microsporangium of *Ginkgo*, as illustrated by photomicrographs, may conveniently be taken up here. Fig. 1 shows a vertical section through one of the microsporangia. At the lower side are spores lying within the sporangial cavity. Transversely in the upper region of the figure runs the termination of one of the fibrovascular bundles of the sporophyll. The bundle is accompanied on its upper side by short transfusion cells, such as are characteristic of the gymnosperms. These appear as reticulated elements. To the right and separated from the transfusion tissue by two elongated unsculptured cells is the bundle proper, with its protoxylem elements uppermost and the larger elements of the metaxylem beneath. Toward the left the bundle as a whole ends in transfusion elements, a situation often present in the gymnosperms. The transfusion cells commonly become imperceptibly merged into the fibrously thickened cells which constitute the mechanical tissues of the sporangium wall. Fig. 2 presents another and less favorable view of the same features.

Fig. 3 reproduces a horizontal section through the petiole of the sporophyll and the two sporangia. Right and left lie groups of spores indicating the position of the spore sacs, while in the center is a fan-shaped mass of tracheids marking the termination of the fibrovascular strands of the sporophyll in a complex of transfusion tracheids. To the right and left of the fan lie cells with elongated nuclei, which mark the position of the two strands of phloem ending in the bases of the sporangial cavities. Fig. 4 reproduces a portion of the right of fig. 3, more highly magnified. The gradual passage of the transfusion elements into the cells which constitute the mechanical system of the sporangium wall can now easily be distinguished. To the extreme right are spores in the cavity of the sporangium, and below these, to the right of the transfusion cells, are to be seen two elongated nuclei belonging to the phloem. Returning now to the radial longitudinal view of the vascular supply of the sporangium, in fig. 5 we see under a somewhat high degree of magnification transfusion elements above tracheids, which are in turn in intimate contact below with the fibrously thickened mechanical elements of the sporangium. Fig. 6 shows still another view of the tracheids, transfusion and mechanical elements demonstrating their continuity with one another at the termination of the bundle. Below lies a group of spores. Fig. 7 further illustrates, under a considerable degree of magnification, the almost imperceptible transition from the tracheary and transfusion elements of the fibrovascular bundle proper to the reticulated cells which constitute the opening mechanism of the sporangium. To the right in the median horizontal line lie two narrow tracheids. Above and also to the left of these are to be seen transfusion cells with reticulated walls of the type characteristic of *Ginkgo* and certain Abietineae. Inferiorly the tracheids abut immediately upon the reticulated elements of the mechanical or fibrous layer.

Fig. 8 represents the vascular system of the microsporophyll in transverse section. Sporangia lie to the right and left with contained spores. The two vascular strands which are present in the terminal portion of the sporophyll of *Ginkgo* end as such at the base of the sporangia. Directly above the sporangial cavities on either hand are masses of small cells, which constitute the phloem of the vascular bundles. The position of the xylem is less apparent and is marked by crosses lying inside the islands of phloem. As the vicinity of the sporangia is reached the fibrovascular strands turn outward, so that the phloem now lies external to the xylem instead of below it, as in the lower region of the sporophyll. In the median region a large amount of mechanical tissue is present, separating the sporangia and continuous with the ends and sides of the xylem of the strands. Fig. 9 represents a more highly magnified and somewhat oblique view of the situation illustrated in fig. 8. To the right may be seen the small elements of the protoxylem of the bundle of the dextral sporangium. On the left the sinstral strand of xylem is obliquely cut, showing pits and reticulations. Farther to the left it passes imperceptibly into the mechanical elements of the wall of the sporangium.

We may now turn with advantage to the diagrammatic fig. 17. Here the fibrovascular tissues are shown as penetrating the stalk of the sporophyll. Dorsal to the strand lie transfusion elements distinguishable by their short length and reticulated walls. The tracheids of the bundles in their upward course become ever shorter, and the spiral or scalariform sculpture of the walls is gradually transformed into the reticulations which characterize the transfusion elements in which the bundle ends. The transfusion cells in turn pass by gradual transition into the mechanical elements, which are responsible for the opening of the sporangium at the time of maturity of the spores. It will be seen that the mechanical cells are not confined to the region of the sporangium wall, but pass up into the median horn, which terminates the sporophyll. In some cases the former actually more or less completely surround the secretory space at the apex. In the mature sporangium the mechanical elements extend to the sporangial cavity, being ordinarily separated from it only by a membrane formed from the disorganized tapetum. The mechanical cells form a continuous jacket, and are only interrupted in the longitudinal inner line corresponding to the stomium (not shown). The figure clearly shows that the mechanical elements are not present in the epidermis. The diagrammatic plane of section of the sporangium and sporophyll is necessarily somewhat to one side of the median line of the termination of the corresponding fibrovascular strand, since the latter continues into and is merged in transfusion elements and mechanical cells in the region of the septum between the two spore sacs. For that reason the upward end of the strand does not appear in the field of view. The explanation of the situation is furnished by reference to the horizontal section shown in fig. 3.

It is clear from the descriptions given in the foregoing paragraphs that the fibrovascular bundles of the sporophylls in *Ginkgo* end in transfusion tissue, which in turn passes, often almost imperceptibly (fig. 4), into the mechanical elements of the sporangial wall. It thus is obvious that in this genus the opening mechanism of the sporangium is an adjunct if not a derivative of the fibrovascular system. This situation recalls the interesting observations made by OLIVER[3] over a decade ago in the case of an isolated

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[3] OLIVER, F. W., On a vascular sporangium from the Stephanian of Grand' Croix. New Phytol. 1:60–67. *pl. 1.* 1902.

sporangium from the Stephanian of Grand' Croix in France. He notes that in the sporangium in question, which he is inclined to refer to the Botryopterideae, there are 6 islands of tracheary tissue lying in immediate contact with the cavity of the sporangium. A true annulus formed from the outer layer of cells likewise is present, and consequently there can be no case of vascular tissue acting as the mechanism for opening the sporangial cavity at maturity. The author notes the rarity of the phenomenon in vascular plants, where as a rule the tracheary elements stop at an interval from the actual sporogenous elements. He calls attention to the similar phenomenon presented by the tracheids found in relation to the embryo sac in the amentiferous genera *Casuarina*, *Castanea*, and *Corylus*. In a later article[4] OLIVER describes the tracheary investment covering the gametophytes and terminating in the pollen chamber of *Stephanospermum akenoides* and *S. caryoides*. He regards the apparatus in this case as destined to supply the pollen chamber (a constant feature of organization of more ancient gymnospermous seeds) with the water necessary to provide for the fertilizing movements of the antherozoids possessed by these extinct types in common with their nearest modern survivors *Ginkgo* and the Cycadales. In the living forms, however, the tracheary device for supplying fluid to the pollen chamber has disappeared. It is of interest to note in the present connection that in the megasporangium (or seed) of *Ginkgo* the transfusion tissue is present in the peduncle of the organ just as it is in the stalk of the microsporangium, but that it dies out sharply as the megasporangium proper is reached. It is present in abundance in the collar, which lies against the base of the seed. The sporangium in *Ginkgo* would seem, in view of the present observations, to be more tenacious of ancestral characters than is the megasporangium.

It is now desirable to consider certain general features of organization of the wall in the sporangia or microsporangia of the lower vascular plants. Fig. 13 illustrates the structure of a sporangium of *Polypodium vulgare*. Here the opening mechanism is provided

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[4] OLIVER, F. W., On the structure and affinities of *Stephanospermum* Brongniart, a genus of fossil gymnospermous seeds. Trans. Linn. Soc. London Bot. II. **6**:361–400. *pls. 41–44.* 1904.

by the annulus, a derivative of the epidermal tissue. A similar situation is presented by the sporangia of other vascular cryptogams. Fig. 14 reproduces the situation for a species of *Selaginella*, one of the Lycopsida. In fig. 15 a microsporangium of *Zamia muricata* is represented. The spore sac is opened by an external annulus, precisely as in the ferns and lycopods. The epidermal character of the opening mechanism is clearly vouched for by the presence of stomata. These are visible on the upper and lower sides of the figure. Noteworthy in this case is the fact that the tracheids of the vascular bundle do not enter into the wall of the sporangium proper, but stop short at its base. Fig. 16 illustrates a surface view of the spore sac much more highly magnified, making clear the position and organization of the stomata. If we compare the condition presented by the sporangia of cycads and vascular cryptogams with that found in *Ginkgo*, a remarkable contrast in the character of the opening mechanism is found. In the lower forms the apparatus for the dehiscence of the spore sacs is known as an annulus and is derived from modified epidermal cells. In the case of *Ginkgo*, on the other hand, no annulus is present, and the opening of the sporangium is provided for by an internal mechanism, obviously an adjunct of the fibrovascular and transfusion tissues, commonly known as the mechanical layer.

It is now convenient to consider the situation in the seed-bearing forms which lie above *Ginkgo* in the scale. The first group to be discussed in this connection is the Coniferales. One of us has pointed out in numerous recent communications that the Abietineae have the strongest claim to a primitive position among the coniferous subtribes. This view is as yet not accepted by the greater number of paleobotanists, but seems to be based on evidence which in the long run is destined to prevail. A most interesting confirmation of the hypothesis of the primitive character of the Abietineae is provided by their microsporangial resemblances to *Ginkgo*. In the first place, the organization of the male cone of *Ginkgo* is similar to that of the Abietineae, since it is composed of sporophylls each bearing two sporangia. One of us has pointed out[5] that the pollen grains of *Ginkgo* possess wings comparable to

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

those of the Abietineae, and having the same relations to the similar internal structures as do those in *Pinus* or *Abies*. The possession of short shoots and the opposite pitting of the secondary wood, bars of Sanio, etc., as well as a number of other characteristics, likewise definitely indicate a closer degree of relationship between the Abietineae and the Ginkgoales than exists between the latter and any other gymnospermous group. It is accordingly of great interest and importance in the present connection to discover that in so many particulars there is the same striking agreement in the organization of the mechanical apparatus of the microsporangium in the Abietineae and the sole surviving genus *Ginkgo*. One of us has pointed out[6] that on cogent grounds the Abietineae may be divided into two subsidiary groups, the Abieteae and the Pineae. The former are without resin canals in the secondary wood, while the latter are provided with ligneous ducts and have an organization of root, leaf, and cone in contrast to that of similar structures in the Abieteae. A further feature of distinction between the Abieteae and the Pineae is the thick wall of the sporangium in the former. The greater thickness of the wall of the microsporangium of the Abieteae makes it more favorable for study than that of the Pineae. Fig. 12 illustrates the organization of the wall of the sporangium in the genus *Pseudolarix*, which, on account of the absence of tanniniferous contents in the cells of the sporangium wall, is the most suitable for illustration. It is clear that the mechanical tissues in this case, as in *Ginkgo*, extend to the cavity of the sporangium. The outward limit of the opening mechanism in this instance is the exterior of the spore sac and not, as in *Ginkgo*, the layer immediately under the epidermis. The same general situation obtains in all the conifers, apparently, for throughout the group the epidermal cells have the same organization as the mechanical elements. Fig. 10 reproduces a part of a vertical section of a sporangium of *Pseudolarix*. On the left of the figure lie certain cells which have reticulated walls. These are the transfusion elements, and further in toward the axis they are joined with the tracheids of the single fibrovascular bundle of the

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[6] JEFFREY, E. C., The comparative anatomy of the Abietineae. Mem. Boston Soc. Nat. Hist. 6:1–37. *pls. 1–7*. 1904.

sporophyll. Toward the right and below in the figure the typical transfusion cells pass directly into elements with a more open reticulation of the wall, the mechanical cells, which constitute the opening mechanism of the sporangium. The jacket of active cells is complete on the free surfaces of the sporangium except where the stomium is present in a terminal position. The septum between the two spore sacs is very largely composed of the fiber cells and the transfusion elements with which they are joined. Fig. 11 reproduces part of a somewhat oblique vertical section of a sporophyll. Here both the sporangia appear at once; toward the right and between them lies the separating partition containing the fibrovascular strand and its associated transfusion elements. Toward the left the transfusion cells can be distinguished, passing directly into elements with much more openly reticulated walls, the mechanical cells. Fig. 18 is a somewhat diagrammatic representation of the anatomical situation in the sporangium of *Pseudolarix*. The tracheary strand comes into the sporophyll and passes outward, giving off a shorter or longer dorsal branch. Abaxially it spreads out into a mass of transfusion elements which are connected with the more coarsely reticulated cells, constituting the mechanical sheath of the microsporangium. Nearly opposite the point of contact of the vascular bundle with the upper wall of the sporangium lies the stomium. The sporophyll ends in a sharp upwardly directed apex, comparable to the less well developed similar structure in *Ginkgo*.

In the Pineae the thin wall of the pollen sac has brought with it such a degree of degeneracy that the topographical relations, seen clearly in the Abietineae, can no longer be discerned. The epidermal layer of the microsporangium is apparently in all cases fibrously thickened, and in this respect differs from the annulus found in the remaining Pteropsida. In the araucarian conifers, which are by many regarded as a primitive subtribe of the Coniferales and more nearly related to the Cordaitales than any other subtribe, significantly enough, the mechanical layer is reduced to the epidermis and has no connection whatever with the fibrovascular system. The same statement is true of the remaining subtribes of the Coniferales other than the Abietineae, which in respect to their sporangial mechanisms, as in so many other features, show themselves more nearly related to the Ginkgoales and consequently to the Cordaitales. In the Gnetales the opening mechanisms of the sporangia likewise are much simplified and in this respect correspond to the higher subtribes of the Coniferales.

In the highest of the seed-bearing plants, the angiosperms, the microsporangium characteristically opens through the agency of an internal fibrous layer, which has been elaborately described in the monumental memoir of CHATIN on the anatomy of the anther. In both the dicotyledons and monocotyledons this layer is, typically at least, well developed, but does not, as in the conifers, extend into the epidermis. Fig. 19 shows the organization of one of the loculaments of the anther in the common garden tulip (*Tulipa Gesneriana*, hybrid?). Toward the top of the figure lies the stomium, meeting with a corresponding structure belonging to the other loculament of the anther on the same side of the connective. The remainder of the wall shows the mechanical layer strongly developed. In the epidermis stomata can be distinguished. Clearly there is no relation between the mechanical layer and the fibrovascular layer in the connective.

Turning our attention now to the dicotyledons, in fig. 20 we find a diagrammatic representation of a loculament of the anther of *Diervilla florida*, the bush honeysuckle. The stomium does not appear in the figure, but the mechanical or fibrous layer of the anther is clearly to be seen, and, like that in the tulip, it does not extend into the epidermis. In this case, as in the other angiospermous sporangium figured, there is no connection between the mechanical tissues and the fibrovascular bundle of the microsporophyll. On account of the unique development of transfusion tissue of a gymnospermous character in the genus *Casuarina*, it seemed possible that there might be here, if anywhere, some connection between the mechanical tissues of the microsporangium and the fibrovascular bundles. Investigation of the stamens of two species of the genus, however, revealed the same general situation as is found in other instances among the angiosperms; namely, the absence of a relation of continuity between the reticulated cells of the sporangial mechanism and the fibrovascular strand. It is to be noted that the megasporangial tracheids described by TREUB and BENSON in *Casuarina*, *Castanea*, *Corylus*, etc., are equally without any relation of continuity with the bundle system of the ovule. In general, in the angiosperms the opening of the cavities of the anther takes place by means of a mechanical device, which is not of epidermal origin, and consequently cannot be regarded as an annulus. The structure in question has clearly originated in the deeper tissues of the sporangial wall, and is in all probability derived from the fibrovascular system, as are similar devices in *Ginkgo* and the Abietineae. In the highest seed plants all relation between the opening mechanisms of the anther and the fibrovascular structures have apparently long disappeared.

It is obvious from the discussion of the pertusing devices of sporangia in the preceding pages that there are two main types of opening mechanisms; namely, the annulus, which is clearly of epidermal origin (in the Cycadales actually containing stomata), and, secondly, the fiber layer, occurring in a more or less complete condition from the Ginkgoales upward. This seems equally clearly to have been derived from the fibrovascular structures. Those sporangia which are opened by the instrumentality of an annulus may be appropriately designated *ectokinetic*, while those which owe their dehiscence to the stresses originated by an internal mechanical system, derived from the fibrovascular tissues, may with equal fitness be termed *endokinetic*. If these distinctions be well founded, they obviously supply us with a valuable additional criterion for the course of evolution in the higher plants. The application of these criteria makes it apparently clear that the angiosperms cannot have been derived from the Cycadophyta, a phylogeny which, moreover, meets many other anatomical difficulties and cannot even be easily reconciled with a reasonable interpretation of the external features of the reproductive parts of the bennettitalean Cycadales and the ranalian angiosperms.

Summary

1. In *Ginkgo* the opening mechanism is clearly an adjunct of the transfusion tissue of the fibrovascular system and is directly continuous with this.

2. A similar condition of relationship between the tracheary tissues and the dehiscing mechanism of the microsporangium is likewise found in the Abietineae among the conifers.

3. In the remaining conifers (and including the araucarians) the sporangial mechanisms are much reduced and no longer have a relation to the vascular bundles of the sporophyll.

4. In the angiosperms the fiber layer of the anther wall is usually well developed and is of internal origin, but has no connection with the fibrovascular system, even in the forms regarded as low.

5. From the Cycadales downward the opening mechanism of the sporangium is of the nature of an annulus and is epidermal in its origin.

6. The facts summarized under the foregoing headings justify the separation of reproductive mechanisms in the vascular plants into two types, the *ectokinetic* and *endokinetic*. The former condition is represented by the annulus and is characteristic of lower forms; the latter is found in the fiber layer, derived from the fibrovascular tissues (particularly modified transfusion elements), and is present characteristically in the microsporangia of existing seed plants (exclusive of the Cycadales).

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EXPLANATION OF PLATES VII–IX

PLATE VII

FIG. 1.—Vertical section through upper part of sporophyll of *Ginkgo biloba*, showing relation of mechanical elements of wall of sporangium to transfusion cells and fibrovascular bundle; ×100.

FIG. 2.—Vertical section of another sporangium showing the same features; ×150.

FIG. 3.—Horizontal section showing relation of two fibrovascular bundles to transfusion tissue and mechanical elements; ×100.

FIG. 4.—Part of the right of fig. 3 more highly magnified, showing merging of transfusion elements into mechanical elements; ×200.

FIG. 5.—Vertical section of sporangium of *Ginkgo*, showing termination of bundle in transfusion elements and mechanical cells; ×150.

FIG. 6.—Vertical section of sporangium of *Ginkgo*, showing termination of bundle in transfusion elements and mechanical cells; ×250.

PLATE VIII

FIG. 7.—Vertical section of sporangium of *Ginkgo*, showing termination of bundle in transfusion cells and mechanical elements; ×250.

FIG. 8.—Transverse section of sporophyll of *Ginkgo*, showing 2 fibrovascular bundles; ×150.

FIG. 9.—Oblique section of sporophyll of *Ginkgo*, showing on the left transition of bundle into mechanical cells; ×200.

FIG. 10.—Vertical section of sporangium of *Pseudolarix*, showing transition of bundle into transfusion tissue and mechanical cells; ×200.

FIG. 11.—Vertical section of sporophyll of *Pseudolarix*, showing termination of transfusion tissue in mechanical elements of the walls of the sporangium; ×200.

FIG. 12.—Section of sporangium of *Pseudolarix*, showing wall consisting of mechanical cells; ×200.

PLATE IX

FIG. 13.—Sporangium of *Polypodium vulgare* as illustration of sporangial mechanism in a typical fern.

FIG. 14.—Diagrammatic representation of opening mechanism of sporangium of *Selaginella*.

FIG. 15.—Semi-diagrammatic view of sporangium in *Zamia*.

FIG. 16.—Surface view of sporangium in *Zamia* showing stomata enlarged.

FIG. 17.—Semi-diagrammatic view of one of the sporangia of *Ginkgo*, showing relation of fibrovascular tissues to opening mechanism of the sporangium.

FIG. 18.—Semi-diagrammatic illustration of organization of the sporangium in *Pseudolarix*, showing relation of fibrovascular tissues to opening mechanism of sporangium.

FIG. 19.—Transverse section of sporangium of tulip, showing mechanical cells.

FIG. 20.—Transverse section of sporangium of *Diervilla*, showing sporangial mechanism.

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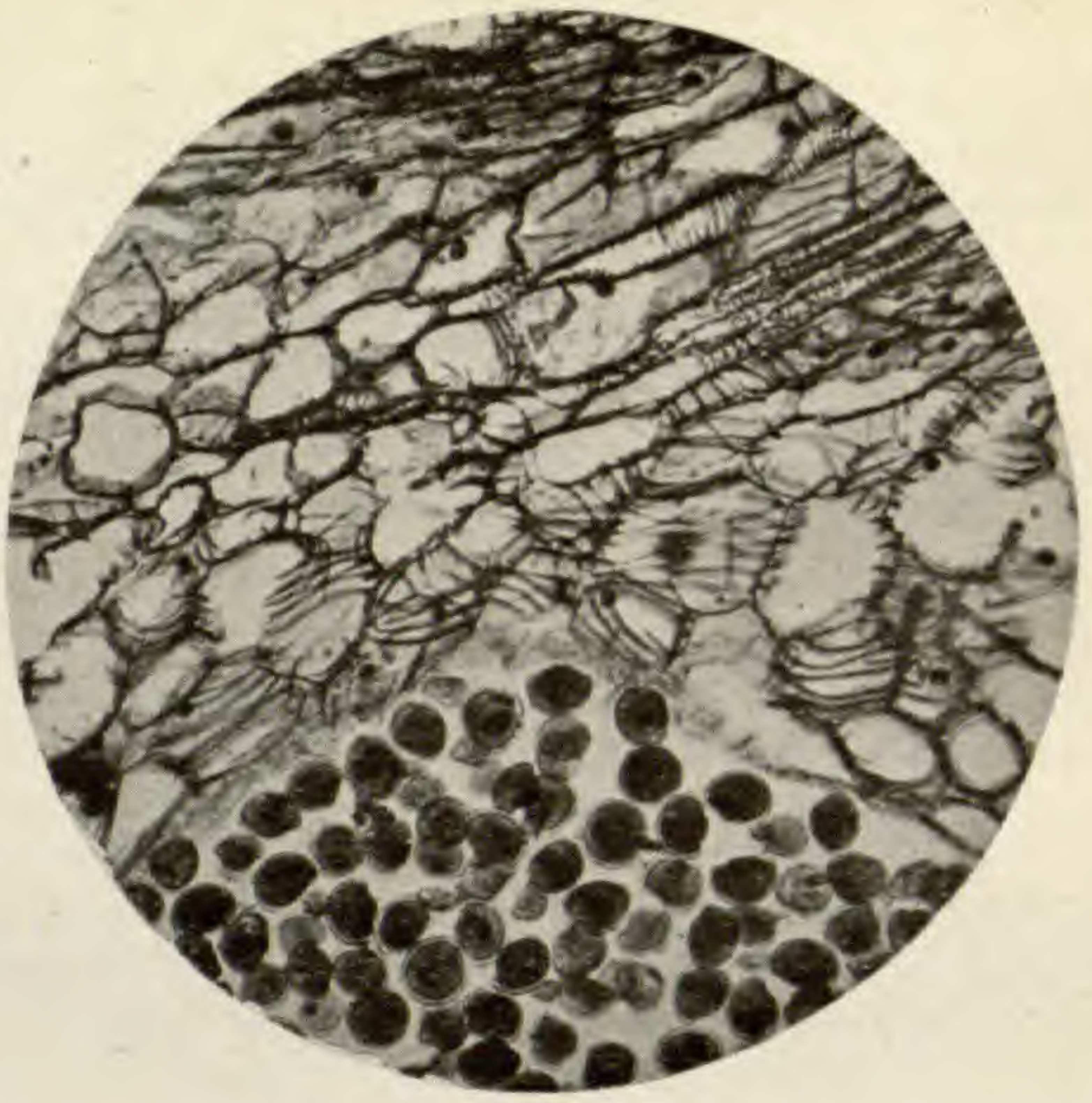

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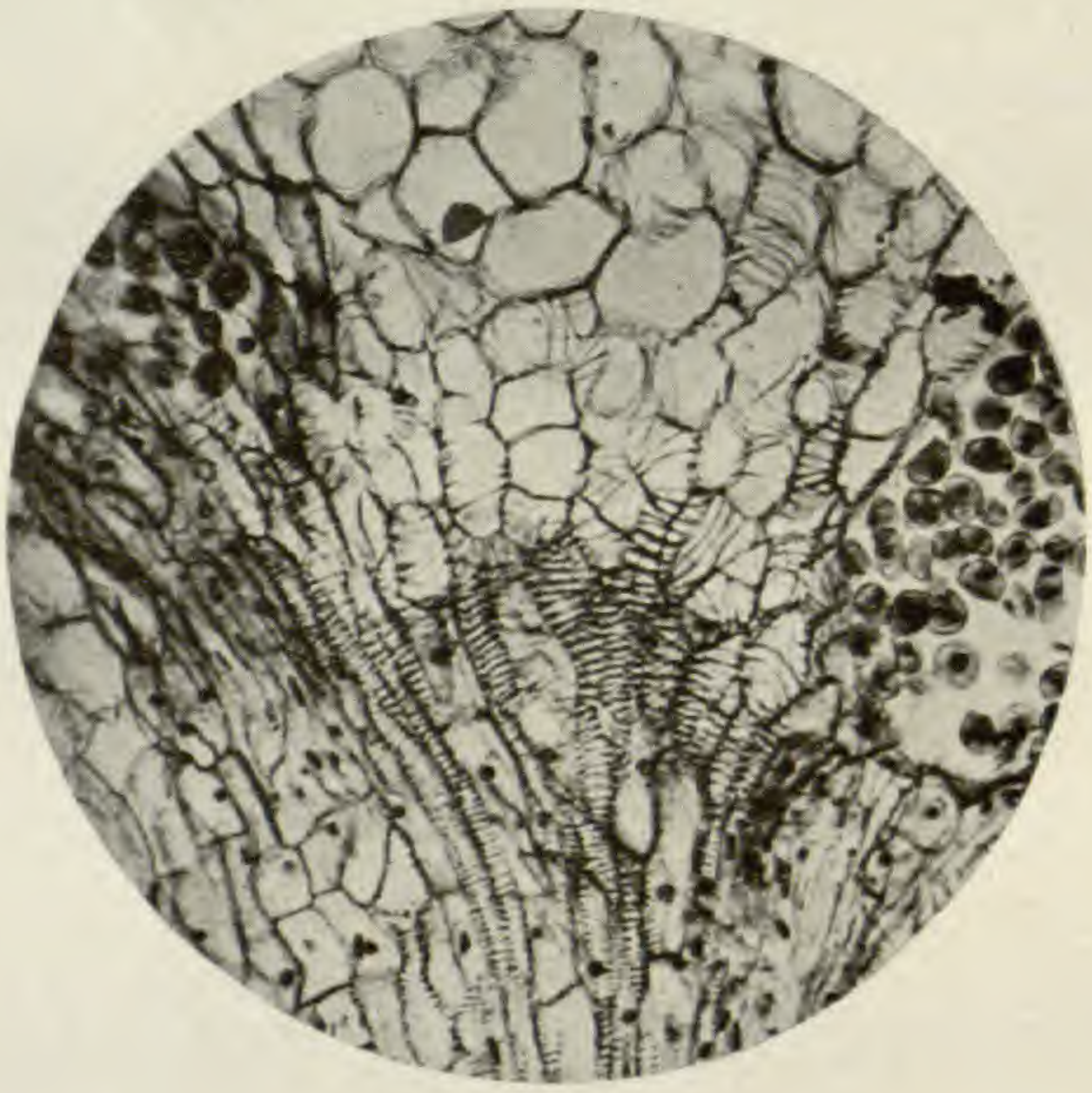

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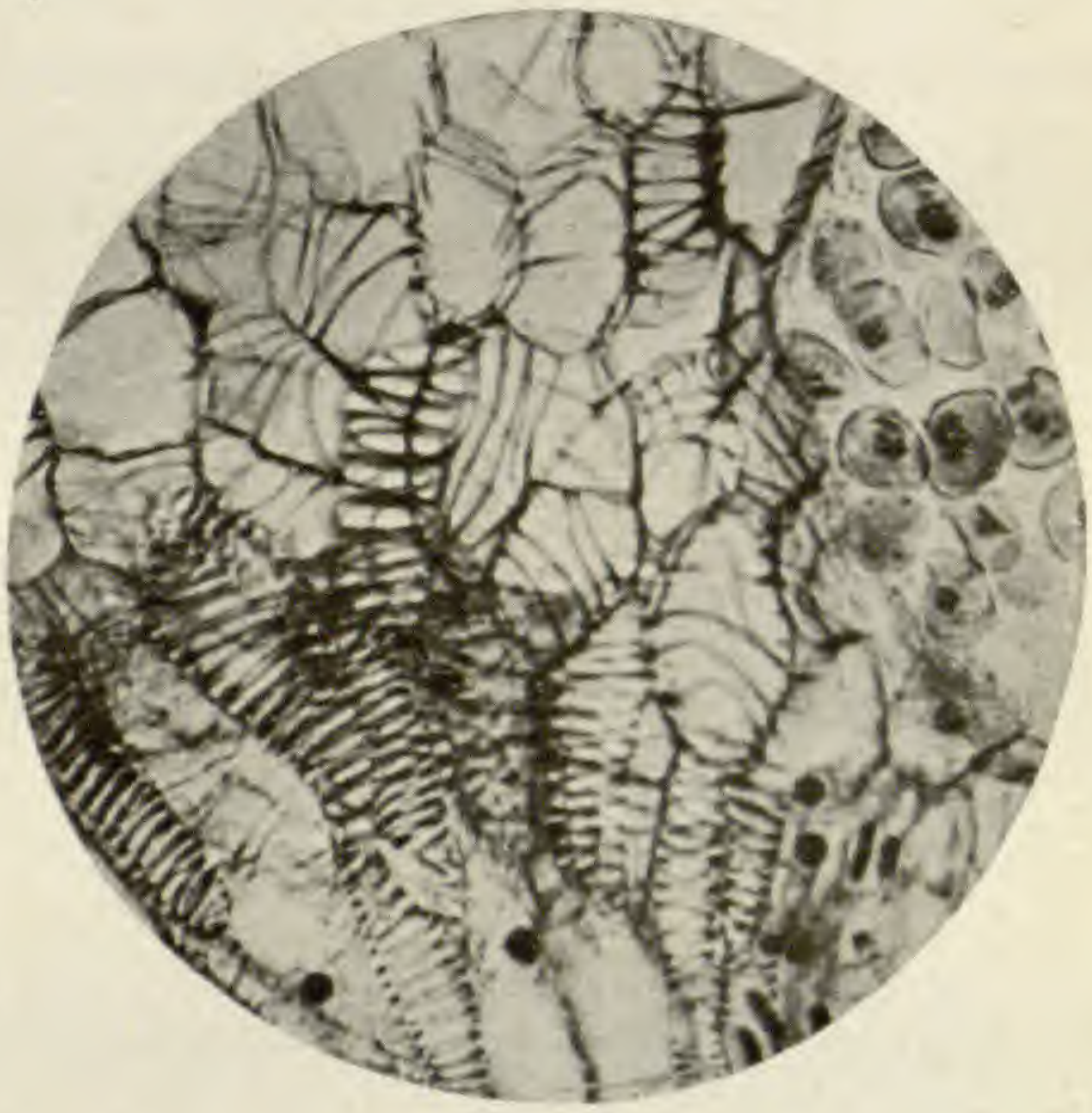

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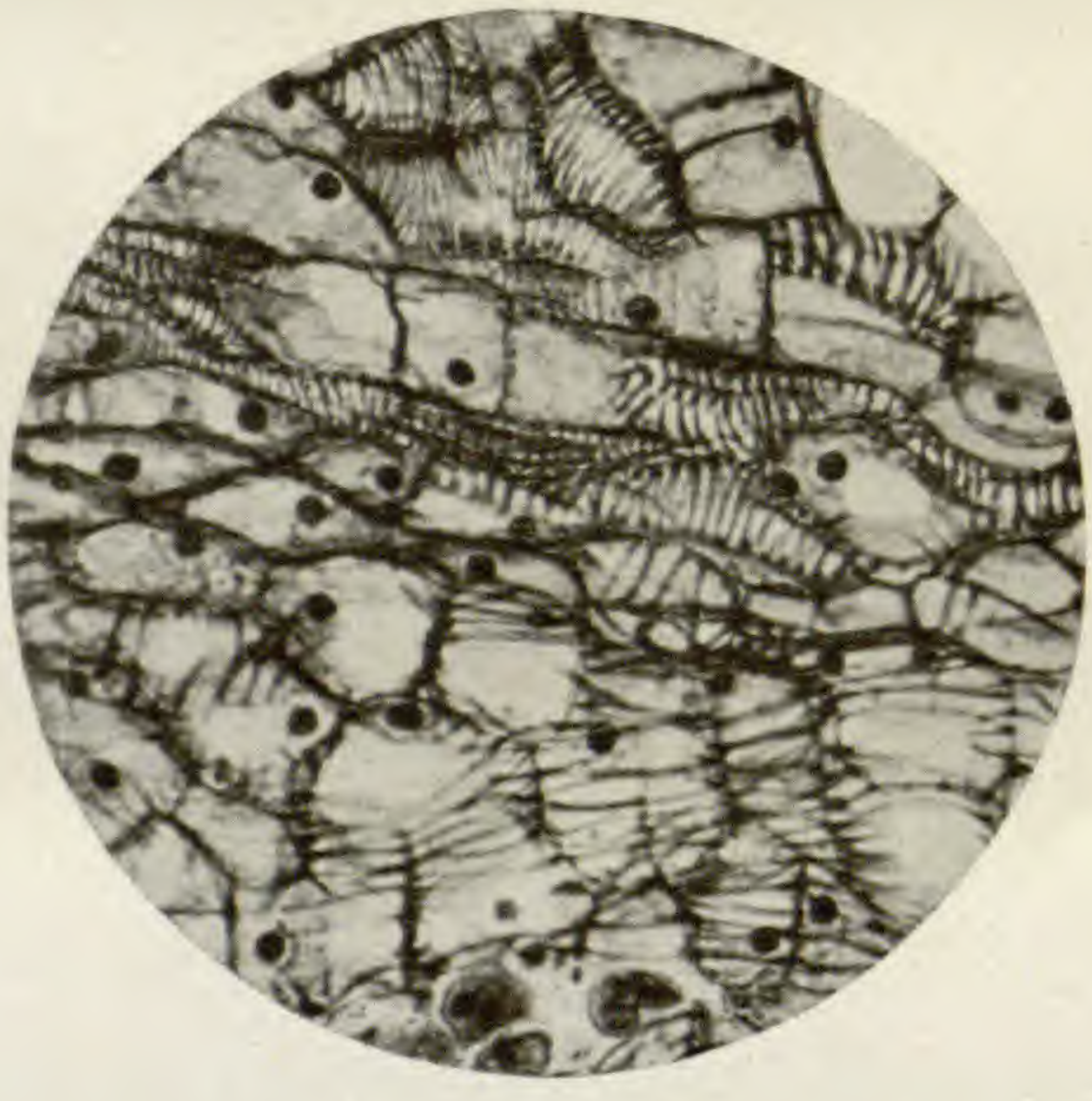

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JEFFREY and TORREY on GINKGO

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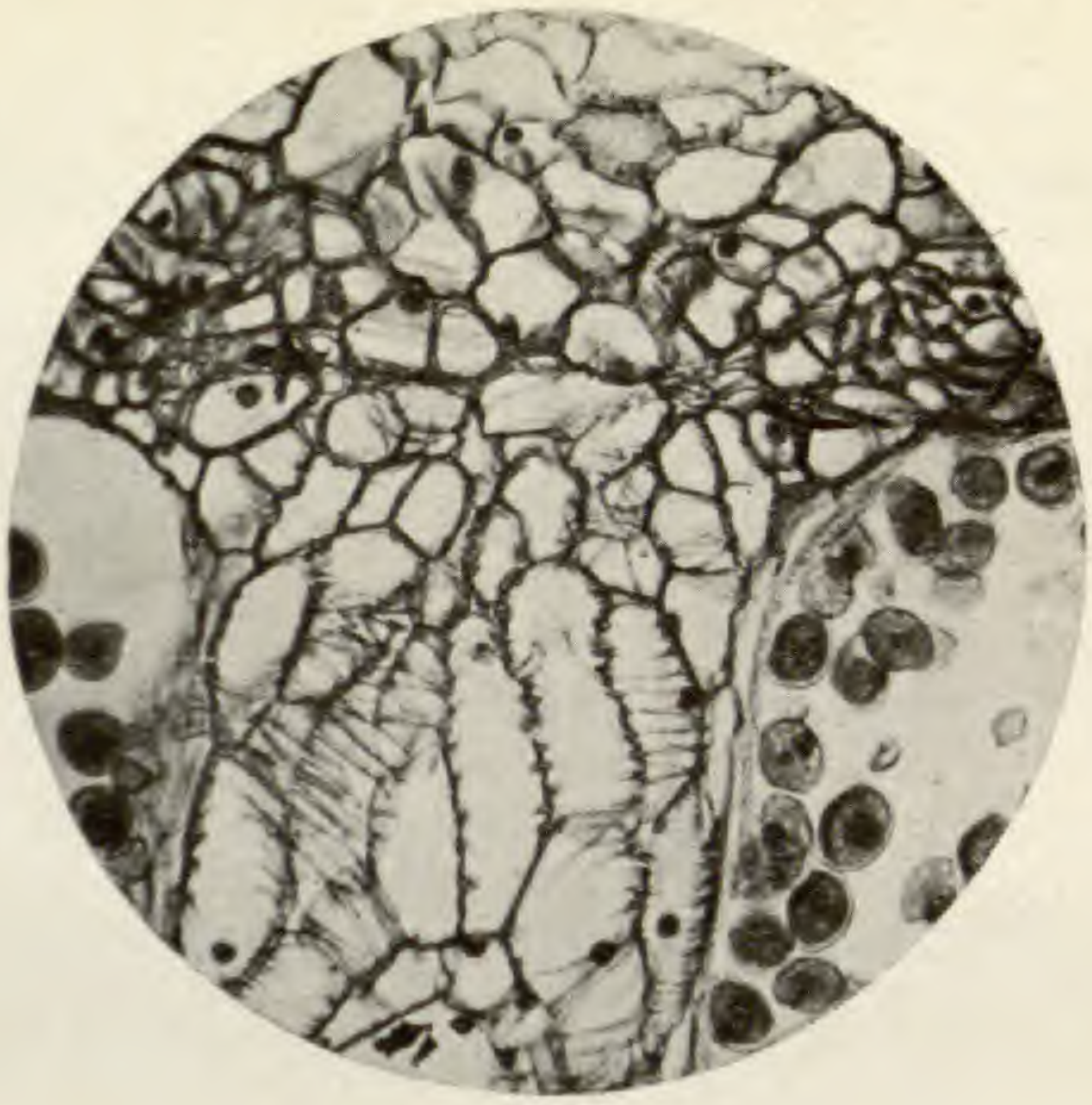

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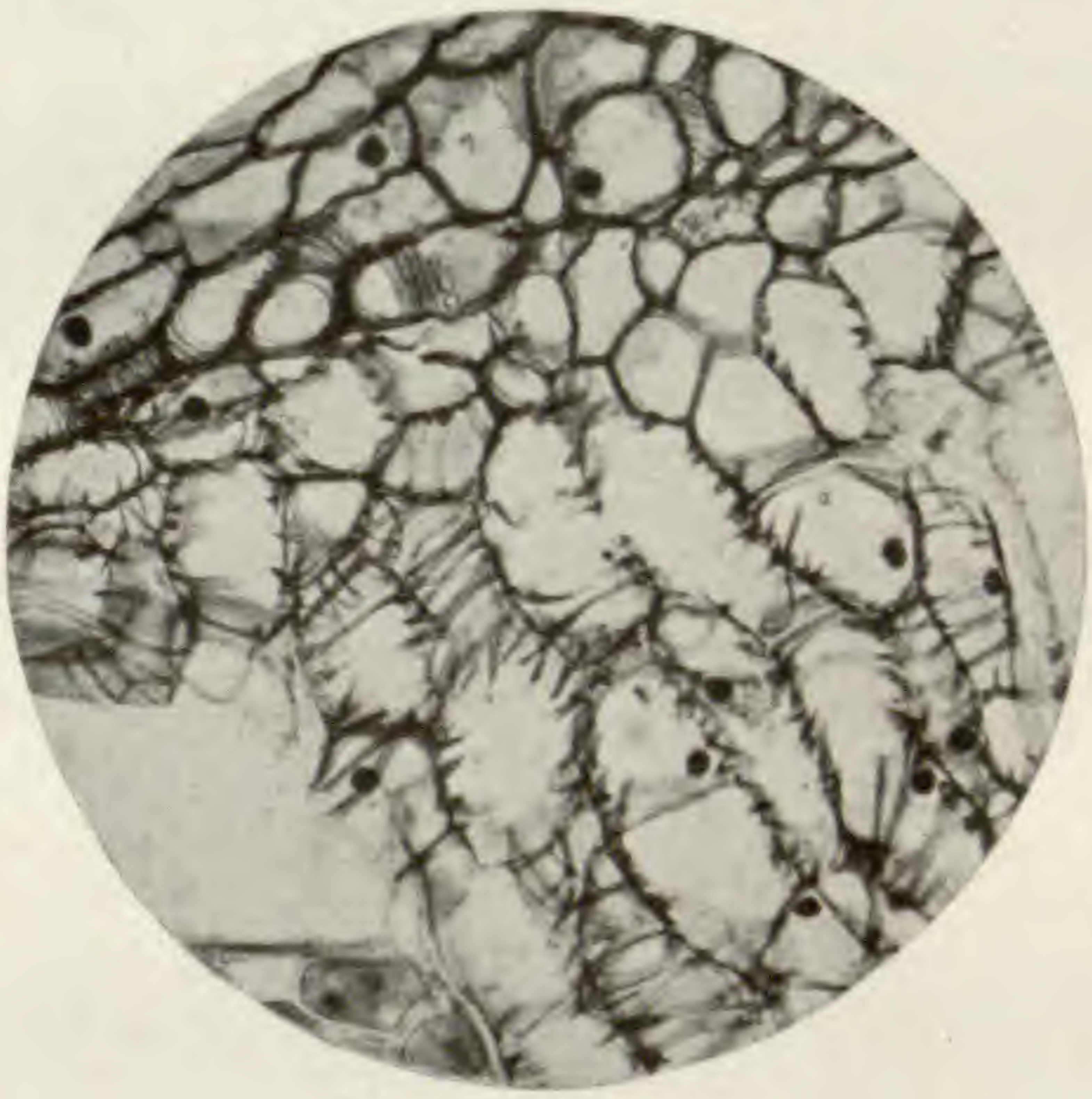

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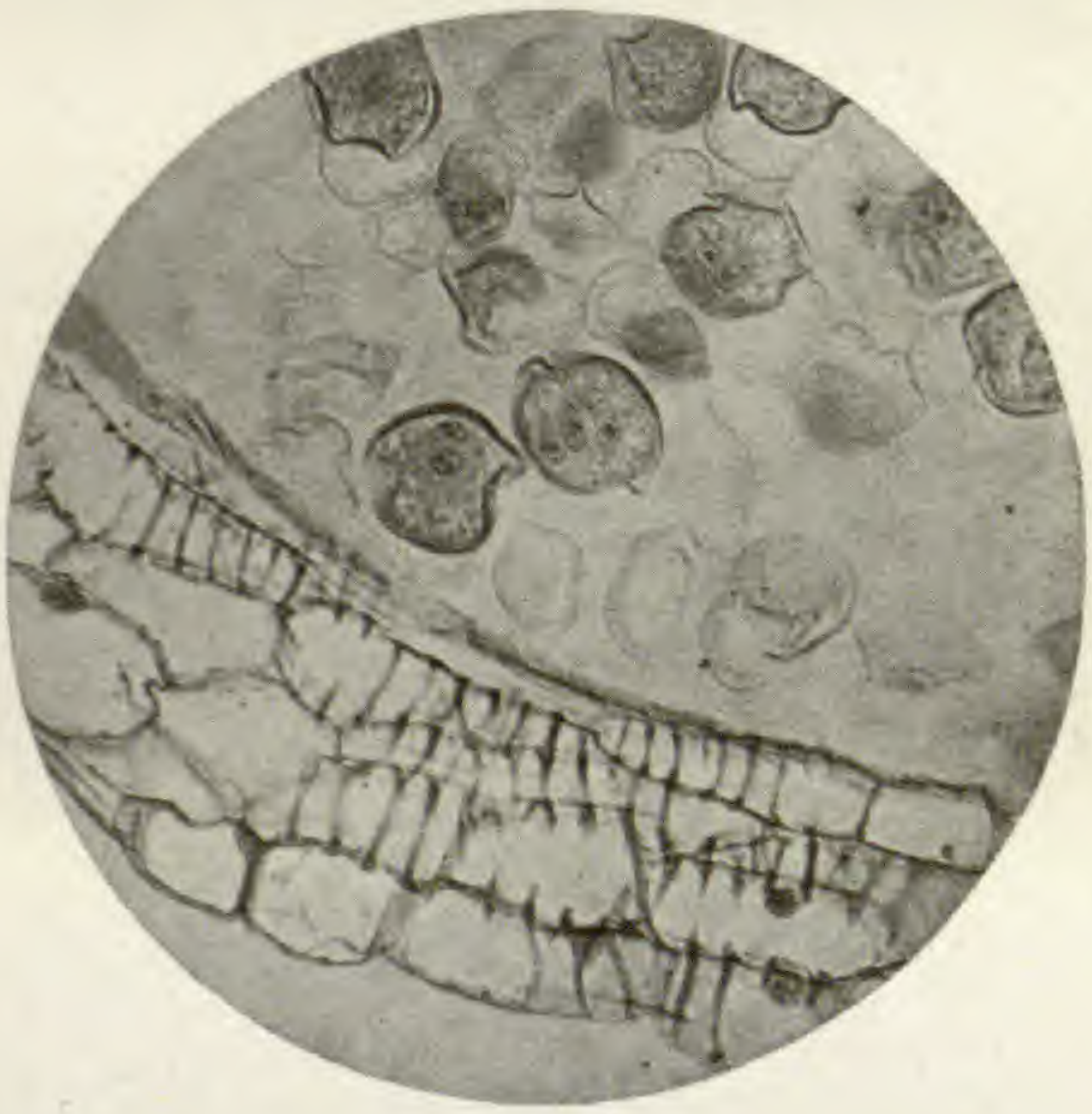

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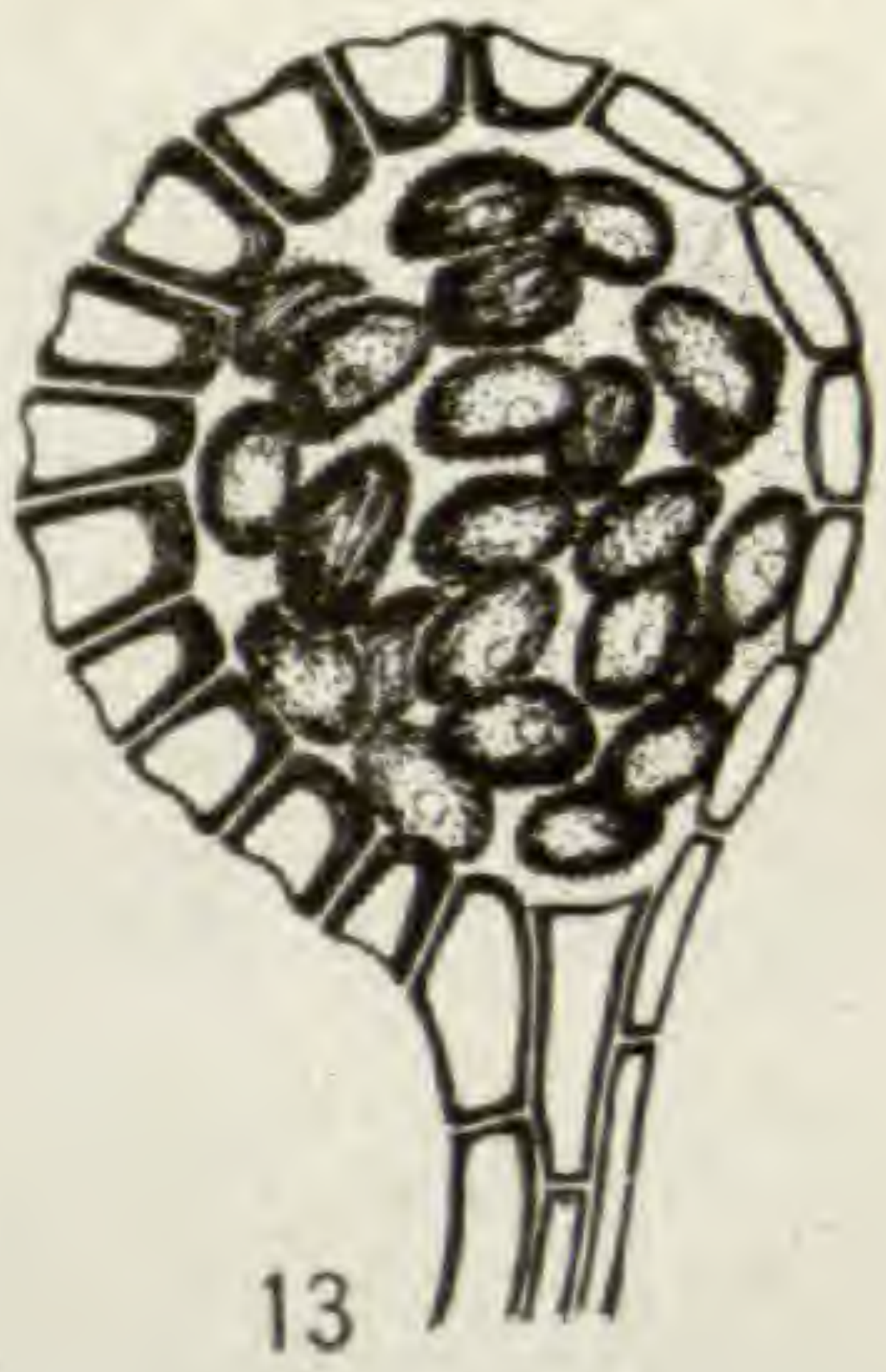
JEFFREY and TORREY on GINKGO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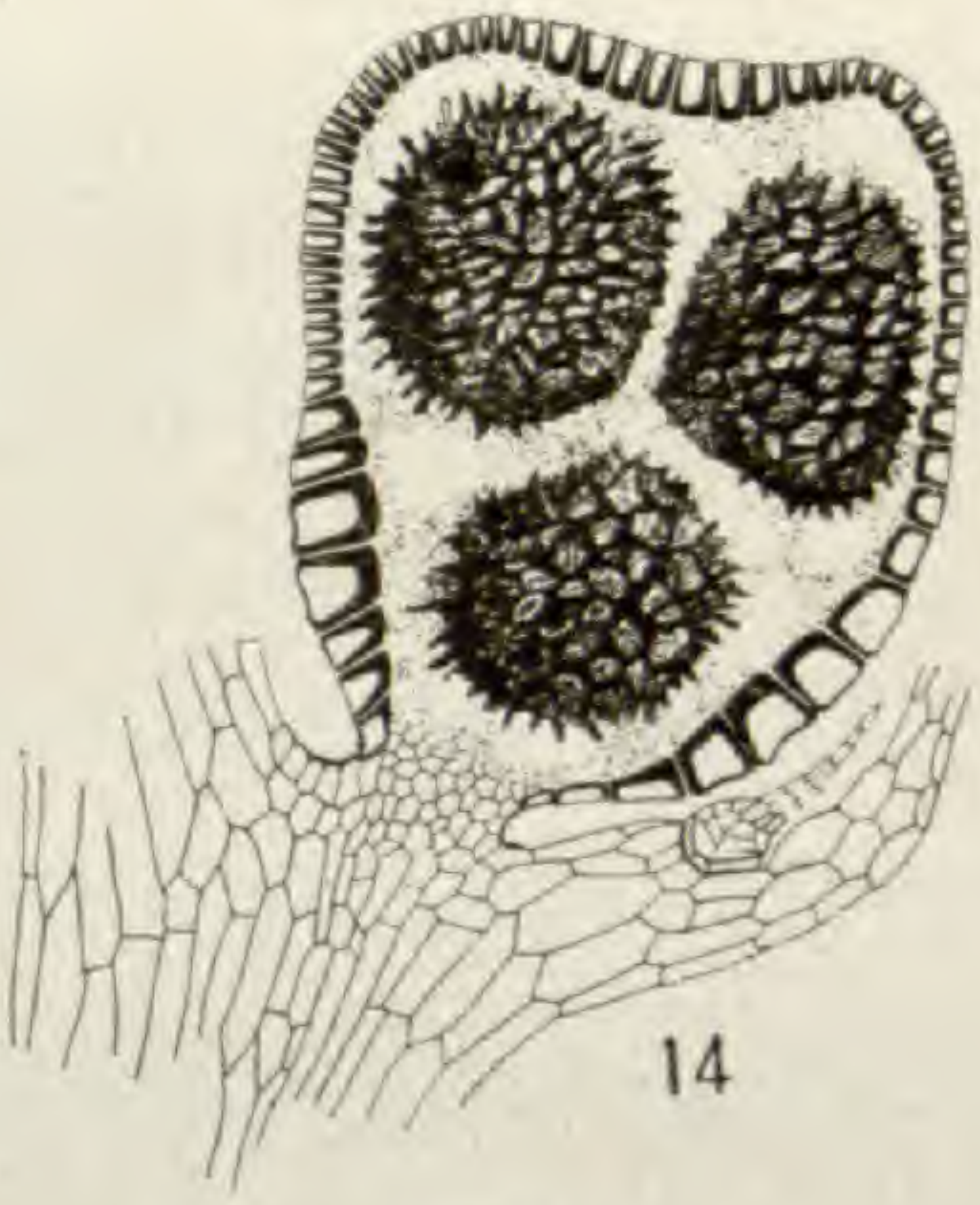
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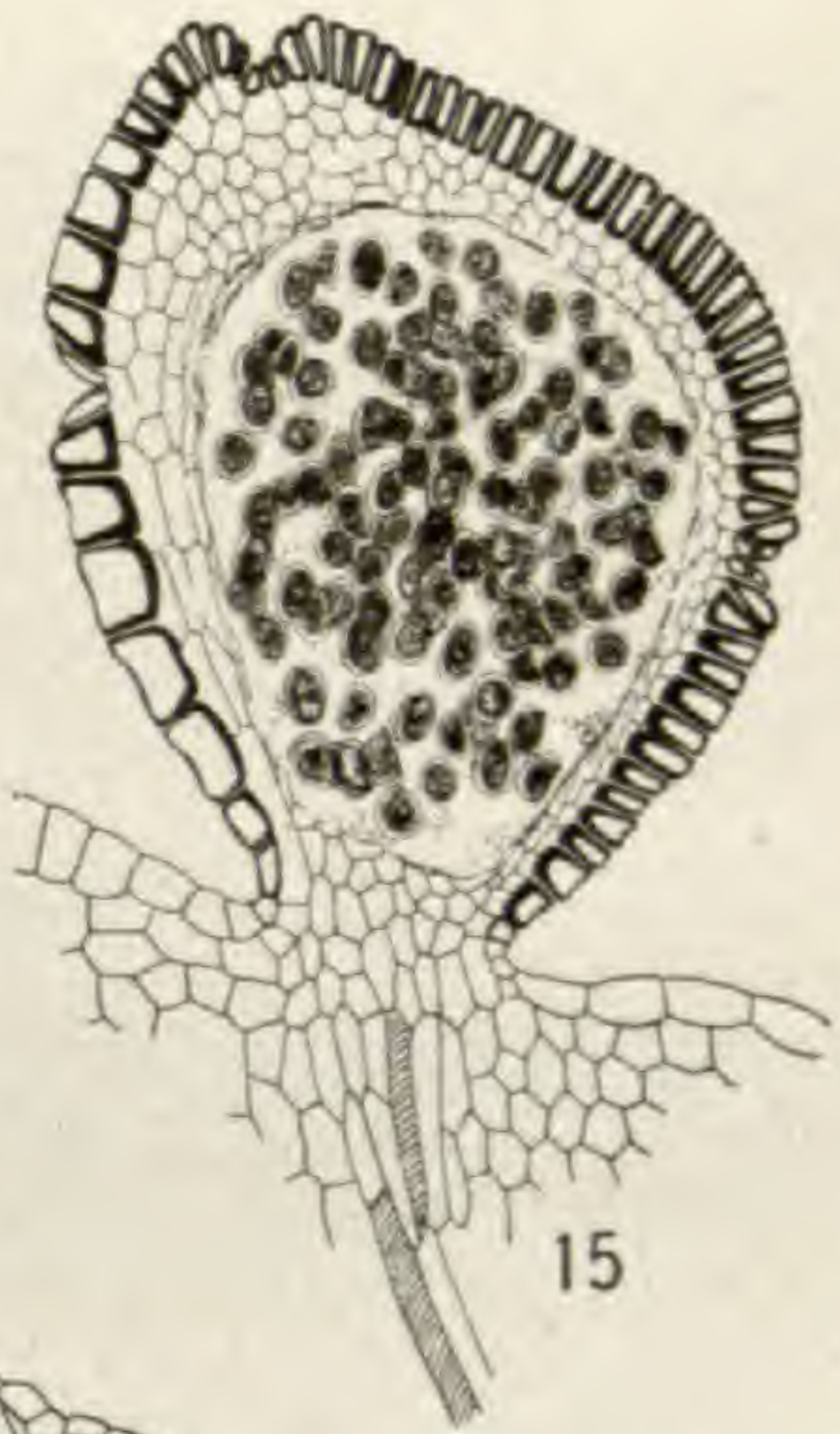
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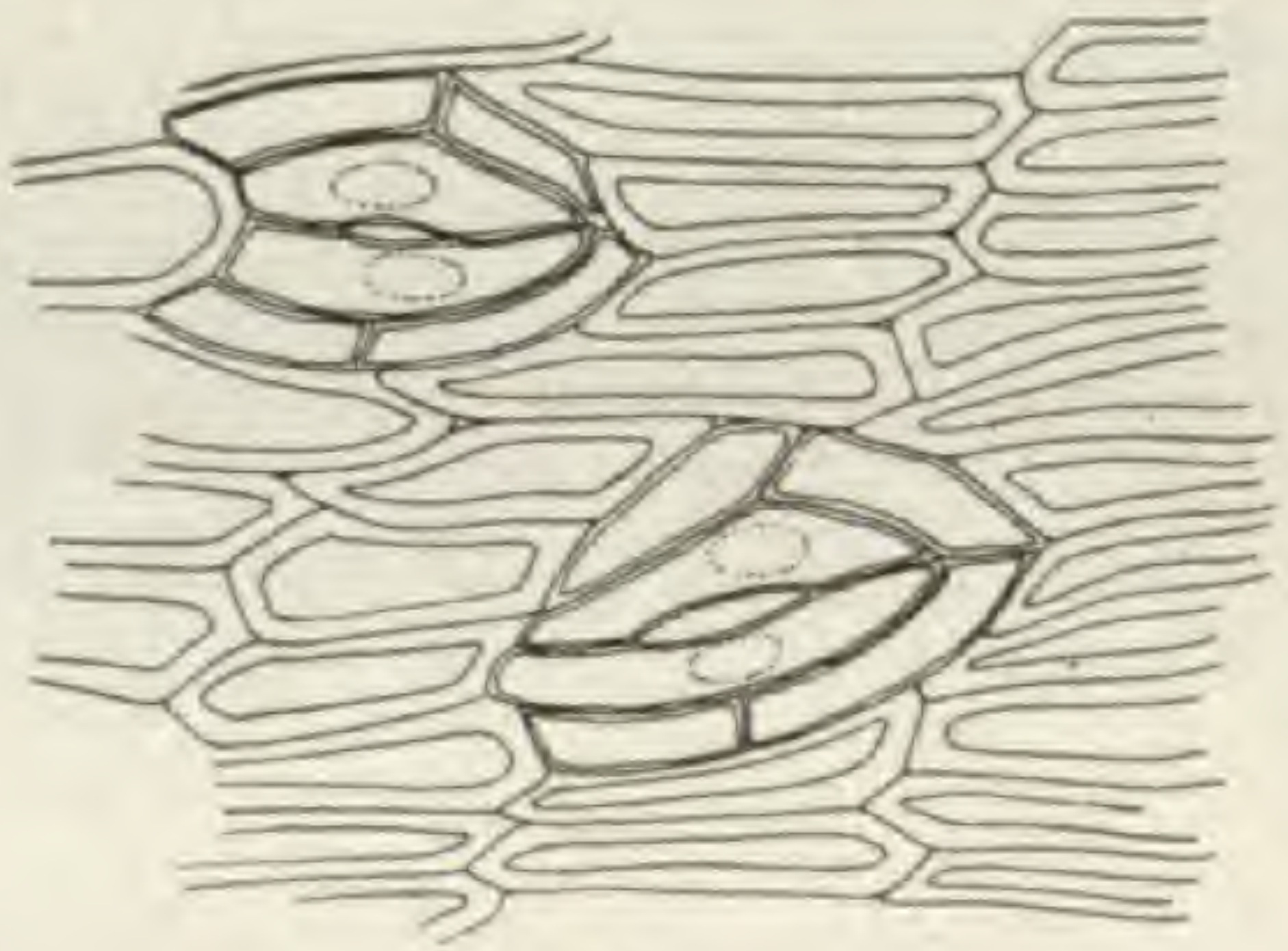
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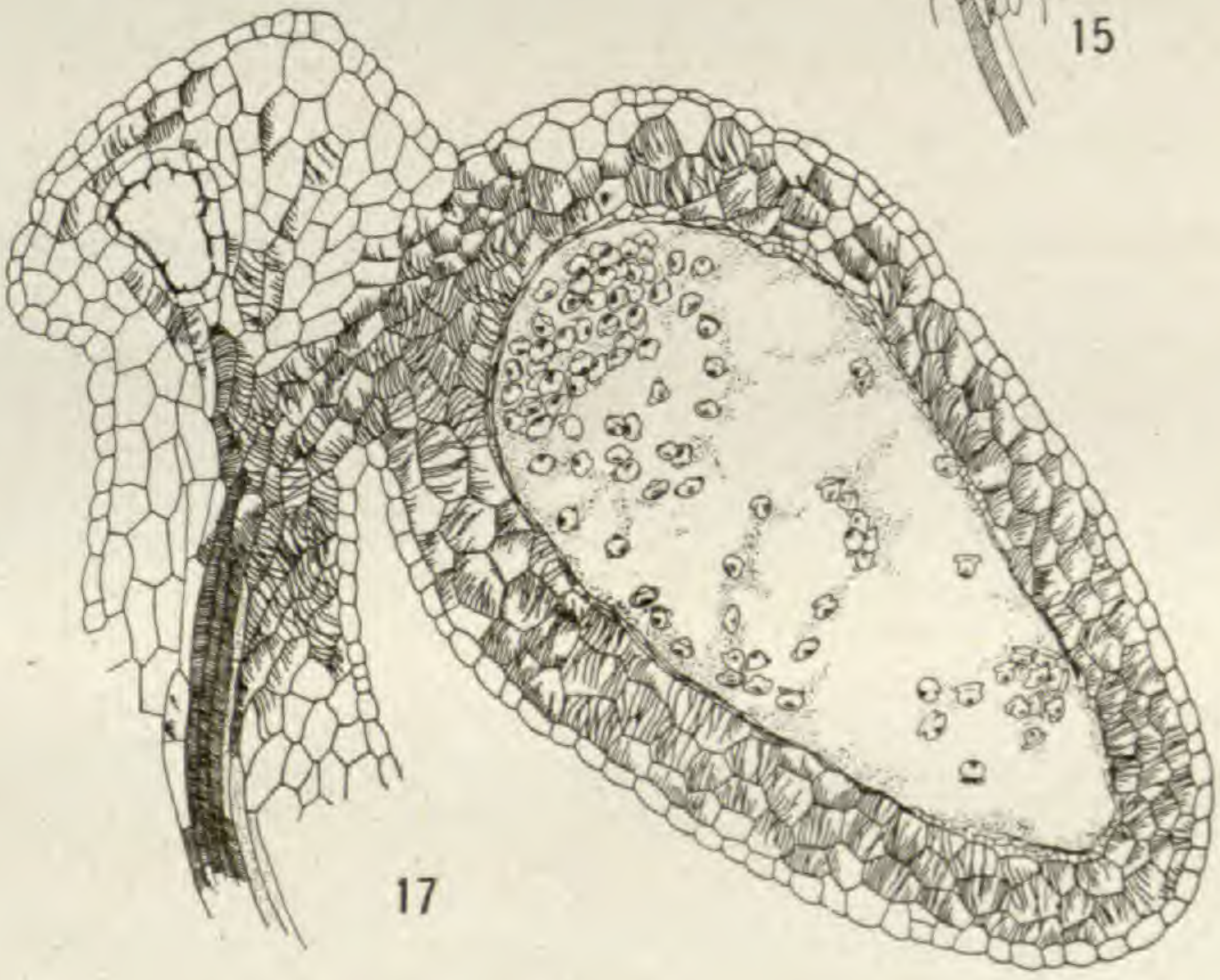
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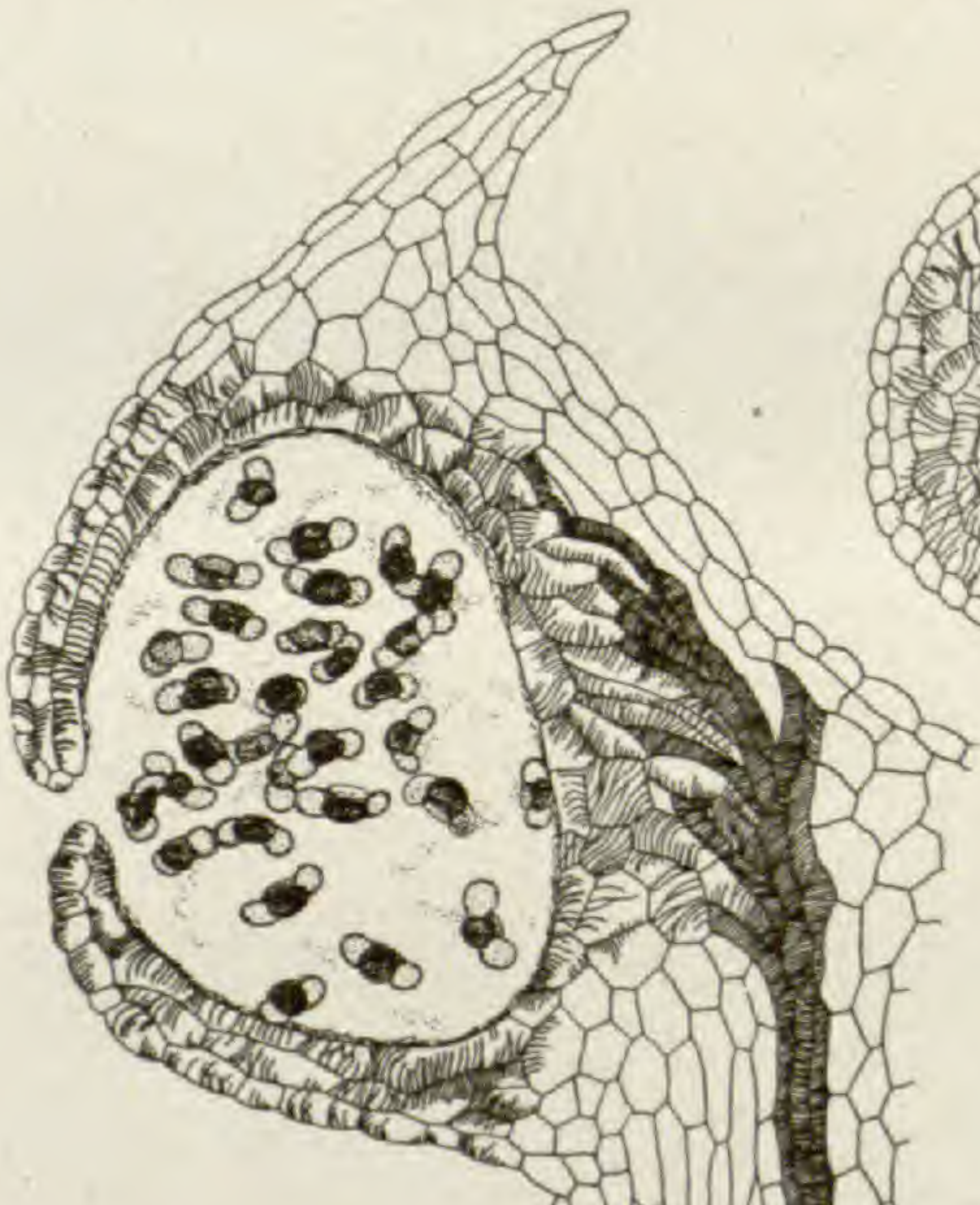
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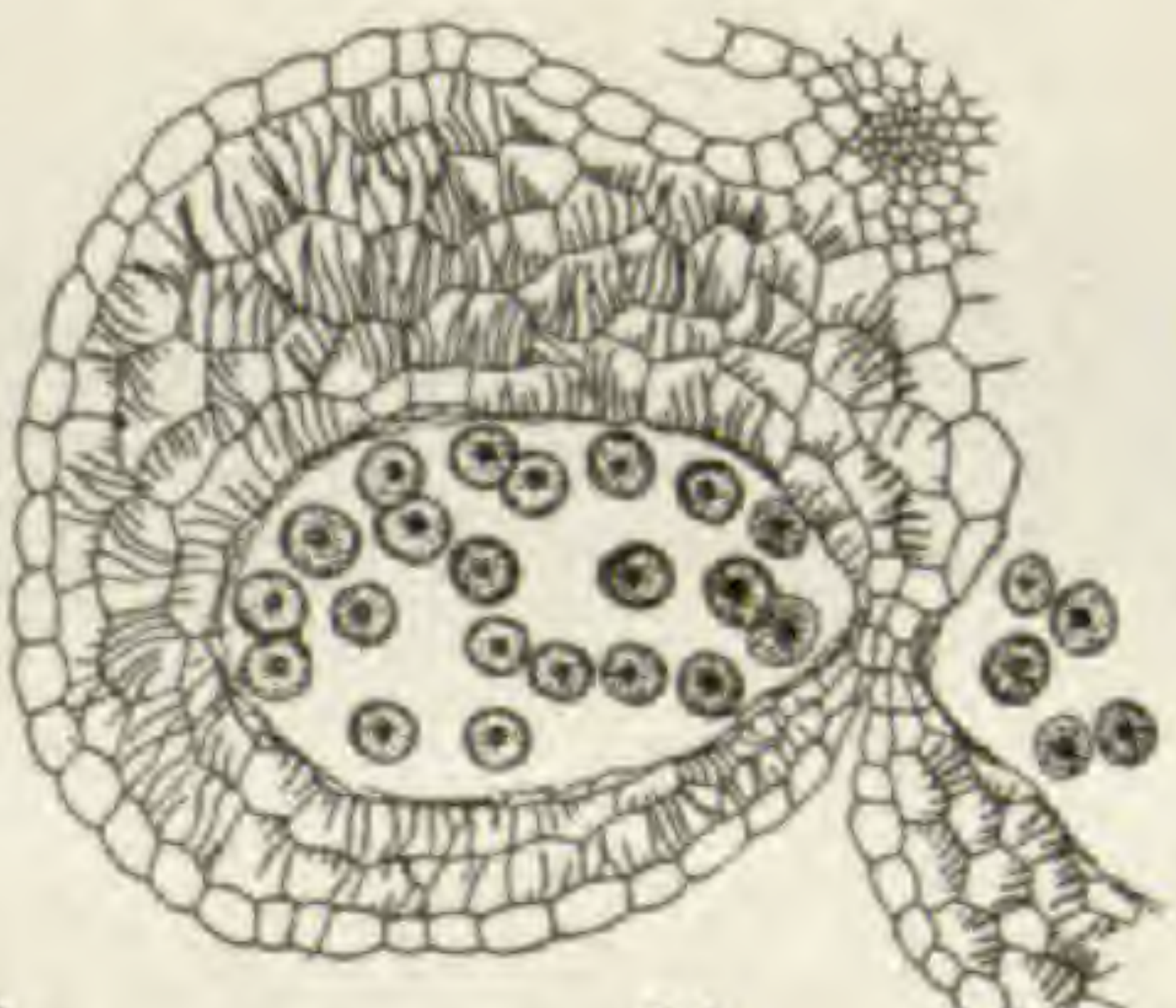
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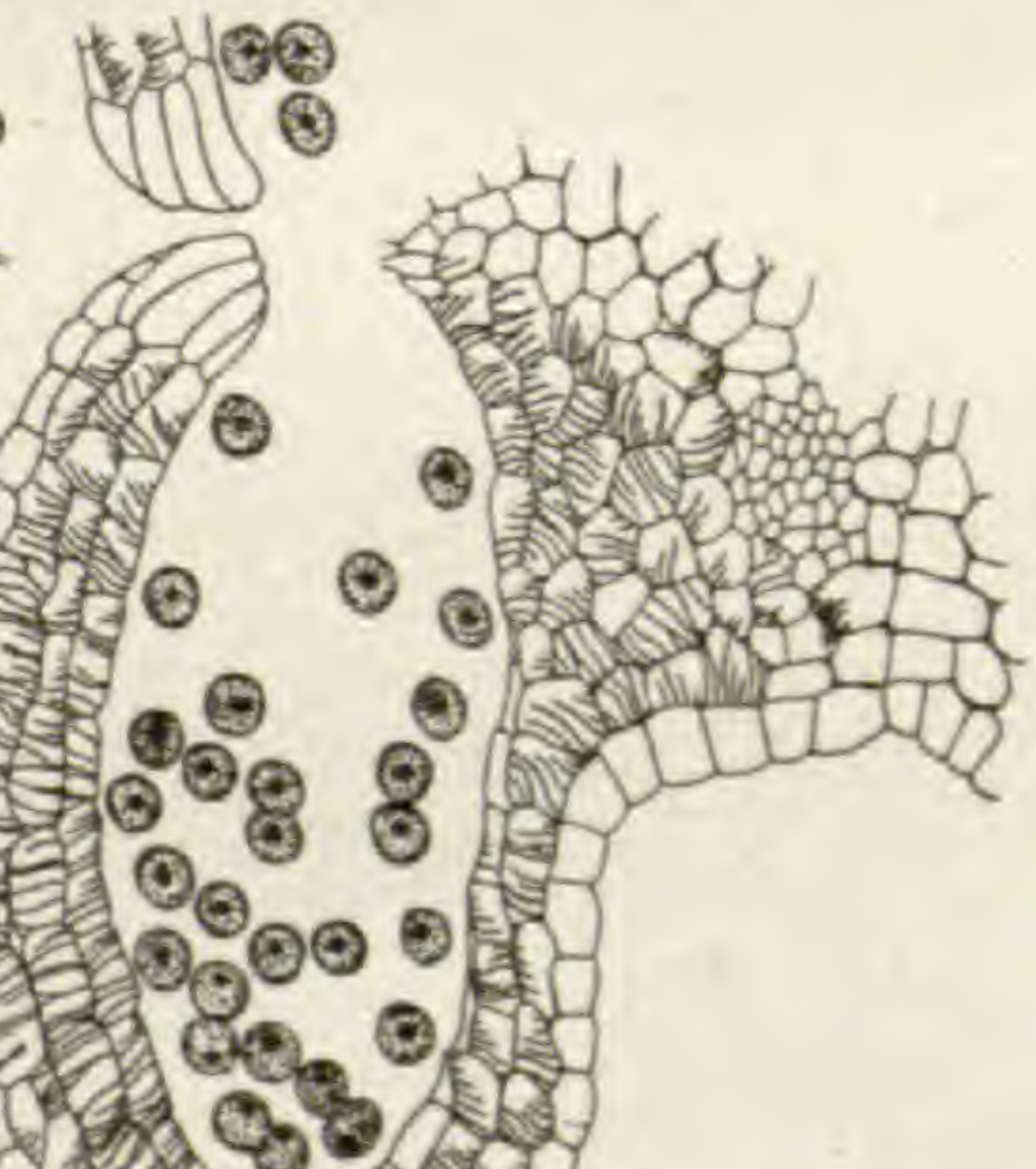
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JEFFREY and TORREY on GINKGO

FURTHER EXPERIMENTS ON CORRELATION OF GROWTH IN BRYOPHYLLUM CALYCINUM

JACQUES LOEB

(WITH SEVENTEEN FIGURES)

The following note contains some additional observations on the phenomena of correlation in *Bryophyllum calycinum*. In his first note[1] the writer had reached the conclusion that a flow of certain substances away from the leaf or its notches prevented roots and shoots growing from these notches, so long as the leaf was connected

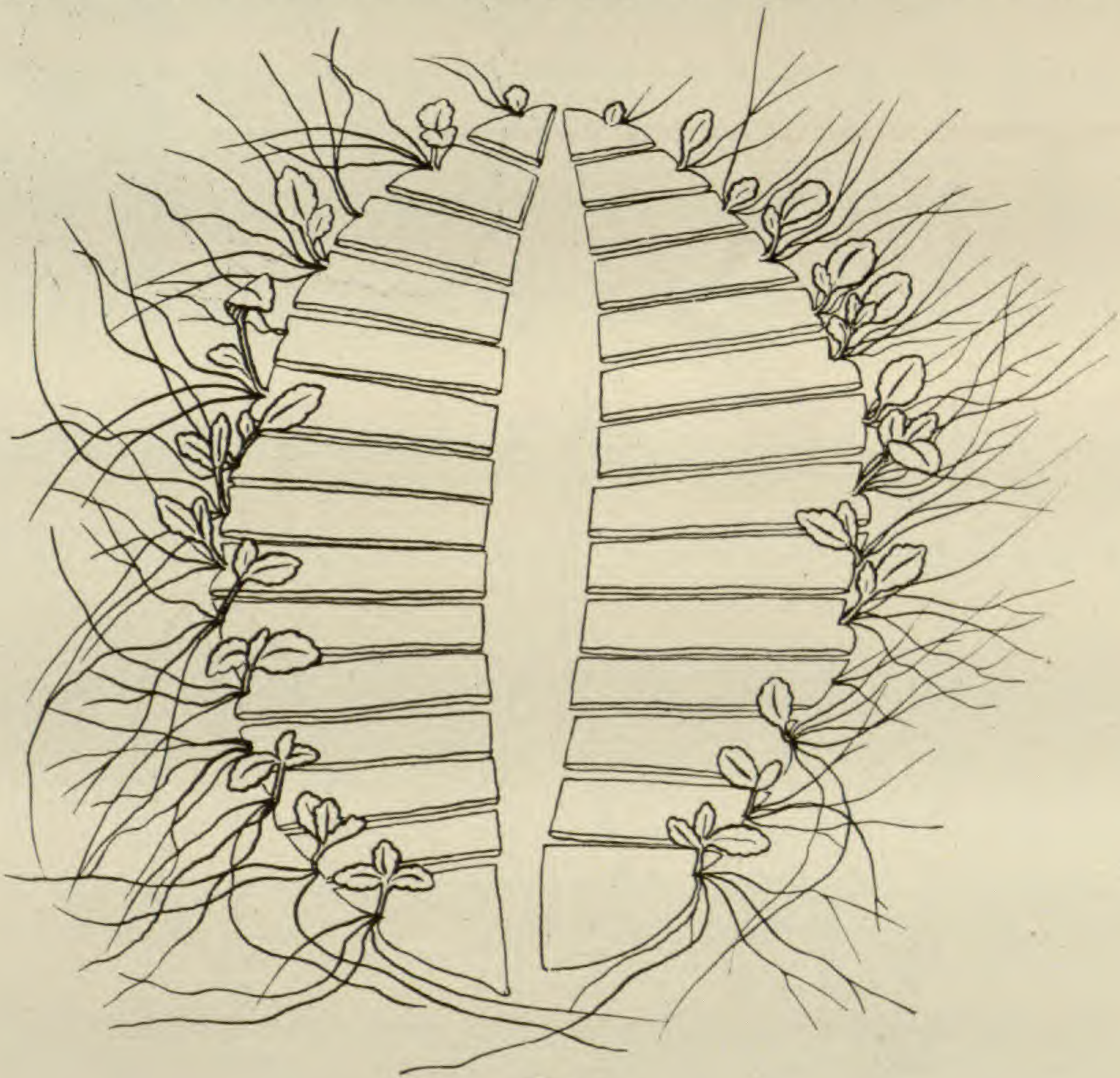

FIG. 1

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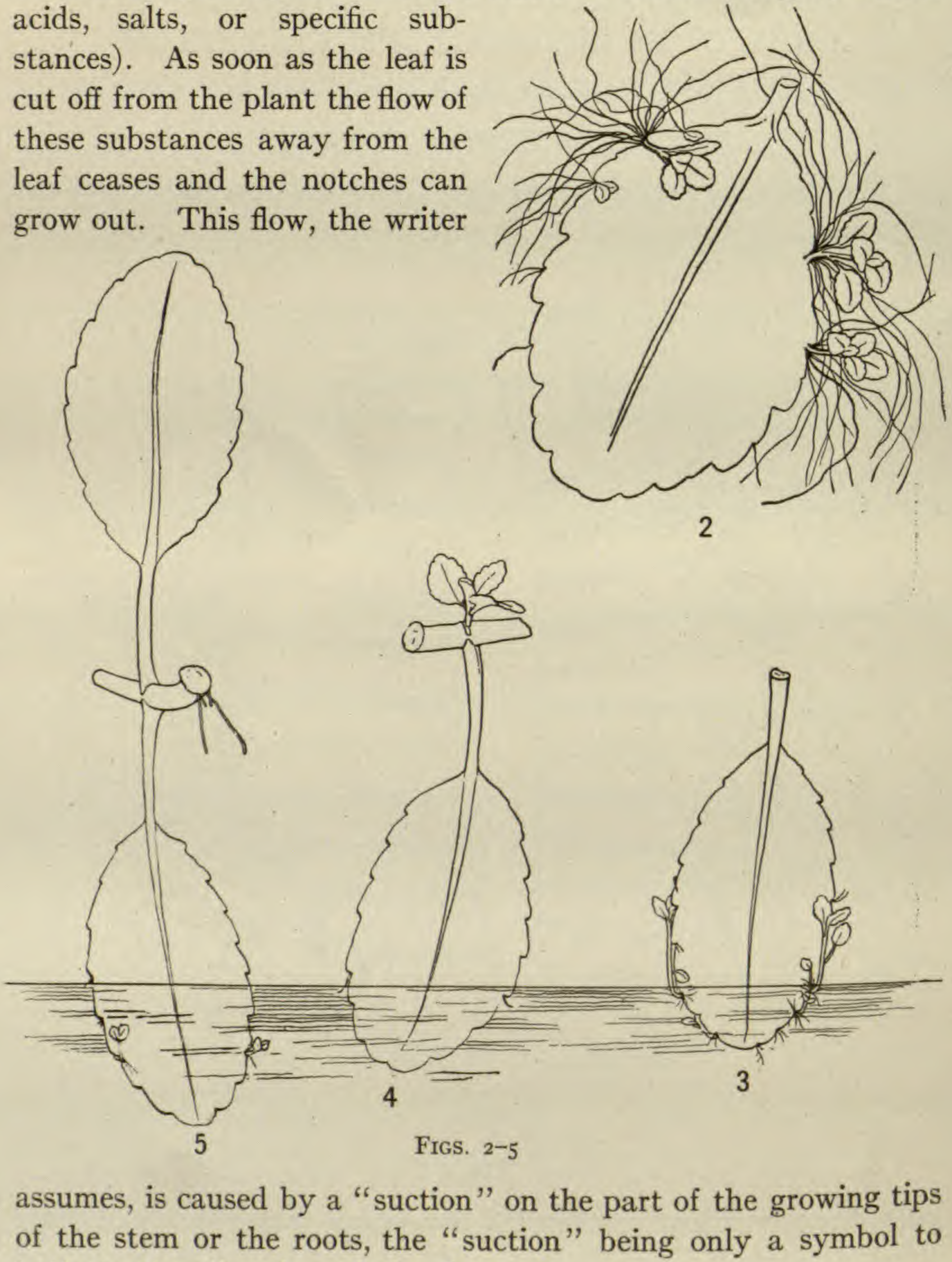
[1] LOEB, J., BOT. GAZ. 60:249–276. *figs. 41.* 1915.

with the normal plant. It is impossible to state at present whether the substances, whose flowing away from the notches prevents these from growing out, are the water or solutes (sugar, amino acids, salts, or specific substances). As soon as the leaf is cut off from the plant the flow of these substances away from the leaf ceases and the notches can grow out. This flow, the writer assumes, is caused by a "suction" on the part of the growing tips of the stem or the roots, the "suction" being only a symbol to indicate the direction of the flow.



FIGS. 2–5

The experiment illustrated in figs. 1 and 2 never fails. Two leaves from the same node are cut off from the plant and put into sufficiently large Petri dishes containing some water. One leaf is cut into as many pieces as there are notches. Every notch gives rise to roots and a shoot (fig. 1). The other leaf is left intact and in this leaf only a few notches will grow into roots and shoots (fig. 2), but the growth is much more rapid in these new shoots than in those of the other leaf. The explanation is simple enough. In the intact leaf (fig. 2) the notches which by chance happen to grow out first act as if they had a suction effect and caused the current of sap to flow away from the other notches, thus preventing their growth. This illustrates sufficiently the principle of correlation by which the notches of a leaf will not grow out as long as it is connected with a healthy plant. In the latter, the suction of the

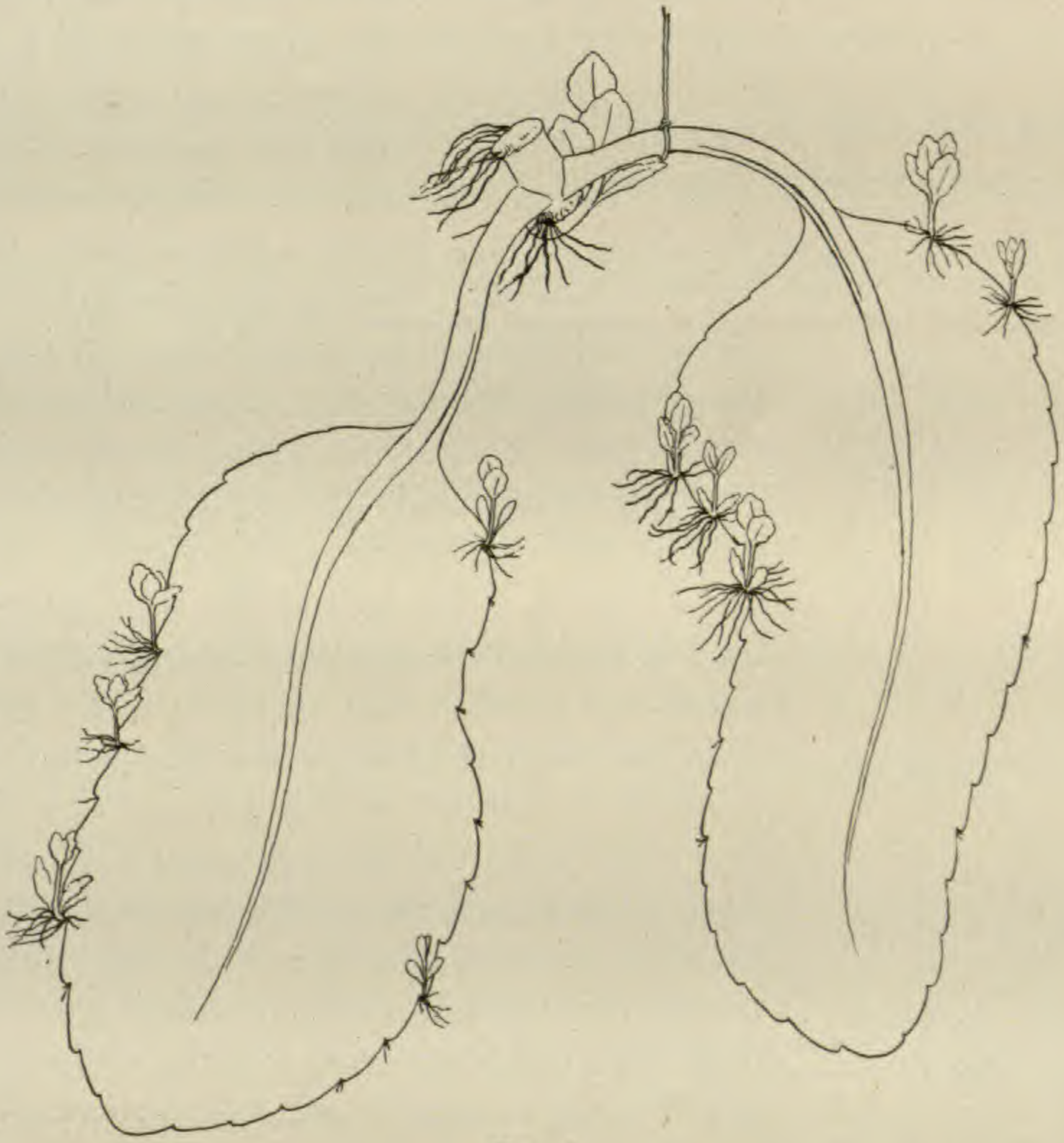


FIG. 6

growing region of the tips of the stem and the roots causes a flow of substances away from the leaf.

In the previous paper the following experiment was described. Three leaves were submersed with their tips in water. One leaf was completely isolated (fig. 3), the second leaf had a piece of a stem attached (fig. 4), and the third piece had the opposite leaf attached in addition to a piece of stem (fig. 5). The drawing was made 25 days later. The completely isolated leaf (fig. 3) had formed two long shoots, the leaf in fig. 4 had only the two tiny roots, and the submersed leaf in fig. 5 had two short shoots. The experiment finds its explanation on the basis of the observation represented in figs. 1 and 2. In fig. 4 the bud of the stem opposite the leaf grew out before even the notches in the completely isolated leaf of fig. 3 could grow. This growth of the bud on the stem acted as a center of "suction" on the leaf and created a flow away from the notches toward the stem. Even when the growth of this bud is prevented the stem has a suction effect. In fig. 5, where two leaves were left, no shoot would grow out from the stem, and hence shoots could grow out from the immersed leaf, but not as rapidly as in the completely isolated leaf (fig. 3), showing that probably, using the symbol of a "suction," some suction must have been caused by the stem, but not as much as if the opposite leaf had not existed.

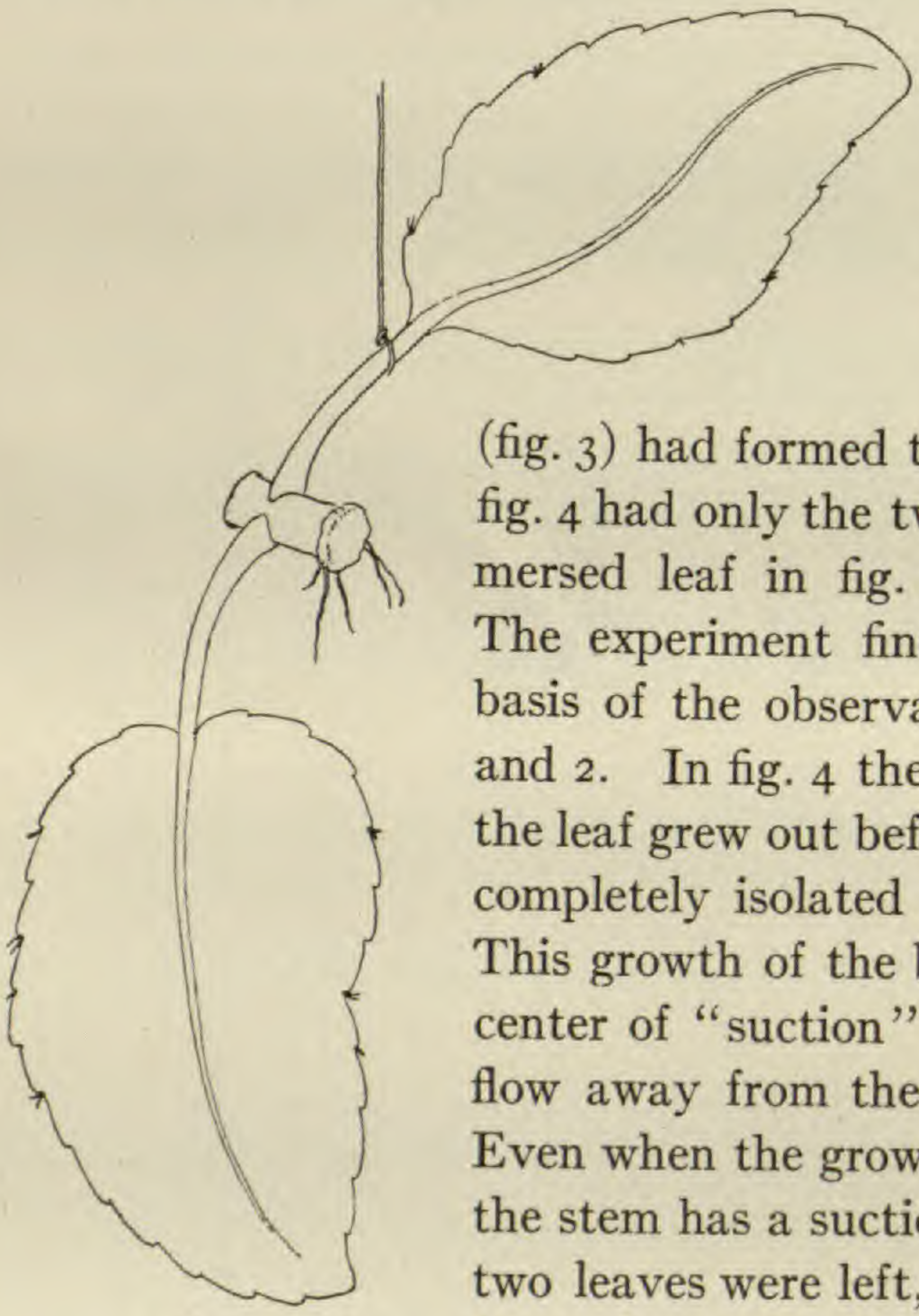


FIG. 7

The writer was anxious to see whether this experiment would not succeed when the whole leaves are suspended in moist air instead of submersing the tips in water. This latter form of the experiment has the drawback that in the leaf with a stem (fig. 4) the growth of the notches in the submersed part is often not entirely inhibited but only delayed; while if such a leaf is suspended in air the growth of the notches is generally permanently suppressed. Hence if it were possible to repeat the experiment with leaves suspended entirely in air the results should be still more striking. It was found that if the two leaves connected by their node are large (and probably rich in sap ?) they will give rise to new roots and shoots in their notches even if suspended in air. In figs. 6 and 7 two such pairs of leaves of different sizes from the same plant were suspended in moist air. The larger leaves (fig. 6) have each formed 5 shoots in their notches, although also one of the axillary buds of the stem had grown out. This latter growth was not able to inhibit the growth of the notches. In the smaller leaves (fig. 7) no growth had taken place in the notches. The drawing was made 19 days after the commencement of the experiment. The moderate "suction" of the stem sufficed to suppress the growth of the notches in the smaller but not in the larger leaves when suspended in moist air.

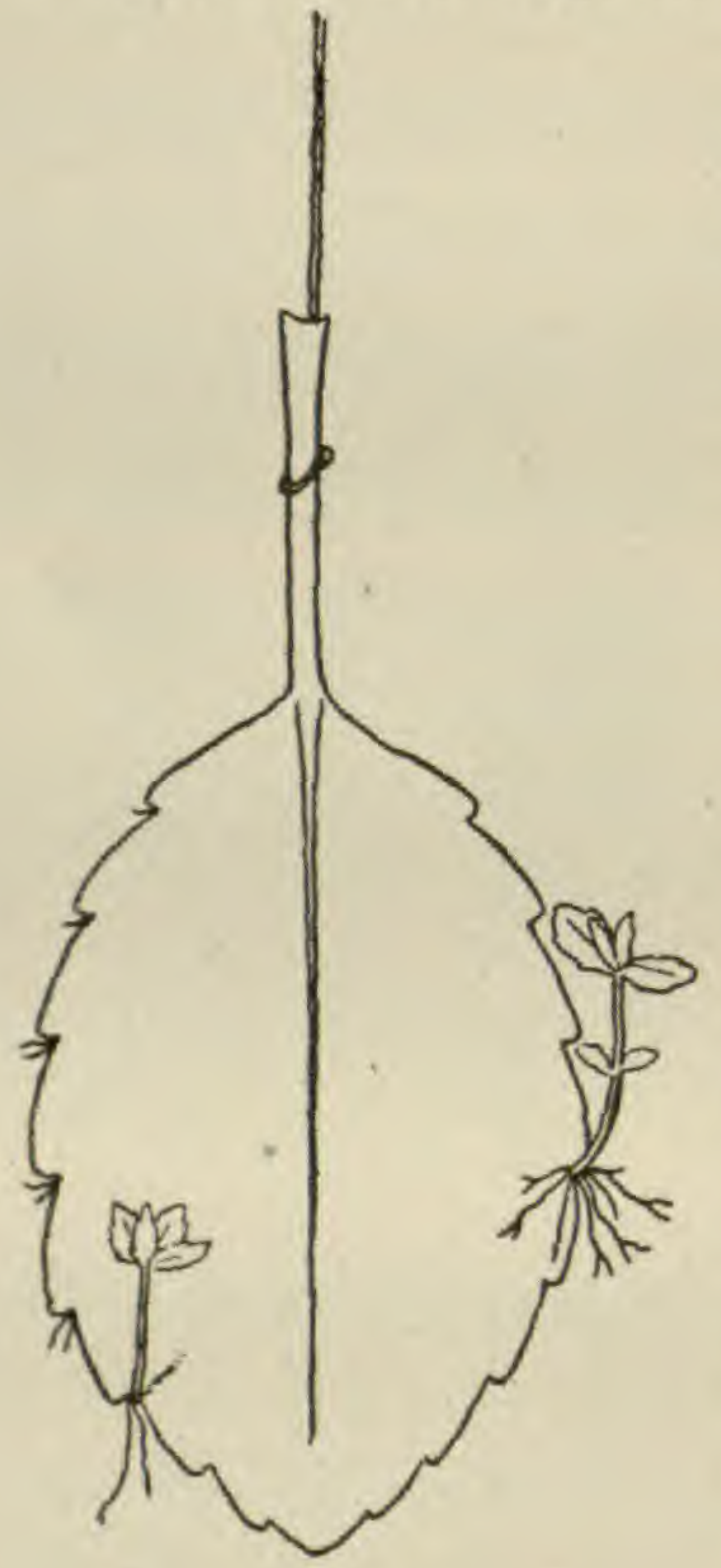


FIG. 8

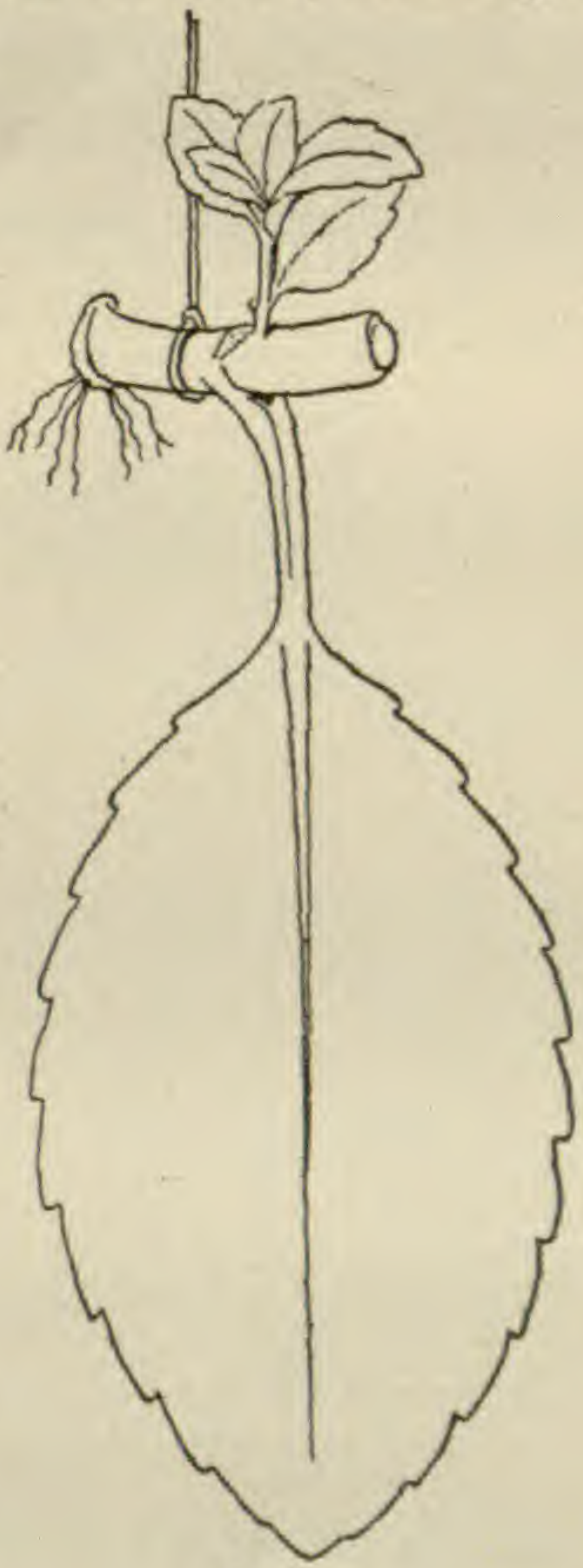


FIG. 9

Figs. 8, 9, and 10 are the replica of the experiment represented in figs. 3, 4, and 5, but with the difference that in figs. 8, 9, and 10 the leaves were entirely in air. The leaves were large and gave a characteristic result. The completely isolated leaf (fig. 8) had formed two large shoots from notches; the leaf of fig. 9 had formed neither roots nor shoots in its notches on account of the complete inhibitory effect of the piece of stem and its bud. In fig. 10 one shoot had been formed in the lower leaf, which had commenced to grow later than the two shoots in the completely isolated leaf in fig. 8. This drawing was made 5 weeks after the beginning of the experiment. These 3 specimens represent 3 different degrees of "suction" away from the notches of the leaf. In the leaf shown in fig. 9 the suction from the stem was too strong and no notches of the leaf grew. In fig. 10 the suction was less and one notch showed growth, which was less rapid than in fig. 8. In fig. 8, the completely isolated leaf, there was no "suction" away from the leaf and the growth of the notches was most rapid.

FIG. 12

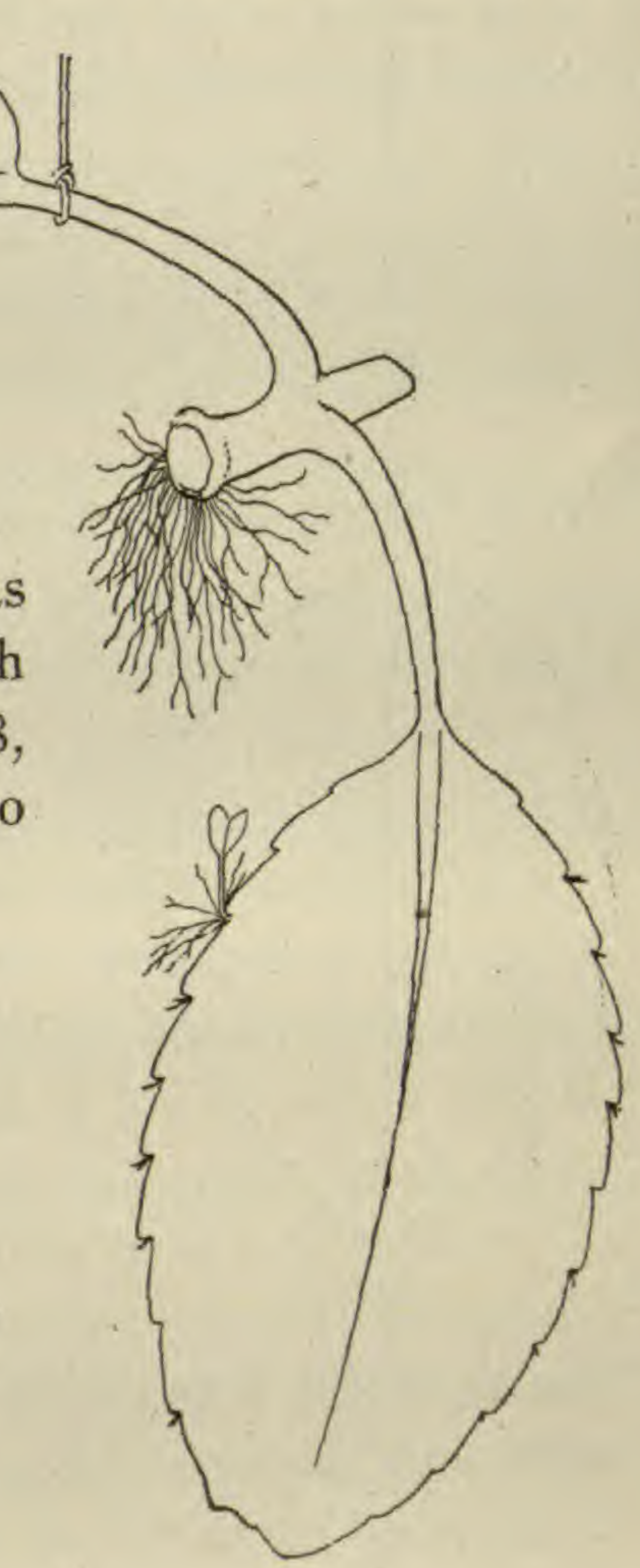


FIG. 10

FIG. 11

A digression is necessary to explain why in fig. 10 only the lower leaf has formed a shoot and not the upper leaf also. The writer is inclined to ascribe the phenomenon chiefly, if not exclusively, to the influence of moisture. The specimens were suspended in a glass trough loosely covered with a glass plate and whose bottom contained a layer of water. The air surrounding the lower leaf was more completely saturated with water vapor than that surrounding the upper leaf. To settle this question control experiments were made in which both leaves were equally near the water (fig. 6); in this case both leaves formed shoots, while when they were suspended higher up in the trough neither formed roots and shoots. Both leaves also formed roots and shoots when put horizontally into the water. The influence of moisture upon the growth of roots and shoots in the notches was very striking in all these experiments. Thus contact with the wall of the trough, where a condensation of water kept some of the notches moist, would favor the growth of roots and shoots in these notches.

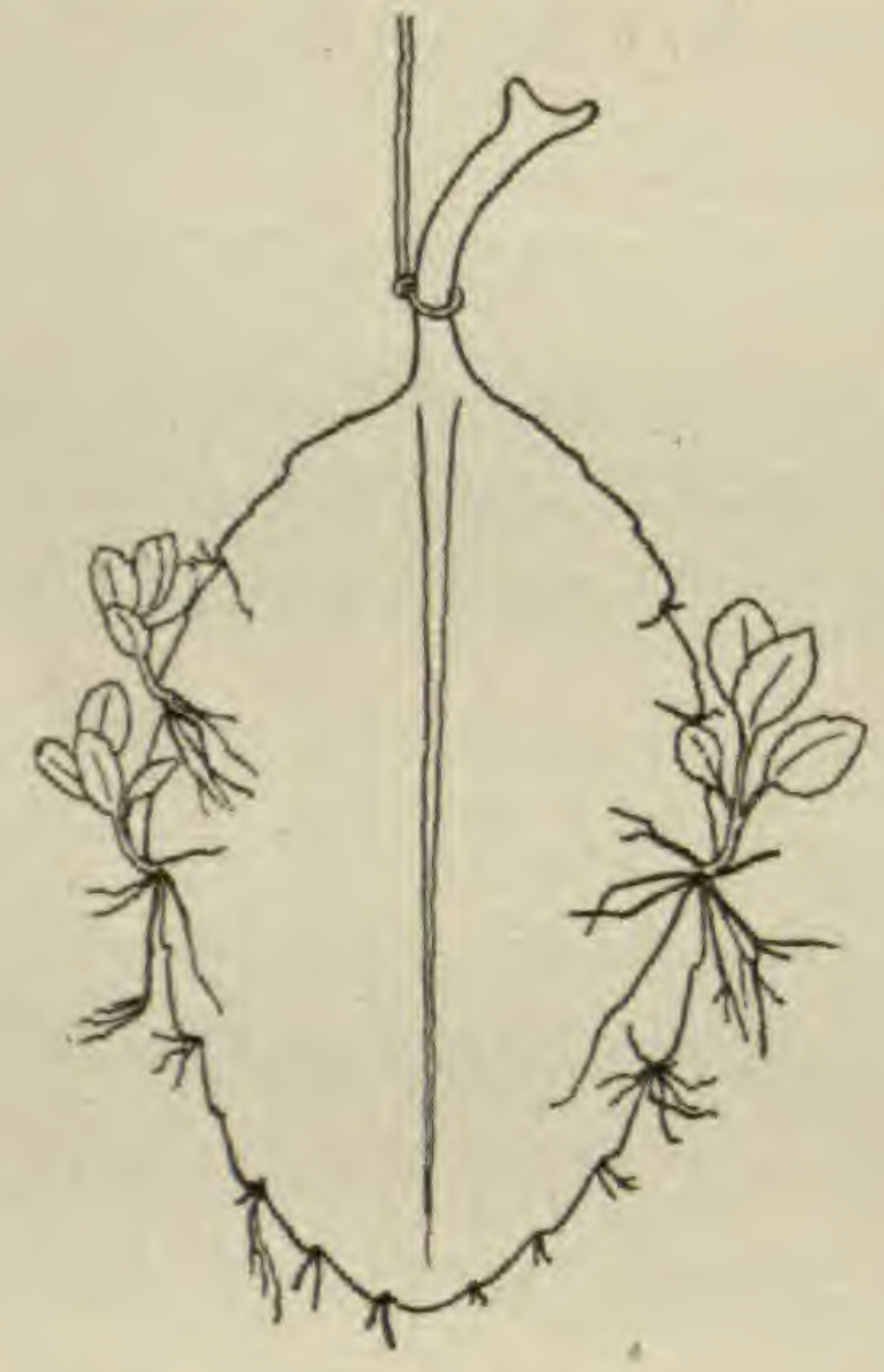


FIG. 13

It has been stated by previous writers that in a completely isolated leaf of *Bryophyllum* the notches grow out because the stalk of the leaf does not form roots. This statement is not strictly correct, as has been stated already in the first article. An example was given in fig. 16, p. 261, where a leaf formed roots and shoots in one notch although roots and a shoot had been formed on the stem, owing to the fact that the piece of the axillary bud was still in contact with the stalk of the leaf. We must assume that in this case the suction from the new growth at the stalk of the leaf was less than if a piece of stem had been there, and this lessened suction permitted one notch of the leaf to grow into a shoot. It occurred to the writer that this assumption might be put to a test. Leaves were prepared being connected by a piece of the cortex of the stem, without the wood (fig. 11), and the behavior of such leaves was compared with the behavior of leaves which were attached to a complete piece of stem (fig. 12). Both sets of leaves were suspended


FIG. 14

in moist air. Leaves of the latter type (fig. 12) never formed shoots in their notches, and at best only a beginning of root formation was noticeable, which never led to roots longer than 1 mm. The bud on the stem opposite the leaf grew out into a vigorous shoot (*S*), and at the lowest point of the callus of the stem, roots (*R*) were formed.

The other type of leaves with only a piece of cortex attached (fig. 11) formed always numerous long roots and quite frequently shoots, while the cortex formed roots (*R*) at the basal end and small leaves (*L*) from the axillary bud of the leaf. The drawing was made after 5 weeks. The growth of the roots and shoots at the cortex did not inhibit the growth of the notches, since in our assumption the suction which the cortex produced was not as strong as if the piece of stem had been complete.

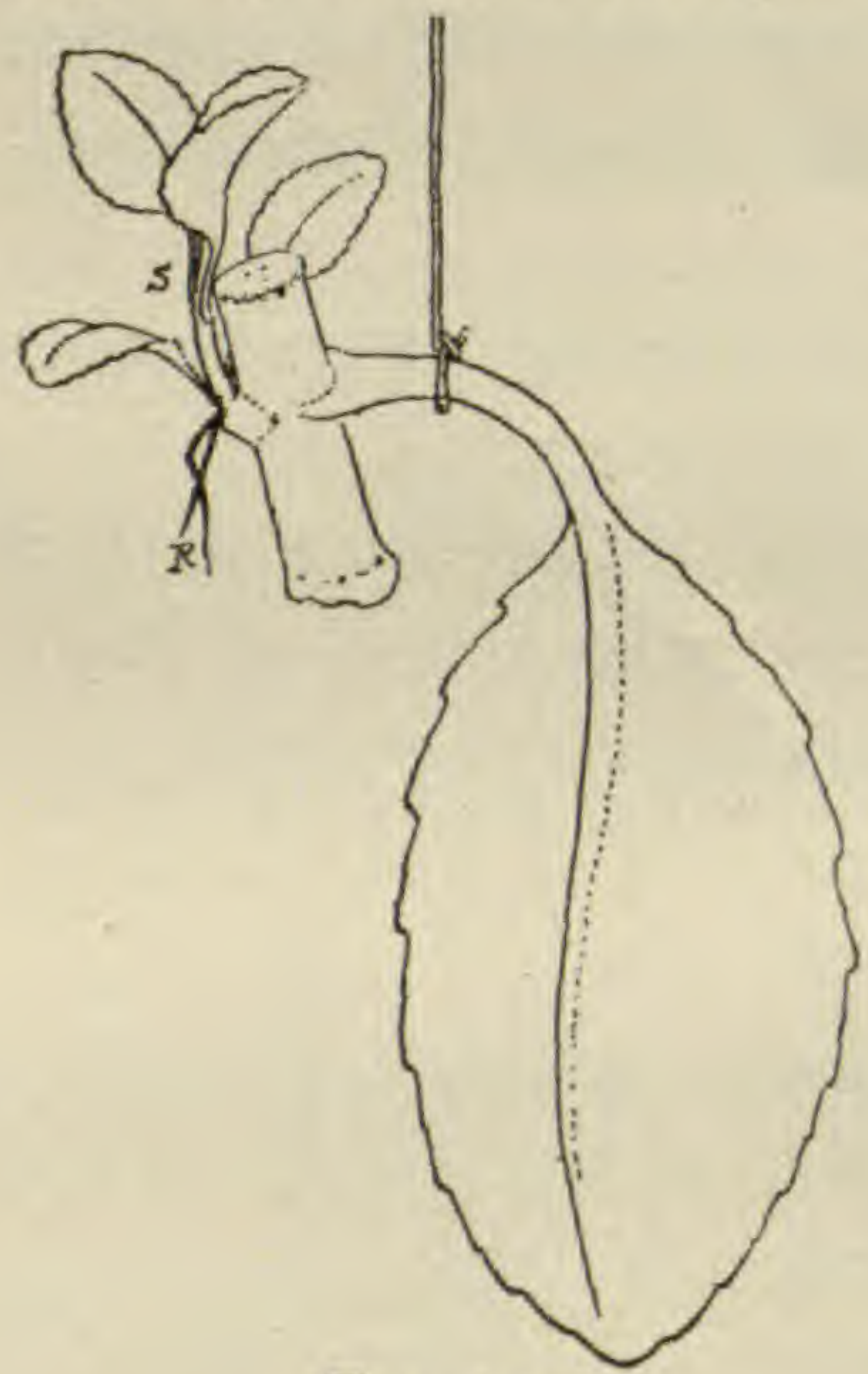


Fig. 15

This furnishes us, therefore, with a method of retarding the velocity of the flow away from the notches of the leaf without interrupting it completely (as in a completely isolated leaf) or without making the flow too strong, as is the case when a whole piece of stem (without the opposite leaf) is attached (as in fig. 12). This difference should find expression in the relative velocity of growth in the notches if the proper experiments were made. Figs. 13, 14, and 15 represent such a series, 3 weeks after the experiment was begun. The leaves were suspended in moist air. The completely isolated leaf (fig. 13) has formed 3 shoots of more than 1 cm. in length and roots of considerable size. The second leaf with a piece of cortex attached (fig. 14) has roots and only one short shoot in the notches, but has a considerable shoot and roots in the cortex. The flow of sap from the leaf to the cortex was not strong enough to completely prevent the growth of the notches, but only to retard it. The third leaf (fig. 15) has a piece of complete stem attached and in this leaf no growth has taken place, and in all probability none will take place in the notches. Instead a strong shoot (*S*) has been formed from the axillary bud of the leaf which had been removed. The "suction" effect of the complete stem or of the growing bud was strong enough to inhibit the growth in the notches of the leaf completely.

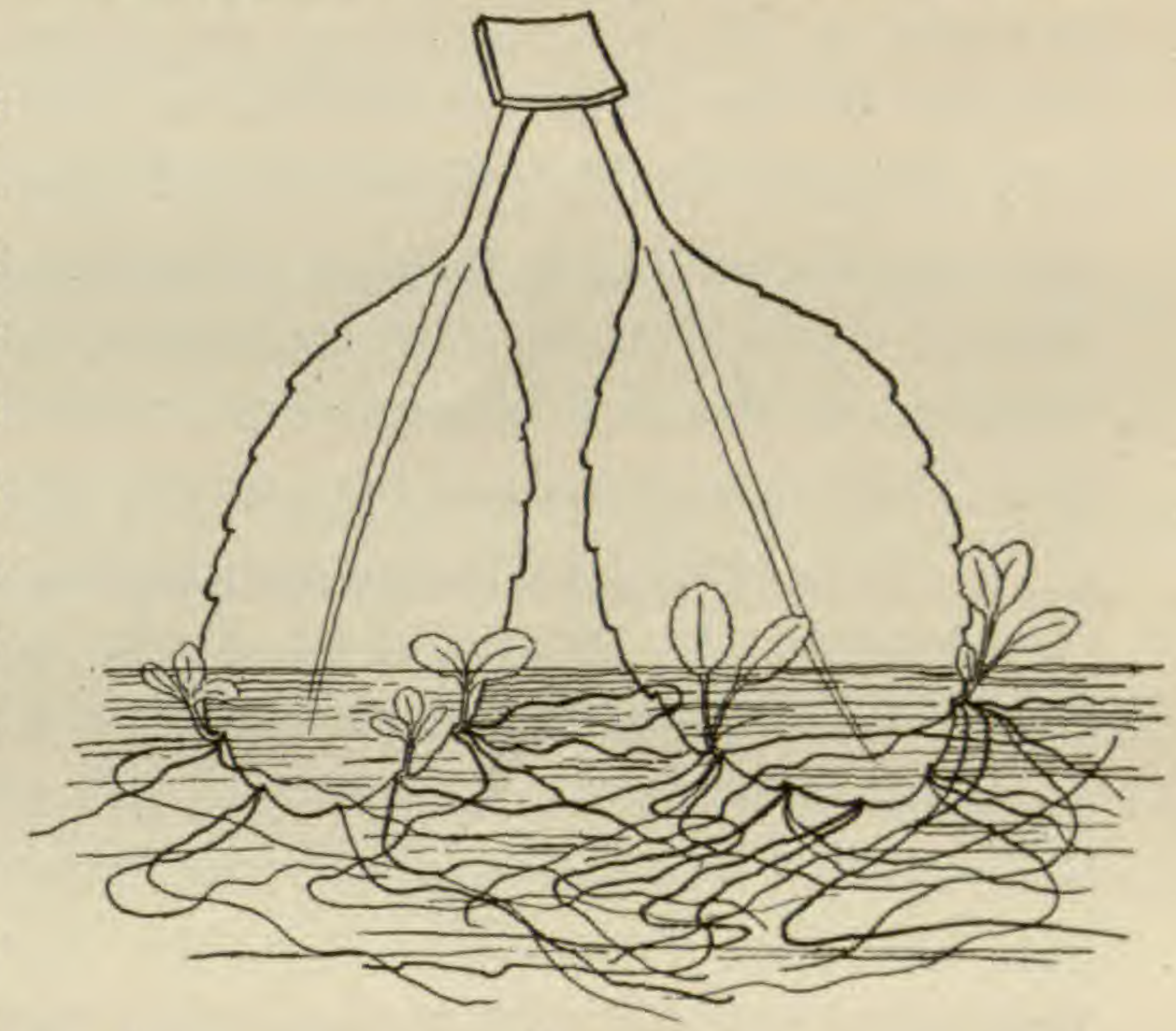


Fig. 16

All these statements can easily be verified. Some variation is met in specimens of the type of fig. 14, inasmuch as very often only the roots will grow out from the notches, while the shoots will not develop beyond tiny beginnings.

In all the experiments we have thus far described the suction effect of the stem inhibited the growth of the notches of the leaf. If the theory be correct that it is only the sap flow to or from a dormant bud which determines whether the latter will grow or continue in a resting condition, the reverse experiment should also be possible; namely, that by accelerating the growth of notches in a leaf it should be possible to inhibit the growth of buds on the stem. From all that has been said this experiment could only meet with success where the suction effect of the stem is moderate. Two leaves of the same node are cut from the plant, the cortex of the piece of stem is cut lengthwise and the wood taken out from the stem (fig. 16). This leaves two leaves connected only by the cortex of the stem (fig. 16). The leaves are placed with their tips under water to induce a rapid growing out of the notches at the tips of the two

Fig. 17

leaves. Other leaves of the same plant are suspended in moist air (fig. 17). Figs. 16 and 17 were drawn after 22 days. The leaves suspended in air (fig. 17) had formed vigorous roots (*R*) and a shoot (*S*) at the cortex. The leaves themselves had formed, as usual in this case, numerous roots and some tiny shoots. The notches of the leaves suspended in water (fig. 16) had formed very powerful shoots and roots and this inhibited the growth of roots and shoots in the cortex. Needless to say, these experiments were all carried out on many specimens, as were all the experiments reported in this and the previous paper. The experiments show that a vigorous growth in the notches of a leaf can act as a center of "suction" which may prevent the flow of sap to the cortex and thus prevent the growth there, if the suction by the stem, or in this case the cortex, is not too strong.

If the whole piece of the stem is left with its two leaves instead of the cortex alone, the experiment may also succeed, but it is not so reliable since the suction of the stem is greater in this case. If the experiment is made with one leaf and a complete piece of a stem and the leaf is put partly in water, the leaf cannot suppress the formation of the shoot on the opposite side of the leaf, since the suction effect of the stem cannot be overcome by the leaf. But when we have only a piece of cortex instead of a piece of complete stem (with wood), the "suction" power of the cortex alone is less than that of a complete piece of stem, and hence can be suppressed if a vigorous stream of sap to the growing notches of the leaves is started, as is the case in fig. 16.

It is hardly necessary to state once more that the term "suction" effect of a growing notch or bud is used only to illustrate the direction and relative velocity of the flow.

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THE SEPARATION OF OXIDASE REACTIONS FROM THE CATALASE REACTION

G. B. REED

(WITH THREE FIGURES)

It is an interesting fact that, with one or two exceptions, the oxidases of plant and animal tissue, even when purified by the best existing methods, still give the catalase reaction, that is, decompose hydrogen peroxide with the evolution of oxygen in its molecular or relatively inactive form. The relation of this catalase action to the physiological activity of the organism is not understood, although several explanations have been advanced.

LOEW[1] was the first to isolate a body capable of decomposing hydrogen peroxide but possessing no other enzyme properties. This body he called *catalase.* Many investigations since this time have proved the correctness of LOEW'S results. SENTER[2] prepared from blood a catalase free from haemoglobin or oxidases. LIEBERMANN[3] has shown that aqueous extracts of the mesenteric fat of hogs and rabbits decompose hydrogen peroxide without showing any oxidase reactions. Catalase in every way similar to that isolated by LOEW has also been prepared by WENDER[4] from yeast, and by PAZZI-ESCOT[5] from various higher plants.

As a result of his observations, LOEW (*loc. cit.*) reached the conclusion that catalase is universally distributed, occurring not only in every organism but in every living cell. He assumed, therefore, that it must have some definite function. Since hydrogen peroxide results from the oxidation of many readily oxidizable substances, he regarded it as at least conceivable that this compound may be produced in the living cell as a result of respiratory processes. The accumulation of such a substance would doubtless

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[1] LOEW, O., Report no. 68. U.S. Dept. Agric. p. 47. 1901.

[2] SENTER, G., Zeit. Physikal. Chem. 44:257–263. 1903; 51:673–705. 1905.

[3] LIEBERMANN, L., Pflüger's Archiv. 104:119–233. 1904.

[4] WENDER, Chem. Zeit. 28:300–301, 322–323. 1904.

[5] PAZZI-ESCOT, Bull. Soc. Chem. Par. 27:280–288. 1902.

prove harmful, and its destruction by catalase would not only prevent injury, but the oxygen liberated would be used again in respiration.

USHER and PRIESTLY[6] believe that hydrogen peroxide is formed in relatively large amounts in photosynthetic processes, and that its decomposition by catalase prevents it from reaching a sufficient concentration to bleach the chlorophyll.

On the other hand, SHAFFER[7] considers that the decomposition of the peroxide is of advantage principally in checking oxidation processes, basing his theory on the fact that the oxygen liberated from the decomposition is a poorer oxidizing agent than the hydrogen peroxide. In this connection BATTELLI and STERN[8] state that catalase retards the oxidation of ferrous sulphate in the presence of animal tissue.

There is very little experimental evidence, however, to indicate that hydrogen peroxide is actually produced in living tissue, except under specialized conditions. Moreover, it seems to be not particularly toxic, since the writer has found that wheat, rice, and radish seedlings develop without ill effects in as high concentrations as 0.5–1 per cent. The hypothesis that catalase is of advantage in checking oxidations is also largely invalidated by the observation of BACH and CHODAT[9] that catalase acts only on hydrogen peroxide, and not on organic peroxides or on the oxygenases; for it is these oxygen compounds rather than hydrogen peroxide that are concerned in respiratory oxidations. The writer has found also that while colloidal platinum decomposes hydrogen peroxide, it has no action on the oxygenases. An extract of the outer tissue of potatoes, which exhibits an active oxygenase in its direct action on gum guaiac (free from peroxide), was shaken for some hours with colloidal platinum, but after removing the platinum by filtration[10]

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[6] USHER, F. L., and PRIESTLY, J. H., Proc. Roy. Soc. London 77:369–376, 78: 318–327.

[7] SHAFFER, P. A., Amer. Jour. Physiol. 14:299–312. 1905.

[8] BATTELLI, F., and STERN, L., Compt. Rend. Acad. Sci. 141:916–918. 1904.

[9] BACH, A., and CHODAT, R., Ber. Deutsch. Chem. Gesells. 37:1342. 1904.

[10] This sample of colloidal metal could be removed from its solution by passing through a hard filter paper; this is shown by the fact that the filtrate from a watery suspension had no action on hydrogen peroxide.

it was found that the oxygenase was quite as active as before the treatment.

From these observations it appears that the function of catalase in protecting the organism against hydrogen peroxide is extremely limited, if indeed it may be said to exist at all.

Finally, it has been suggested many times, and supported by a considerable body of experimental data, that the action of catalase is associated with the reactions of the oxidases. Although the isolation of catalase has shown that the oxidases are not of necessity concerned in the decomposition of hydrogen peroxide, as was originally supposed, the idea is still held generally that the action of the oxidases is in some manner connected with or dependent upon the catalase reaction.

This association of oxidase action with the decomposition of hydrogen peroxide was suggested at an early date by SCHOENBEIN. He found that plant and animal tissues were usually capable of effecting the decomposition of hydrogen peroxide and activating many oxidation processes. In studying the relative power of various organs of the animal body to break down hydrogen peroxide, SPITZER[11] found them to stand in about the same order in which ABELOUS and BIARNÈS[12] had arranged them in respect to their ability to oxidize salicylic aldehyde. From this correlation SPITZER was led to believe that both processes have the same cause, and he, therefore, used the rate of evolution of oxygen from hydrogen peroxide as a measure of the oxidizing ability of tissues and tissue extracts.

These observations have been extended by LOEVENHART and KASTLE,[13] who have also studied the behavior of certain inorganic catalyzers in this connection. They report a striking parallelism between the rate at which a number of substances activate the oxidation (by hydrogen peroxide) of formic acid or of formic aldehyde, and the rate of hydrogen peroxide in the presence of these substances. They accordingly conclude that catalase action

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[11] SPITZER, W., Pflüger's Archiv. **67**:615–656. 1897.

[12] ABELOUS and BIARNÈS, Arch. de Physiol. **8**:311. 1896.

[13] LOEVENHART, A. S., and KASTLE, J. H., Amer. Chem. Jour. **29**:397–437, 563–588. 1903.

and the activation by enzymes of oxidations in the presence of hydrogen peroxide (peroxidase action) are closely correlated and may be referred to a common cause.

A study of the relations existing between the catalase and oxidase actions of colloidal platinum and of certain plant extracts has led the writer to a different conclusion. The present paper contains an account of the experiments with colloidal platinum.

In a previous paper[14] it was pointed out that the *direct* oxidase action (that is, in the absence of peroxides) of colloidal platinum, is dependent upon the amount of oxygen combined with it; that the reaction can be completely inhibited by electrolytic reduction, and again renewed by recharging the platinum with oxygen at an anode. It was also pointed out that the peroxidase action is essentially the same process; namely, in the presence of hydrogen peroxide the metal is kept fully charged with oxygen, while it is at the same time actively giving up oxygen to the substance undergoing oxidation.

The amount of oxygen which could be combined with the platinum electrically was only sufficient to bring about a very small amount of oxidation as compared with that effected by a low concentration of hydrogen peroxide. It was expected, therefore, that the amount of oxygen in combination with the platinum, when it was introduced into a solution of hydrogen peroxide and an oxidizable substance, would have very little effect, either on the rate of reaction or on the amount oxidized. Several measurements proved this to be the case.

Two platinum disks of exactly the same size, 2 cm. in diameter, were cut from the same piece of foil, sealed into glass tubes for convenience in handling, and platinized by the usual method. Care was taken to continue the platinizing for the same length of time with the same current, so that equal amounts of colloidal platinum were deposited on each electrode. These were then attached to opposite poles of a 110-volt circuit delivering one ampere and connected by placing in the opposite arms of a U tube containing dilute salt solution. In this way one electrode was subjected to active oxygen, the other to active hydrogen.

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[14] REED, G. B., BOT. GAZ. **62**:53–64. *figs. 4.* 1916.

The peroxidase activity of the two preparations was then compared by removing them from the electrolytic apparatus, washing them quickly by dipping them several times into a considerable volume of distilled water, and placing them separately in flasks, each of which contained 100 cc. of the following mixture: 0.1M formaldehyde, 0.03M NaOH, and approximately 0.1M H_2O_2. The solutions were kept well stirred and at a constant temperature of 18° C. on a water bath. At frequent intervals portions consisting of 2 cc. each were removed and titrated with 0.05M HCl for the amount of formic acid produced from the oxidation of the formaldehyde. The results are indicated in fig. 1, where the curve representing the action of the oxidized platinum is seen to be identical with that which represents the action of the reduced platinum. The points indicated by an ⊙ represent the reaction of oxidized platinum, and the points indicated by a × represent the reaction of reduced platinum. It is thus apparent that the two preparations of platinum are equally effective in this oxidation. Oxidation

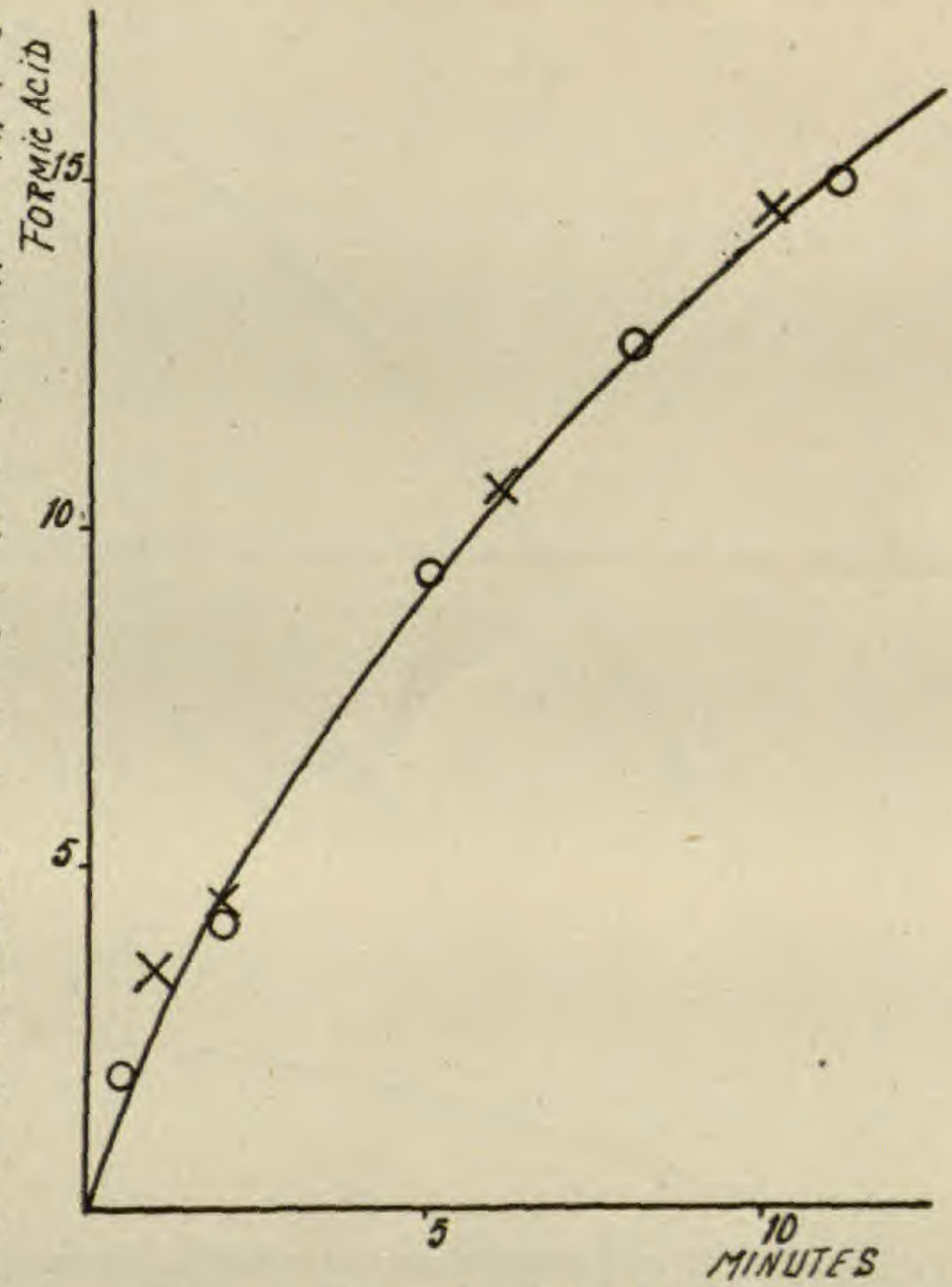

FIG. 1.—Curve showing oxidation of formaldehyde by hydrogen peroxide in presence of colloidal platinum: points indicated by ⊙ are from an experiment in which the platinum had been subjected to active oxygen just previous to placing it in the formaldehyde mixture; points indicated by × are from a second experiment in which the platinum had been previously subjected to active hydrogen; ordinates represent amounts of formic acid formed (expressed as the difference in number of cc. of 0.05M HCl required at the beginning and during the course of the experiment); abscissae represent time in minutes.

(in the presence of hydrogen peroxide) of potassium iodide, pyrogallol, and SPITZER's reagent was likewise found to be equally catalyzed by platinum, whether it had been treated with active oxygen or with hydrogen.

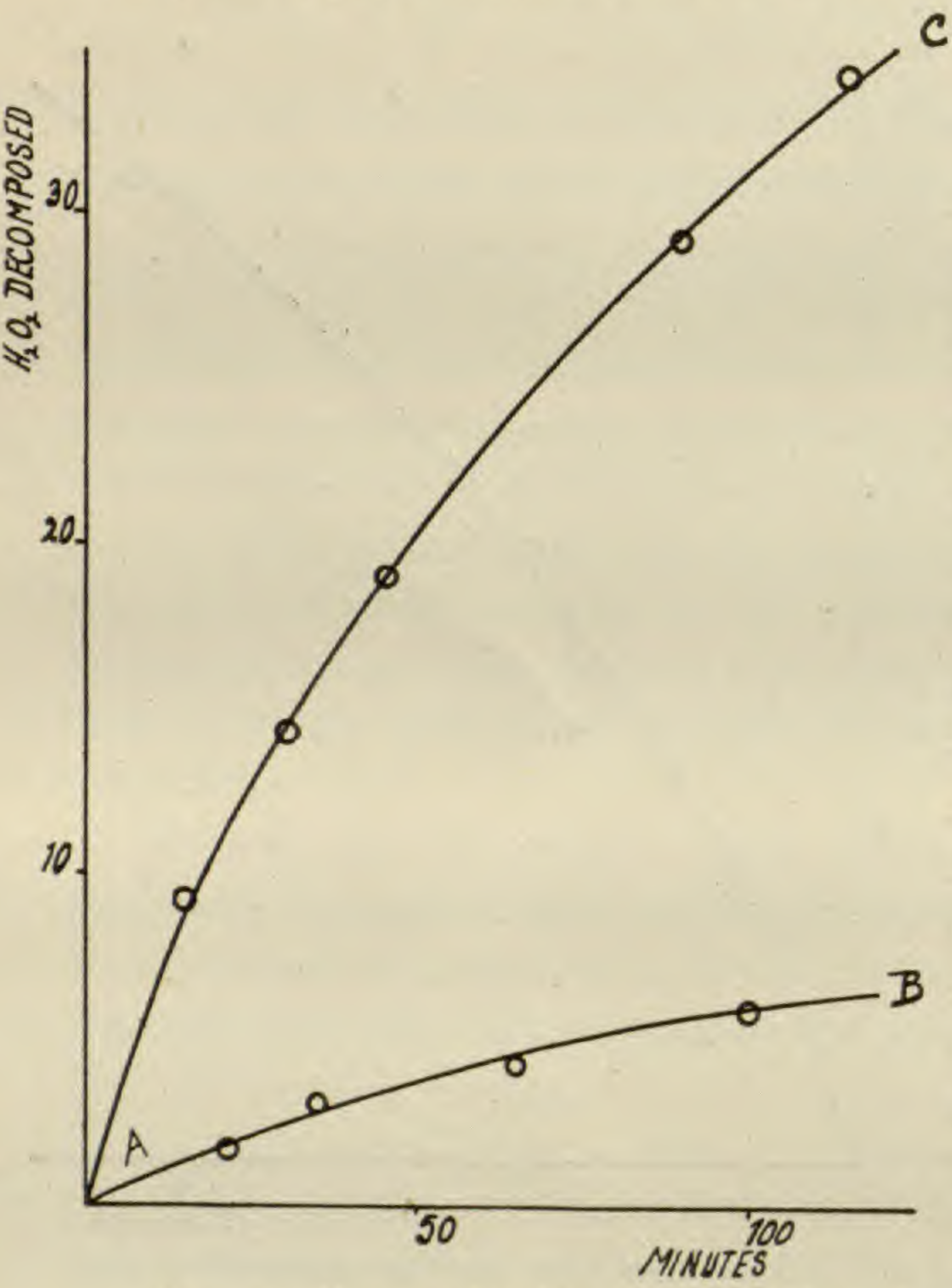

FIG. 2.—Curves showing decomposition of hydrogen peroxide: *AB* shows activity of platinum black which had previously been subjected to active oxygen by using it as an anode; *AC* shows action of platinum black which had previously been subjected to active hydrogen by using it as a cathode; ordinates represent percentage of hydrogen peroxide decomposed; abscissae represent time in minutes.

The action of platinum black in decomposing hydrogen peroxide, however, was greatly modified by its previous treatment. The two electrodes, freshly platinized and subjected to active oxygen and hydrogen as before, were placed separately in flasks, each of which contained 100 cc. of 0.1M hydrogen peroxide, to which a mixture of phosphates (5 cc. of 0.1M NaH_2PO_4 and 10 cc. 0.1M Na_2HPO_4) had been added in order to preserve the neutrality of the solution. As the decomposition of the peroxide proceeded, portions consisting of 2 cc. each were pipetted out, acidified with H_2SO_4 and titrated with potassium permanganate for the amount of hydrogen peroxide left in the solution.[15] The results are shown in fig. 2, where *AB* indicates

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[15] Since hydrogen peroxide reduces acidified potassium permanganate quantitatively, the amount of the latter used in a titration is a measure of the unchanged peroxide present in the solution. EULER (Hofmeister's Beiträge 7:1. 1908) has employed this method in the study of catalase.

the rate at which hydrogen peroxide is decomposed by platinum black which had previously been subjected to active oxygen (by using it as an anode), and AC indicates the rate obtained with platinum black which had previously been subjected to active hydrogen (by using it as a cathode); it is apparent that the latter treatment greatly accelerates the catalase action. Thus while electrolytic oxidation or reduction of the platinum apparently has no effect on the peroxidase action of the metal, the catalase action is very greatly modified. That this treatment has no effect on the peroxidase action may be explained by following the oxidation potential of the platinum during the reaction. In a previous paper (*loc. cit.*) it was pointed out that in oxidations by hydrogen peroxide, where colloidal platinum played the part of a peroxidase, the peroxide served to maintain the potential on the catalyzer at a maximum, although the substance undergoing oxidation would tend to pull down the potential. If this be true (since previous treatment with either active hydrogen or oxygen has no effect on the peroxidase action) the same potentials should be

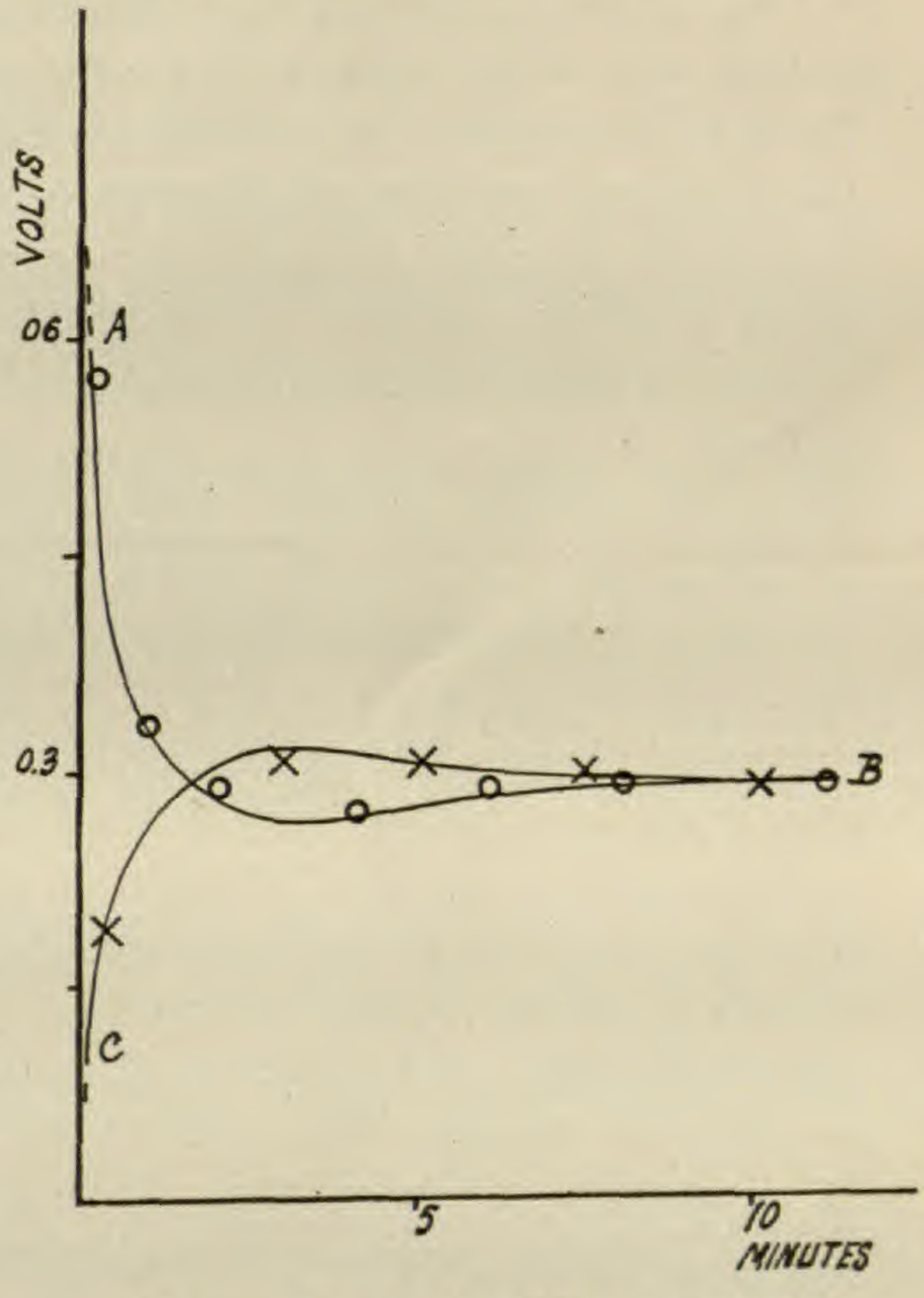

FIG. 3.—Curves of oxidation potential of a platinum cathode in a 0.1M solution of hydrogen peroxide: upper curve AB represents drop in potential on an electrode which had previously been treated with active oxygen by using it as an anode; lower curve CB represents rise in potential on an electrode which had previously been subjected to active hydrogen by using it as a cathode; ordinates represent volts; abscissae, time in minutes.

reached in the peroxide solution, whatever the previous condition of the platinum. The following experiment shows this to be the case.

One of the platinized electrodes which had previously been treated with active oxygen, was connected to the system: $Hg-Hg_2Cl_2-KCl-H_2O_2-Pt$, and the oxidation potential determined.[16] The hydrogen peroxide solution was of the same concentration as used in the previous decomposition experiments (0.1M), and contained a similar solution of phosphates to preserve neutrality. The rate of drop in potential is shown by the curve *AB*, fig. 3. The platinum electrode was then treated with active hydrogen and placed in a similar solution of hydrogen peroxide. In this case the initial potential was low but rapidly increased, as shown by *CB*, fig. 3, to the same equilibrium as reached in the previous case. By comparing these curves it will be apparent that the previous treatment has no effect on the potential assumed by the platinum after it has been a short time in the peroxide.

Although the potential changes on the platinum indicate why previous oxidation or reduction should not alter the peroxidase activity, it makes more complicated the problem of the changes in catalase activity. It is apparent, however, that factors which have no effect on the peroxidase activity do alter the catalase action. In other words, the peroxidase action is quite independent of the rate of hydrogen peroxide decomposition, and the two reactions may be regarded as quite separate. Further experiments by the writer indicate that a similar separation of the oxidase from the catalase reaction may occur in the living cell. These experiments will be discussed in a subsequent paper.

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[16] REED, G. B., BOT. GAZ. **61**:523–527. *figs. 2.* 1916.

SOME FACTORS INFLUENCING NITROGEN FIXATION AND NITRIFICATION[1]

BRUCE WILLIAMS

Progress in the knowledge of soil bacteriological investigations must necessarily depend upon an acquaintance with the individual factors that determine the efficiency of a particular group of soil organisms. Once the relative importance of these factors is determined, the unessential discriminated from those which are of paramount importance, a constructive policy for the promotion of bacterial activity may be announced that will be reflected in an increased fertility of agricultural lands. Investigations on the two general processes, nitrogen fixation and nitrification, have been reported recently from these laboratories.[2] These reports have announced the effects of various organic and inorganic fertilizers in developing certain bacterial floras; the stimulus exerted by cultivation on the two processes just mentioned has been noted; and the comparison of a number of soil types with reference to their bacterial activities was emphasized in the investigations. The results of this work were such as to suggest a number of interesting possibilities to account for unusual conditions that were here and there encountered. Accordingly, an effort has been made to detect some of the influences that are responsible for these conditions and to place certain factors in the relative positions of importance. The data in this paper, which are in every case somewhat preliminary are closely connected with those of the previous publication and are accumulative toward establishing certain contentions which have heretofore been suggested.

The moisture content of a soil with relation to its free nitrogen fixing power is a matter which it would be well to investigate. The rather meager information on this subject has recently been aug-

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[1] Paper 44 from Lab. Plant. Path. and Bact. Va. Agric. Exp. Sta.

[2] REED, H. S., and WILLIAMS, BRUCE, Nitrogen fixation and nitrification in various soil types. Va. Agric. Exper. Sta. Tech. Bull. 3. 1915.

mented by an interesting article by LIPMAN and SHARP,[3] giving the point at which maximum fixation is reached in a certain soil and suggesting that there are really two maxima of fixation with reference to moisture content, the one that is most favorable to aerobic bacteria, and the other at which the anaerobic forms most actively flourish. These tests measured nitrogen fixation under the influence of varying percentages of moisture. Of significance also is the effect of various periods of drying on the vitality of the nitrogen fixing flora. The ability of those organisms to withstand long exposure to drying serves somewhat as an index to their activity in arid regions and in more temperate climates after long periods of drought.

It is true also that if any progress is ever to be made in attempts to introduce cultures of free nitrogen fixing organisms, *Azotobacter*, for instance, artificially into soils, the persistence of such species under exposure to drying will be an important determinant in their efficiency. Such phases of the problem as these received attention in the present tests.

Soils which had been in the laboratory 12–18 months, inclosed in glass jars and practically air dried, were employed. All of these soils had been tested originally for this nitrogen fixing power, giving additions of nitrogen ranging from 2.3 to 16.7 mg. per 1 gm. of mannite consumed. *Azotobacter* had been isolated and grown in pure culture from probably 25 of the soils. All of these soils were now taken and 5 gm. inoculations were made into 25 cc. of Ashby's medium in order that qualitative examination might be made for the organism. Examinations made after 4 days revealed *Azotobacter* in only 4 of the soils. Attempts to isolate the organisms from these were unsuccessful, although it is probable that if the efforts had been repeated the organism would finally have been obtained. A number of soils from which it was positively known that *Azotobacter* had been isolated when first brought to the laboratory were then taken, their moisture content brought up to 20 per cent, and the soils kept in the incubator room at 28° C. for 3 weeks. An opportunity was then afforded for the bacterial flora to revivify

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[3] LIPMAN, C. B., and SHARP, L. T., Effect of moisture content of a sandy soil on its nitrogen fixing power. BOT. GAZ. **59**:402–406. 1915.

itself and regain something of its original status, but similar qualitative tests at the end of 3 weeks showed *Azotobacter* present in only 3 soils, and from one alone was the organism successfully isolated. It appeared from these tests that one at least of the free nitrogen fixing organisms had materially deteriorated through the processes of drying attendant upon 10 months' storage of the soil in the laboratory.

To determine to just what extent drying affected the fixing power of the soils as a whole was the object of the next series of tests. Four soils were selected which had exhibited rather extraordinary fixing powers when first brought from the field, but which had been in the laboratory for 15 months. Fixation tests were made with them, the solution method being employed, which uses 10 gm. of soil in 100 cc. of Ashby's medium, incubated for 21 days, with total nitrogen analysis for increase at the end of that period. The films which formed on two of the flasks were good and gave evidence of the presence of a vigorous culture of *Azotobacter*, but the two remaining ones did not exhibit this characteristic evidence. In table I the efficiency of the soils in question for fixing nitrogen in solution is given.

TABLE I

EFFECT OF DRYING ON THE NITROGEN FIXING POWER OF CERTAIN SOILS

No.	MOISTURE CONTENT AFTER 15 MONTHS	MG. NITROGEN FIXED PER 100 CC. ASHBY'S MEDIUM		
		November 1913	January 1915	Percentage of decrease
1..........	1.8	10.6	7.5	30
2..........	1.3	13.4	8.1	40
3..........	1.0	15.4	11.6	24
4..........	3.8	13.9	7.8	43

The soils lost 24–43 per cent of their original efficiency for fixing nitrogen during the period of 15 months' storage. Whether this falling off in nitrogen fixing power is due particularly to attenuation of aerobic or anaerobic forms is difficult to say, but from evidence previously obtained on the persistence of *Azotobacter* under drying, as previously noted, it is probable that the vitality of this organism was materially lowered, and to this condition may be referred in large measure the decrease in addition of nitrogen.

A rich garden soil was obtained in the spring of 1915. It was known to have a vigorous *Azotobacter* flora and was examined to determine the effect of short periods of drying on its fixing power. Tests were made at various intervals up to 30 days. The results are recorded in table II.

TABLE II

THE EFFECT OF SHORT PERIODS OF DRYING ON NITROGEN FIXING POWER OF GARDEN SOIL

Percentage of moisture	Date of tests	Mg. nitrogen added by 10 gm. soil in 100 cc. Ashby's medium (after 21 days)
9.1..........	June 7	16.4
6.3..........	" 8	17.0
2.9..........	" 9	14.8
1.5..........	" 18	10.1
1.2..........	July 7	10.1

The gradual decrease in the moisture content of the soil was not reflected in any notable falling off in its nitrogen fixing power until the test made after 10 days' drying. After 30 days, the moisture content remaining stationary, there was no further diminution. Up to this time the nitrogen fixing flora had retained practically 60 per cent of its original efficiency for fixing nitrogen.

The nitrogen fixing flora significantly decreases in its activity under the influence of drying, as the foregoing tests indicate. This deterioration is manifested as early as two weeks after removing the soil from the field and exposing it to the ordinary laboratory conditions of drying. After 15 months, however, a number of soils retained an ability to fix nitrogen that was somewhat surprising, which indicates that some species at least have considerable resistance to drying. A number of qualitative tests for *Azotobacter* in soils kept in storage for 15 months indicate that this organism is more easily attenuated than some other species.

The striking failure of some soils to fix nitrogen lends itself to speculations as to the underlying causes for such a condition. It appears, however, that the nitrogen fixing flora is so intimately connected with the humus content of a soil that this latter factor is by far the most important influence in connection with the process. The possibility that certain other factors lend a depressing effect has been eliminated to a degree from consideration in this laboratory by results of certain work with reference to toxic conditions of the soil. Whatever may be the inimical effects on higher plants of a toxic condition, if such in reality often exists, it is extremely doubtful whether similar effects are exerted on the bacterial flora. At least a number of compounds which are supposed to be responsible for soil toxicity have been shown to exhibit little deleterious effect on the growth of *Azotobacter*.[4] Evidence from further tests goes to show that the soil extract itself probably does not carry substances which would retard fixation.

Three soils which possessed conspicuously low fixation powers were selected and extracts made therefrom. The extracts were made into Ashby's medium and fixation tests with pure cultures of *Azotobacter* were carried out. Along with the flasks containing the extracts were controls using Ashby's solution made with distilled water. The same strain of pure culture was used for inoculating all the flasks. After 21 days the contents of the flasks were analyzed for total nitrogen. The nitrogen fixed by the culture grown on the extracts from the 3 soils was 6.5, 7.6, and 6.7 mg. nitrogen respectively. Those grown on the control flasks gave 6.8 mg. There was certainly no depressing effect registered here from using extract from soils deficient in nitrogen fixing power in substitution for distilled water in the culture medium.

The studies on nitrification (*loc. cit.*), which up to this time had included an examination of some 93 soils, were continued along with the work on fixation. Among the soils examined were a number which had exhibited signal evidence of poor nitrifying power. These in the majority of cases would be classed as poor soils from a practical agricultural standpoint, and their bacterial activity was closely correlated with this condition. Previous tests with lime had emphasized the stimulus which it exerts on nitrification, and to measure its efficiency on these soils, particularly deficient

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[4] REED, H. S., and WILLIAMS, BRUCE, The effect of certain organic soil constituents upon nitrogen fixation by *Azotobacter*. Va. Agric. Exper. Sta. Tech. Bull. 4. 1915.

in their nitrifying power, would be a matter of considerable interest. Since it is likely that the humus content of a soil most intimately of all factors controls its nitrifying efficiency, it was decided to observe the effect of the addition of sugar to these soils in comparison with that produced by the addition of lime. Accordingly, two 100 gm. portions of each soil were measured out into glass jars, the moisture content brought up to 20 per cent, and the jar placed in the incubator room. To one portion of the soil 0.15 per cent $CaCo_3$ was added, and to the other 2 per cent of mannite. After 3 weeks, a period allowed for the lime and sugar to exert some influence on the bacterial flora, an optimum moisture content being maintained, 0.1 per cent ammonium sulphate was added to measure the nitrifying power of the soils. All of these soils, it will be recalled, had been tested for nitrifying power without the addition of lime, and a threefold comparison was thus afforded. Table III gives the quantities of nitrates found with and without lime.

TABLE III

COMPARATIVE NITRIFYING POWER OF CERTAIN SOILS WITH AND WITHOUT LIME

No.	MG. OF NITRATE NITROGEN PER 100 GM. SOIL		No.	MG. OF NITRATE NITROGEN PER 100 GM. SOIL	
	Without $CaCO_3$	With 0.15 per cent $CaCO_3$		Without $CaCO_3$	With 0.15 per cent $CaCO_3$
1	...	...	13	...	2.12
2	...	1.5	14	0.6	7.6
3	...	...	15	1.8	7.2
4	...	...	16	...	1.4
5	3.3	8.8	17	1.4	8.8
6	5.1	1.2	18	...	1
7	3.0	...	19	1	1
8	7.2	12	20	3	5
9	...	...	21	1	1
10	6.5	1.6	22	...	Trace
11	...	2.2	23	1	1
12	...	1			

Without the use of lime a number of soils completely failed to nitrify ammonium sulphate, and few exhibited what might be termed an average nitrifying power as judged by the method employed. While under the influence of lime there is an increase in the majority of the soils, although the stimulation is not especially noteworthy. It appears that where there is some development of the nitrifying flora in the soil originally the effects of lime are decidedly more evident than in those soils apparently devoid of nitrifying power. The results of these tests are not to be construed as evidence depreciating the effect of lime on nitrification. They are contributory to the fact that the nitrifying flora of a soil cannot be developed merely by the use of lime in the absence of other factors more fundamental in their influence than is lime itself.

There was no formation of nitrates whatever under the influence of 2 per cent mannite. It not only failed to afford a source of energy for the nitrifying ferments, but exerted a depressing effect on the activities of such as were present. Smaller quantities of various sugars (0.5 per cent) have in some instances exerted a beneficial effect on nitrification.[5] The quantity used in these tests was possibly large enough to have a toxic effect on the soil organisms. It seems well established, however, that a satisfactory pabulum for nitrifying organisms cannot thus be so readily established.

Virginia Agricultural Experiment Station
Blacksburg, Va.

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[5] Coleman, L. C., Untersuchungen über nitrifikation. Centralbl. Bakt. **20**^{2}:401. 1908.

BRIEFER ARTICLES

THE STAINING OF WOOD FIBERS FOR PERMANENT MICROSCOPIC MOUNTS

In the preparation of wood fibers for permanent microscope mounts there is some difficulty in fixing a deep enough color, especially in the vessels of the hardwoods, when simple solutions of aniline dyes, such as safranin, are used; and the method of using such a reagent as Haidenhain's haematoxylin involves much time and loss of some elements due to washing. To avoid these difficulties a saturated solution of nigrosin in picric acid may be employed.

To macerate the fibers the original green or dry wood is soaked in a mixture of 5 per cent nitric and 5 per cent chromic acids in equal parts, with heating to about 70° C. if there is little time. A somewhat stronger solution may be used cold, and in practice has resulted in sufficient maceration in the course of one or more days, depending largely upon the kind of wood being treated. It is not necessary to macerate the complete piece of wood, as radial slices may be removed and the fibers carefully separated out with needles long before complete maceration is accomplished. After washing once in distilled water, the fibers are immediately put into the stain. To prepare the stain, saturate distilled water with picric acid and then saturate the solution of picric acid with nigrosin, and in applying to the fibers use a 25 per cent solution of the stain in distilled water. Fibers prepared according to this method are sufficiently stained, almost immediately, to a very fine light blue, which becomes darker until a deep blue-black is reached as they remain longer in the stain. In case the fibers do not take up the color, which seldom happens in the case of wood fibers, but is often the case in certain textile fibers, a stronger solution may be used. Even with the full strength, certain textile fibers, apparently those like cotton which contains no lignin, will not stain after a prolonged treatment. After staining, the material may be dehydrated and mounted in balsam in the usual manner. Benzol balsam is the kind we have employed, although there appears to be no reason why xylol balsam should not be used. The color will not hold in glycerin jelly mounts. In case a precipitate appears it may be removed by adding a drop of concentrated hydrochloric acid to the distilled water in which the fibers are washed just after staining.

To secure permanent mounts showing a differentiation between groundwood and sulphite pulp as ordinarily found in newsprint, the following modification is of value. Leave the newsprint, which has been reduced to pulp, in a moderately strong solution of fuchsin for some hours, dehydrate with absolute alcohol and transfer to benzol, transfer back to a moderate solution of nigrosin in absolute alcohol, and after a few minutes transfer again to benzol. Superfluous stain may be washed out with either fresh absolute alcohol or with benzol. Mount in balsam. The groundwood shows red while the sulphite is blue. Bordered pits in some of the tracheids of the sulphite show a red torus and the pit openings are often reddish.

Further studies are being carried on to determine the qualities of this method for staining other types of material.—H. N. LEE, *Forest Products Laboratories of Canada, Montreal, Canada.*

CURRENT LITERATURE

BOOK REVIEWS

Individuality in organisms

Continuing his important and illuminating contributions to philosophical biology, CHILD[1] presents in clear and masterly fashion a new conception of the nature of the individuality of organisms which he has developed through many years of investigation of development and reproduction in the lower animals. The book deals with the very fundamental problem of the nature of the unity and order in the organism, the constancy and course of development, the maintenance of individuality in a changing environment, and the processes by which physiological isolation and reproduction occur. As the new theory of individuality applies to plants as well as to animals, and as much supporting evidence has been obtained from the study of plants, the discussion will prove to be extremely valuable to botanists as well as zoologists, and to all who are interested in the philosophical aspects of biological discussion.

The first chapter discusses the problem of the nature of individuality, which resolves itself into the problem of the nature, origin, and maintenance of the definite space and time relations existing among the reactions occurring in the fundamental reaction system, the protoplasm. A new dynamic theory of the origin and nature of organic individuality is presented in the second chapter. The way is prepared for it by a critical consideration of various theories which have been advanced in the past, such as the corpuscular theories of WEISMANN and others, which necessitate the assumption of an ordering principle or entelechy, the dualistic theories, physico-chemical hypotheses, chemical correlation hypotheses, theories of polarity and symmetry, etc.

The organic individual is conceived to be a system of relations between the protoplasmic substratum and the chemical reactions of metabolism; and the foundation of organic unity, according to CHILD, is the transmission of dynamic change of some kind through the protoplasm. The essential features of this dynamic conception of individuality are simple, and are presented with convincing force and clearness. An undifferentiated mass of protoplasm which has a uniform rate of metabolism in all its parts, and which is capable of becoming an individual, forms the starting-point. This mass is acted upon at some point by external factors which act as stimuli, with the result that metabolism is increased at the point of stimulation. In this purely mechanistic

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[1] CHILD, C. M., Individuality in organisms. 12mo. pp. x+213. The University of Chicago Press. 1915. $1.25.

way we have established a region of relatively high metabolic rate in the undifferentiated protoplasmic mass. From this point of high metabolic rate there is a radiation of dynamic change over the surface or through the mass of protoplasm. It is a transmissive, not a transportive, change, and in some way involves an increase in metabolic activity as it spreads over or through the protoplasm. In other words, there is a wave of increased chemical activity spreading away from the point of impinging stimulus.

This metabolic change cannot be transmitted to indefinite distances, for the intensity of the metabolic change decreases with transmission. This transmission-decrement partly determines the limits of transmission. Other factors upon which the limit of transmission depends are the intensity of the original stimulus and the relative conductivity of the protoplasm. The limit of transmission determines the size limit of the individual, for parts that lie beyond the transmission limit remain physiologically isolated.

Momentary action of the stimulus may lead only to a temporary increase in metabolic rate at the point of origin, but prolonged or frequent excitation may lead to more or less permanent changes in the protoplasm, chief among which is an increased irritability. The greatest increase in irritability occurs in the region of highest metabolic rate, which is the region of stimulus, and is progressively less in regions of lower and lower metabolic rates, reaching zero at the limit of transmission. Thus we may have established a gradient in the condition of the protoplasm, an irritability gradient, which constitutes the substratum for a persistent metabolic gradient independent of the local stimulus. For as soon as a persistent irritability gradient is established, uniform stimulation will be followed by a graded metabolism dependent upon the degree of irritability of each axial level. Such persistent metabolic gradients are looked upon as the starting-point of organization. They are factors in determining the direction of growth and differentiation, and are therefore the basis of the space and time relations of development.

The phenomenon of dominance of one part or region over other regions of the organism during development is simply a result of these differences in metabolic rates, levels of high rate dominating and controlling those of lower rate, and the interrelations of the parts of the organism, depending thus upon differences of metabolic rate, constitute the foundation of the unity and order exhibited by it. From this standpoint the organic individual is viewed as consisting of one or more gradients in a protoplasmic mass of specific physicochemical constitution; and the process of becoming an individual is merely the process of establishing the necessary gradients. Such gradients, which arise *de novo* in response to external conditions, and cannot *originate* in any other way, may nevertheless become hereditary and persist through many generations when once established.

This dynamic theory is distinguished by its satisfactory interpretation of organic unity in terms of differences in rate of metabolism and of transmitted change, rather than in terms of hypothetical organization and transportation of chemicals. And, being entirely mechanistic, it has the great merit of offering the possibility of experimental advancement toward a complete solution of the problems of individuality.

The three succeeding chapters present some of the evidence which supports this conception of the origin and nature of individuality. The third chapter presents the evidence for the existence of metabolic gradients. This evidence consists mainly in the demonstration of susceptibility, respiratory, and electric potential gradients in the organism coinciding with the supposed metabolic gradients. Embryological evidence for the existence of axial metabolic gradients, and the developmental gradients in cases of asexual and experimental reproductions are considered. The evidence is varied and extensive, and comes from a study of both animals and plants. It all agrees in showing that axial gradients in dynamic processes are characteristic features of organic constitution.

Chap. iv considers the evidence for the existence of physiological dominance in the process of individuation, in support of the conception that "the organic individual is fundamentally a dynamic relation of dominance and subordination, associated with and resulting from the establishment of a metabolic gradient or gradients." The evidence is obtained principally from experimental reproduction in plants and animals, and demonstrates the independence of apical regions in the developmental process, and the control of the development of other levels of the major axis by this independent apical region. The apical regions are the regions of most rapid metabolism, and they are physiologically dominant over the other subordinate levels.

Chap. v gives evidence bearing upon the problems of range of dominance, physiological isolation, and experimental reproduction. The experiments deal with the control of the spatial relations of parts and of the range of dominance by altering the length of metabolic gradients; the obliteration and redetermination of axial gradients; the extension of dominance during development; and physiological isolation and reproduction in plants. The final chapter is a general discussion of individuality from the new viewpoint, and considers such topics as the nature of dominance and inhibition, the origin of metabolic gradients and dominance, the relation of morphological differentiation to metabolic rates, the fundamental reaction system (protoplasm), the nature of agamic and gametic reproduction, and closes with a brief but trenchant discussion of such large problems as heredity and evolution from the dynamic standpoint.

An interesting contrast is drawn between animals and plants in discussing the fundamental reaction system. In animals the independent self-determining apical region begins the process of differentiation, and if the self-determined changes go far enough, they result in the formation of a nervous system, physiologically the most stable structure of the whole animal body. The greatest differentiation occurs in the region of highest metabolic rate. But in the plant the apical region remains undifferentiated, and the degree of differentiation increases with increasing distance from the apical point, a situation exactly the reverse of that found in animals. This difference between the animal and plant is traced to a difference in physiological stability of their respective protoplasms. In animals the stable molecules which form nervous tissue accumulate in spite of the high metabolic rate, while in plants the degree of physiological stability is low, and the high metabolic rate of the apical point may mobilize the whole substratum. It is only in regions of lower rate that the accumulations of visible structure can occur. In this contrast we see one of the fundamental differences between the two classes of organisms.

The reader is favorably impressed by the thoughtful style and illuminating manner in which the philosophical discussion proceeds. The dynamic conception of individuality presented in this work seems so reasonable, and the marshalled evidence is so clear and convincing, that it is bound to exert a profound influence on biological thought and theory. The author modestly states that this conception is "manifestly far from being a complete solution of the problem of organic individuality." It is an excellent working hypothesis, however, suggests many lines of experimental approach toward a final solution, and offers an attractive substitute for the unsatisfactory vitalistic conceptions which have held until now so prominent a place in our philosophy of organic life.—CHARLES A. SHULL.

NOTES FOR STUDENTS

Linkage and crossing-over in oats.—SURFACE[2] is continuing in *Genetics* a series of studies in oat breeding of which two former papers have appeared in the Annual Report of the Maine Agricultural Experiment Station. It will be very gratifying indeed if this new journal makes so strong an appeal to geneticists that work of high grade will no longer be buried with such ephemeral literature as that which makes up the bulk of nearly every station report.

In crosses between strains of wild and cultivated oats (*Avena fatua* and *A. sativa*), SURFACE has studied particularly the inheritance of qualitative characters in the flowering glumes. The base of the fertile floret in the wild oat is characterized by a broad callus associated with ease of abscission from the spikelet, whereas the lack of the callus in the cultivated oat is associated with persistence of the floret. First generation hybrids present the callus in an intermediate condition, and in subsequent generations typical Mendelian segregation takes place. There are, therefore, three types of bases, called, for short, "cultivated," "intermediate," and "wild." Perfectly correlated with the "cultivated" base are the almost complete absence of awns on the lower flowering glume, their complete absence from the upper flowering glume, the absence of pubescence from the rhachilla, the slight pubescence at the base of the first floret, and its complete absence at the base of the second floret. Conversely, the "wild" base is perfectly correlated with the presence of well de-

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[2] SURFACE, FRANK M., Studies on oat breeding. III. On the inheritance of certain glume characters in the cross *Avena fatua*×*A. sativa* var. *Kherson*. Genetics 1:252–286. 1916.

veloped awns on both flowering glumes, a densely pubescent rhachilla, and dense pubescence at the base of both fertile florets. The "intermediate" base, as would be expected, is associated with an intermediate development of the other characters. Thus, in some spikelets the first floret has a medium sized awn, but the second has none; likewise, the first floret has pubescence at the base, but the second is glabrous. It is on the basis of differences between the first and second florets that SURFACE is able to correlate 7 putatively distinct characters with the "cultivated" type of base, but those whose view of Mendelian inheritance is less formal than the author's will wish very clear evidence before admitting them as distinct. SURFACE himself has suggested, following NILSSON-EHLE, that characters may be genetically present in an individual, but not manifest because of environmental conditions. In some strains of oats, for example, awns are potentially present, but develop only under certain external conditions. What is true of a population, acted upon by varying environmental factors, may equally well be true of homologous parts of a single individual, conditioned in development by their position on that individual.

Turning to a concrete case, the spikelet of *Avena* is composed of 2, or at most 3, fertile florets, presenting a gradation from the first, which is large and in every way the most highly developed, to the third, which is ordinarily a sterile or even vestigial structure. In a reduction series composed of so few members, and with so steep a gradient, we have ideal conditions for a variable character to be manifest in the first member and latent in the second. At any rate the reviewer would look for a physiological rather than a genetical explanation for the situation as regards awns and pubescence in the spikelet of *Avena*. If the first flowering glume is awned, it is a fair assumption that the second is potentially the same, even though its position be such that manifestation of the character is a physiological impossibility. Perhaps an underlying basis for the whole group of correlations might be found in the diminution of the vascular supply between the base and apex of the spikelet.

In addition to the characters showing perfect correlation, SURFACE discovered two pairs of characters showing partial linkage. The factor for pubescence on the back of the first flowering glume showed linkage with the factor for black color of the glume, and the pubescence of the back of the second flowering glume showed linkage with the "wild" base. In respect to these characters the data are too involved to be repeated here, but as interpreted by SURFACE they show (1) that the presence of pubescence is a dominant character in the case of the first flowering glume, but a recessive character in the case of the second glume, and (2) that the factor for pubescence of the lower glume is a basic pubescence factor, in the abscence of which the factor for pubescence of the second glume is without effect. The bare facts upon which SURFACE bases these conclusions are as follows. The first hybrid generation has the lower flowering glume pubescent, the upper smooth. In the one parent, *Avena fatua*, both glumes are pubescent; in the other, *A. sativa*, both are smooth. In no case does segregation in the second or third hybrid generation result in spikelets with the first glume smooth and the second pubescent.

It is not easy to accept a hypothesis which limits the operation of a Mendelian factor to one of two identical and adjacent morphological structures, nor to believe that the character of pubescence is in the case of one of these structures due to a dominant and in the other case to a recessive factor. Furthermore, unless the reviewer has failed to comprehend the data, a much simpler hypothesis will account for the situation. Let us postulate a single dominant factor for the glume pubescence; this factor is linked with the factor for black glumes. A second factor, linked with the factor for "cultivated" base, may be conceived of as a partial inhibitor of the pubescence factor. In accordance with the idea of position effect, it would operate most powerfully upon the second glume, resulting in a first generation hybrid with the pubescence only partially suppressed on the first glume, but completely suppressed on the second. According to this conception, and taking no account of crossing-over, all black-glumed plants in the second hybrid generation would have at least the lower glume pubescent. Both blacks and non-blacks would have undergone segregation with regard to the inhibiting factor, but since blacks alone carry the pubescence factor, a complete loss of the inhibitor could be manifested only by blacks, one-fourth of which would appear, in accordance with expectation, fully pubescent on both glumes. It appears to the reviewer that these considerations apply to the numerical data as well as one could expect.

The cross-overs detected by SURFACE were exceptions to the linkages between the factors for pubescent glumes and black (about 0.7 per cent), and the inhibitor of glume pubescence and the factor for "cultivated" base (about 1.5 per cent).

It seems unfortunate that the author has not used the conventional terminology of the grass inflorescence in his otherwise exceedingly well presented paper. Although he defines his terms, the paper is a long one, and if merely glanced over, the unusual terminology might be perplexing if not misleading. "Head" is used for panicle; "grain" for the caryopsis with its adhering lemma and palea; "pedicel of lower grain" for the portion of the rhachilla between the two fertile florets; "pedicel of upper grain" for the portion of the rhachilla above the second floret; "flowering glumes" for the flowering glume and lemma of the same floret; "dorsal side of grain" for the lemma; "ventral side of grain" for the palea.—H. H. BARTLETT.

Cohesion of water and the rise of sap.—From theoretical considerations RENNER[3] draws the following important conclusions: (1) The vapor pressure

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[3] RENNER, O., Theoretische und Experimentalles zur Kohäsionstheorie der Wasserbewegung. Jahrb. Wiss. Bot. 56:617–667. 1915. PFEFFER's Zeitschrift.

in fully turgid cells is equal to that of pure water, and of plasmolyzed cells to that of their cell sap. The vapor pressure of plasmolyzed cells with 100 atmospheres osmotic pressure is 7 per cent less than that of pure water. (2) Water movement in parenchyma is dependent upon difference in degree of turgescence and independent of difference in osmotic pressure. (3) If in a wilted leaf the osmotic pressure of the parenchyma is P atmospheres, there exists in the adjacent vessels a tension of P-1 atmospheres, and the vapor pressure of the water in the vessels is correspondingly reduced. (4) The energy potentials for moving water in the plant are the potential differences in imbibitional energy, osmotic energy, and hydrostatic pressure. These potentials originate from the transformation of a part of the energy potential which exists between the surface cells and the atmosphere in the way of vapor pressure differences. The rest of the primary potential is usable by transpiration alone.

In the experimental part RENNER makes use of the annulus of fern sporangia (*Polystichum Filix-mas*) for measuring the tensile strength of water under the conditions in which it exists in plant cells. The walls of these cells are impermeable to cane sugar in aqueous solution, and to certain substances in very low concentration within the cells, but they are more or less permeable to salts. In a solution of cane sugar with an osmotic pressure of 200 atmospheres the annulus cells are deformed and the sporangia opened, but no gas bubbles are formed within the cells (there is not a throwing movement of the sporangia), although the water within is stretched equivalent to about 200 atmospheres. Since the osmotic pressure of sugar solutions was not sufficient, and the walls were permeable to salts, RENNER turned to the vapor pressure method of determining the tensile strength of the water within these cells. Over a saturated solution of sodium chloride almost all of the sporangia spring with the formation of gas bubbles in the annulus cells. In the few not springing, the water in the annulus cells must be stretched approximately equivalent to the osmotic pressure of the solution, 368 atmospheres. There was little springing of the sporangia until the solutions used exceeded 300 atmospheres osmotic pressure.

Working independently, but with similar methods, URSPRUNG[4] concludes that the water in annulus cells of fern (*Pteris, Scolopendrium*) sporangia are under a tension (a stretch) of somewhat móre than 300 atmospheres at the time most of the sporangia spring. These determinations of cohesion exceed the values obtained by DIXON[5] in his measurements in glass tubes.

HALLE,[6] a student of RENNER, finds that generally when plant cells lose water by evaporation the protoplasm is not immediately withdrawn from the

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[4] URSPRUNG, A., Über die Kohäsion des Wassers im Farnannulus. Ber. Deutsch. Bot. Gesells. **33**:153–162. 1915.

[5] BOT. GAZ. **60**:74, 75. 1915.

[6] HALLE, HANS, Untersuchungen über Welken, Vertrocken, und Wiederstraffwerden, Flora **108**:73–126. 1915. Written by RENNER after the death of HALLE at the front.

wall, but the wall is deformed with the protoplast, owing to the cohesion of the contained water. Very considerable negative pressures or tensions of water can thus be developed in plant cells before bubble formation occurs; 250 atmospheres in the stellate hairs of *Verbascum thapsiforme*, less than 20 atmospheres in the hairs of *Lychnis coronaria*, and still less in the ringed velamen cells of orchids and the pith of orchid and *Sambucus* stems. This is independent of living protoplasm, for it occurs in dead cells. In thin-walled parenchyma the cells may be completely crushed without gas appearing, while in thick-walled parenchyma there is a considerable deformation of the walls before gas accumulates within the cells. In fully wilted leaves of *Alliaria officinalis* the tracheae are still free from gas, which means that the water within them must be under negative pressure equal to the osmotic pressure of the parenchyma. Vessels in storage organs withstand little negative pressure, while those of actively conducting tissue develop great negative pressure without bubble formation within them. If in wilted tissue air is admitted to a vessel by a wound, it does not pass through the side walls to intact vessels, although they bear water under considerable tension. When both sides of the cell wall are water free, air accumulates within rapidly even under slight negative pressure; while the cell is full of water, air does not accumulate negative pressure within even under many atmospheres.

In another article URSPRUNG[7] raises the question of the cause of bubble formation in "Tonometern," any apparatus used for the qualitative or quantitative determination of fluid cohesion. For water he believes bubble formation is not determined by the limits of cohesion, for the cohesion of water as measured by such instruments falls far below the cohesion calculated by VAN DER WAALS on the theoretical basis. The great variation in the tension at which bubbles appear, even under like conditions, also argues against the limits of cohesion being the cause of bubble formation. Since all evidence indicates that adhesion of water to the wall exceeds its cohesion, URSPRUNG believes that the limits of adhesion do not determine bubble formation. GERNEZ found that in supersaturated solutions of gases bubbles collect on the surface of containing or inserted solids and not within the liquid itself, because these surfaces bear adsorbed gas as inoculation centers. URSPRUNG believes that adsorbed gases (in very thin layers of submicronic or amicronic thickness) on the walls of the "Tonometern" cause bubble formation. Hence all attempted measurements of cohesion of water by such instruments give values far short of the actual cohesion. They measure rather the tension at which gas bubbles form under the influence of inoculating centers of adsorbed gases and the (gas) supersaturated condition of the water in such instruments. As bearing upon cohesion within tracheae, URSPRUNG emphasizes the fact that wood tissue adsorbs gas readily. The author uses considerable space in criticizing DIXON's experiments upon cohesion of water.

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[7] URSPRUNG, A., Über die Blasenbildung in Tonometern. Ber. Deutsch. Bot. Gesells. 33:140–153. 1915.

The four articles reviewed indicate that cohesive stretching of water is of great significance in plants. They show the magnitude of force thus available for work and give much insight into the conditions under which it is operative; also they add evidence for the cohesion theory of the rise of sap.

COPELAND[8] has devised a very simple but ingenious piece of apparatus for determining various facts concerning the movement of water through stems of lianas. From his measurements he concludes that the resistance offered by stems to water flow through them is very much less than claimed by other workers in this field; that the tension in the water column of the stem assumed by the cohesion theory does not exist; and that the living cells along the stem play no essential part in the movement of the water through it. COPELAND believes that the main problem of rise of sap remains, as he earlier stated,[9] "Why is this atmospheric pressure exhausted so slowly with the ascent of the tree that whatever height is reached, and however rapidly and forcibly water may be drawn from the wood, some pressure always remains?" Notwithstanding the ingenious character of these experiments and the suggestiveness of the results, much more work of an analytical sort is needed to establish a new theory of the rise of sap or to dispose entirely of the cohesion theory.—WILLIAM CROCKER.

Structure of the Boleti.—Very few studies on the finer structural details of the Boleti have been published. These have been fragmentary observations occasionally employed to supplement specific diagnoses drawn from the grosser features, or to interpret certain surface characters. A comparative study of the structure of all parts of the plant in a large number of species is greatly needed, and especially a study of the origin and differentiation of its parts is much to be desired. A contribution to the former phase of the subject is now presented by YATES[10] in the study of 6 Californian species, supplemented by two eastern ones.

In general, there is not such a marked differentiation of the tissues, when the size of the plants is considered, as one might expect who has not given some attention to these plants. According to the author's interpretation, the tissues of the pileus and stipe are differentiated into two main zones: an outer, the cortex ("rind"); and an inner, the trama ("context"). In some species the cortex of the pileus consists of elongate vertical hyphae, which in dry species give the pileus a tomentose appearance, as in *Boletus chrysenteron*. In the viscid species the outer layer of the walls of these hyphae is more or less gelatinized and the pileus is not tomentose, as in *B. granulatus*, *B. luteus*, and *B. cali-*

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[8] COPELAND, E. B., Über das Saftsteigen. Jahrb. Wiss. Bot. **56**:447–459. 1915. PFEFFER's Zeitschrift.

[9] COPELAND, E. B., BOT. GAZ. **34**:161–193, 260–283. 1902.

[10] YATES, H. S., The comparative histology of certain Californian Boletaceae. Univ. Calif. Publ. Bot. **6**:221–274. *pls. 21–25.* 1916.

fornicus (Murrill),[11] the last being subtomentose. In others the gelatinous matrix is firm and the pileus not viscid, or slightly so when wet, as in *B. edulis* and its variety *separans*.

Forms with a somewhat similar structure of the pileus present three zones: (1) in *B. Eastwoodiae* (Murrill),[12] form *A*, there is an outer zone of vertical interwoven brownish hairs which sometimes gelatinize to such an extent as to disappear, an intermediate zone of compactly woven hyphae, and the looser trama; (2) in *B. Eastwoodiae*, form *B*, the outer zone consists of rather distant hairs arising from a compact brown rind of interwoven hyphae; while (3) in *B. chrysenteron*, form *E*, there is a zone of densely interwoven, small brown hyphae between the palisade of hairs and the loose trama. In other species the structure of the pileus is more homogeneous, consisting of interwoven hyphae, larger and with a more open mesh in the trama, smaller and more compact and dark colored in the "rind." Such species have a glabrous pileus, as in *B. auriflammeus* and *B. Frostii*, there being 3 zones of different density.

The hymenophore (=stratum of tubes) is very uniform in the species studied. Cystidia are said to be wanting in most of those examined. In *B. granulatus* and *B. californicus* the cystidia form tufts of clavate cells which arise in the trama and project beyond the surface of the hymenium. They also occur on the dissepiment edges and on the stem.[13] In *B. luteus* the tufts of cystidia are smaller.

The general structure of the stipe is quite uniform. The hyphae in general are parallel with its axis, in some species almost strictly parallel, in others more or less interwoven. The central portion is loose and the hyphae of larger diameter; the outer portion compact and the hyphae of smaller diameter.[14] The surface of the stipe, then, is often characterized by outgrowths of short hyphae, cylindrical, clavate, or capitate cells, either in tufts, or covering the entire portion of the stem, thus forming a palisade layer. In the latter case the author speaks of this palisade as the "rind" (in *B. auriflammeus*). The "context" here then includes all the hyphae which extend more or less parallel with the axis of the stipe, that is, both "rind" and "context" of species lacking the palisade layer.

This palisade layer on the stipe in *B. auriflammeus* forms the tomentum, and in the opinion of the reviewer represents merely the vesture of the stem, not the cortex ("rind"). Cortex and medulla ("context") are of the same character as in those species with a smooth stipe, then, or where the vesture of the stipe is composed of isolated tufts of cells. This interpretation of the tomentum on the stipe of *B. auriflammeus* finds confirmation in the fact that the vesture of the stem is usually "reticulate" and represents very likely

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[11] *Rostkovites californicus* Murrill, Mycologia 7:44. 1915.

[12] *Suillellus Eastwoodiae* Murrill, N. Am. Flora 9:152. 1910.

[13] Absent or very rare in *B. brevipes* Pk., which MURRILL unites with *B. granulatus*.

[14] A general feature in the stipe of most agarics.

a depauperate condition of the hymenophore extending down on the stem, as in *B. edulis* described by YATES (form *B. pl. 22. fig. 10*) and by PATOUILLARD (Tab. Analyt. Fung. no. 9, 1883). Besides a few basidia, bearing spores, in this reticle on the stem, PATOUILLARD states that cystidia are also present similar to those in the normal hymenium of this species, while YATES says that cystidia are not present in the forms he examined, although one would suspect their presence from his figure. This vesture on the stem of some species may grade from a reticle above to an even tomentum below, or to tufts more or less crowded, or without a reticle tufts more or less crowded or disparate over the stem (*B. luridus*, for example). Similar examples of depauperate hymenia are abundant in the Basidiomycetes, and even occur in the Ascomycetes, in *Gloeoglossum difforme*, for example (see DURAND, Ann. Myc. **6**:421. *figs. 160–162.* 1908).

Some of the different forms of *B. chrysenteron*[15] described may be interpreted as due to variations in the vesture of the stipe, the tomentum of the pileus, and the color changes resulting from the presence of oxydases, due to age, vigor of growth, relation of the dissepiment edges to the stipe surface during the origin of the hymenophore, etc., which cannot be enlarged upon here. However, the author makes no mention of the structural features of the usually sterile dissepiment edges, which in many species of agarics, at least, bear a very interesting relation to the vesture of the stipe.

The author very properly remarks that, by authority of the Third Botanical Congress (Brussels, 1910), FRIES's *Systema Mycologicum*, 1821, is the starting-point for the nomenclature of the Hymenomycetes. But he follows MURRILL's nomenclature[16] of the Boleti, perhaps unaware that this author does not recognize the rules adopted by the Vienna and Brussels congresses, the two largest and most representative legislative assemblies of botanists ever held. One of the deplorable results of this hunting for names by groping in the mystic and heroic age of mycology is well exemplified by the attempt to introduce the generic name *Ceriomyces* for the section containing the largest number and most typical species of the genus *Boletus*, and citing as the type of this genus *Ceriomyces crassus* Battarra.[17] It is not likely that well informed botanists will discard the name *Boletus edulis*, which is in strict conformity with the international rules,[18] and use in its stead *Ceriomyces crassus*, a con-

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[15] The form *C* is very likely *B. subtomentosus*. Some students unite *B. chrysenteron* and *B. subtomentosus*. For some of the varieties of these two species, see BATAILLE, F., Les bolets, classification et determination des espèces. pp. 1–30. Besançon. 1908.

[16] N. Am. Flora **9**:133–161. 1910.

[17] BATTARRA, J. A., Fungi Agric. Arim. Hist. 62, 63. *pl. 29.* 1755.

[18] The generic name *Boletus* was properly retained by HENNINGS (ENGLER und PRANTL, Die Nat. Pflanzenfam. 1^{1**}:191. 1898) for the section containing the larger number of species, including *B. edulis*, his action being entirely in accordance with the principle of the international rules adopted at Vienna in 1905, and at Brussels in 1910 (see Article 45).

fusing medley of species, for European mycologists long since an undecipherable rebus.—GEO. F. ATKINSON.

Patrogenesis.—COLLINS and KEMPTON,[19] working on intergeneric hybridism in the tribe Maydeae, have had the great good fortune to bring to light a new case of the phenomenon discovered in *Fragaria* by MILLARDET, and termed by him "hybridation sans croisement ou fausse hybridation." The hybrid *Tripsacum dactyloides*×*Euchlaena mexicana*, carried through 3 generations, has shown no trace of characters derived from *Tripsacum*, aside from the fact that the original hybrid seed was "an unmistakable *Tripsacum* seed." Characters of *Euchlaena* appeared with the first leaves, and in further stages of development the plant was an almost normal *Euchlaena*.

In attempting to explain the complete exclusion of maternal characters from the hybrid, the authors have discussed two hypotheses: (1) that the *Euchlaena* characters have completely masked those of the *Tripsacum*, and (2) that the embryo of the original hybrid developed only from the male nucleus, which must be regarded as having dispossessed the female nucleus with which it would have been expected to unite. COLLINS and KEMPTON adopt the latter hypothesis, and look upon their hybrid as exemplifying a new type of inheritance, which they call "patrogenesis" in order to place the phenomenon in what they regard as a proper contrast with parthenogenesis. We may well doubt, in the absence of cytological data, whether this explanation is probable enough to warrant the introduction of a new term. BATESON'S terms monolepsis and amphilepsis, indicating hybrids in which the characters are brought in respectively from only one or from both parents, have already been introduced, and have the advantage that they imply nothing as to cytological conditions.

Maternal monolepsis is well known in the Orchidaceae, especially in the intergeneric hybrids between *Zygopetalum Mackayi* and species of *Odontoglossum*, *Lycaste*, and *Oncidium*. Paternal monolepsis is a rarer phenomenon, and its discovery in intergeneric grass hybrids is most interesting. That it is to be explained by merogony, however, should not be too incautiously assumed. It will be recalled that a similar explanation of DEVRIES' patroclinic hybrids in *Oenothera* was brought forward, with even some show of cytological evidence, but was afterward disproved.

In the first hybrid generation COLLINS and KEMPTON had only one plant of their *Tripsacum*×*Euchlaena*. There is no evidence, therefore, that this generation may not consist normally of more than one type, as in the case of many *Oenothera* hybrids. Certain evidence obtained by COLLINS and KEMPTON themselves almost tempts one to predict that twin hybrids will be found if it is possible to get large enough progenies.

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[19] COLLINS, G. N., and KEMPTON, J. H., Patrogenesis. Jour. Heredity 7:106–118. 1916.

Euchlaena and maize are rather closely related to one another, and cross spontaneously. Seed of the former imported from Mexico is often obviously hybridized. That from Durango, whence COLLINS and KEMPTON got their strain, is so impure that the parent of their hybrid is not free from suspicion of contamination. A priori, therefore, we should expect similarity of behavior in the hybrids between these two closely related plants and the distantly related *Tripsacum*. Nevertheless, all the plants of *Tripsacum*×*Mais* thus far raised have shown maternal characters only, in marked contrast to the paternal characters of the corresponding hybrid *Tripsacum*×*Euchlaena*. More first generation plants are greatly to be desired, but there is difficulty in getting them because the parent species can seldom be brought into flower simultaneously.

The authors lead us to expect cytological data bearing on the question raised by their very important discovery.—H. H. BARTLETT.

Negative osmosis.—In osmotic experiments it has been found that the flow of water is not always from the less concentrated toward the more concentrated solution. Several cases have been reported in which an opposite flow of water occurred, or in which a movement of water was observed in dealing with solutions with the same osmotic pressure. This is called negative or abnormal osmosis. FREUNDLICH[20] has given us a discussion of these experiments, has defined the conditions under which abnormal osmosis can take place, and has dealt with the cause of it. It appears that such movements of water are caused by the development of an electric current through the membrane, and the water moves in an electroendosmotic manner in the current. The generation of this current and the consequent electroendosmosis can take place in two sets of conditions: (1) when the membrane is permeable to both solvent and solute the ions of the electrolyte are adsorbed by the membrane, their transport numbers changed, and a difference of electric potential on opposite sides of the membrane established, which leads to the starting of an electric current; (2) when the membrane allows only one of the ions of the electrolyte to pass through, the other ion being held back. This semipermeability toward one ion leads to a difference of potential, an electric current is established, and water moves across the membrane by electroendosmosis. This can take place only when the electrolytes on opposite sides of the membrane are different and are such that the ions may react with each other and set free electrical changes.—F. E. DENNY.

Growth in Laminariaceae.—Miss FALLIS[21] reports experimental data on the growth of several species of Laminariaceae. She worked on species of *Laminaria*, *Agarum*, *Cymathaere*, *Egregia*, *Alaria*, and *Nereocystis*. She found

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[20] FREUNDLICH, H., Über abnorme Osmosen. Kolloid. Zeitschr. **18**:11–16. 1916.

[21] FALLIS, ANNIE L., Growth in some Laminariaceae. Puget Sound Marine Sta. Publ. **1**:137–155. *pls. 25–28.* 1916.

by experiment that the plants grow as well suspended from a raft by means of cloth strings as they do when attached naturally to the rocks by their own holdfasts. She found also that the removal of the holdfast and even of a considerable portion of the stipe does not affect the growth of the remainder of the plant. The removal of the tip of the blade produces only a negligible effect so long as the basal portion is left intact. Pieces of the blades even as small as 1 mm. square were found to grow when placed in a cloth bag attached to a raft. In kelps having a very short stipe it was found that the region of greatest growth in the post-juvenile stages is near the base of the blade, the main growth of the stipe occurring during the younger stages. She found that the kelps experimented upon grow almost twice as rapidly during the daytime as during the night.

The results reported coordinate well with the small degree of physiological specialization of parts that is found in such genera as *Laminaria* and other leaflike kelps. The data are interpreted largely from the viewpoint of their bearing on the location of the region of greatest growth. Only slight attention is given to the interpretation of the data in their relation to regeneration, and none at all to their relation to coordination.—GEORGE B. RIGG.

Taxonomic notes.—BRANDEGEE[22] has described new species in *Aristolochia* (2), *Jatropha*, *Lycium*, *Galvezia*, *Maximowiczia*, and *Orobanche* from Lower California; in *Aristolochia* and *Asclepias* from Mexico; and in *Sedum* and *Antirrhinum* from California.

MAXON,[23] in continuation of his studies of tropical American ferns, has presented three groups of *Polypodium*, "whose species have for the most part been greatly misunderstood." One of these groups is *P. trichomanoides* and its American allies, including a critical discussion of 26 species. Another group is *P. furfuraceum* and related species, including 21 species, 5 of which are new. The third group is *P. squammatum* and its allies, including 17 species, 5 of which are new. In addition to the new species in these groups, 5 additional new species of *Polypodium* are described, and 2 new species of *Notholaena*.

MILLSPAUGH,[24] in continuation of his studies of North American Euphorbiaceae, has recognized the following segregates from *Euphorbia: Chamaesyce* S. F. Gray, with 9 new species and 84 old ones; *Eumecanthus* Kl. and Gke., to which 41 species are transferred; *Aklema* Raf., to which 19 species are transferred. New species are also described in *Acalypha* (2), *Croton* (3), and *Tragia*.—J. M. C.

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[22] BRANDEGEE, T. S., Species novae vel minus cognitae. Univ. Calif. Publ. Bot. 6:357–361. 1916.

[23] MAXON, WILLIAM R., Studies of tropical American ferns. no. 6. Contrib. U.S. Nat. Herb. 17:541–608. *pls. 32–43.* 1916.

[24] MILLSPAUGH, C. F., Contributions to North American Euphorbiaceae VI. Publ. Field Mus. Nat. Hist. Bot. Series 2:401–420. 1916.

Floral evolution.—In a series of papers published in *New Phytologist* during 1911 and 1912, WERNHAM has discussed floral evolution, especially with reference to the phylogeny of the Sympetalae. The last paper[25] presents the summary and conclusions, and it is announced that the series has now appeared in book form. The immense amount of material presented cannot be mentioned in a review, but the general conclusion is that the Sympetalae are polyphyletic, having been derived from the Archichlamydeae along 7 distinct lines. The names given by the author to these lines will indicate the relationship of the various sympetalous orders to those of the Archichlamydeae, The lines are as follows: (1) Centrospermae-Primulales; (2) Geraniales-Ericales; (3) Guttiferales-Ebenales; (4) Geraniales (Plantaginales)-Contortae-Tubiflorae; (5) Calyciflorae-Umbelliflorae-Rubiales-Dipsacales; (6) Calyciflorae-Campanulatae; (7) Calyciflorae-Passiflorales-Cucurbitales. There is also a very useful discussion of the method of determining plant affinities and of the principles of classification.—J. M. C.

Resistance against attack of dodder.—By growing *Cuscuta Gronovii* on a great variety of hosts, GERTZ[26] has studied various means of protection possessed by plants against the attack of dodder. The main means found are mechanical resistance to penetration, high concentration of oxalic acid, and presence of latex, certain alkaloids, or ethereal oils. Various alkaloids and ethereal oils, in the concentration existing in the hosts tested, vary greatly in their effectiveness from indifferent to rather highly toxic. In comparing his results with rather fully reported results of other workers, GERTZ concludes that different species of dodder vary greatly in their resistance to a given protective substance. He notes the presence of the 3 characters of dodder often reported: resorption of the basal part of the sprout, ability to act in part as an autotroph by chlorophyll development, and impermeability of haustoria to certain toxic materials. This subject is in need of thorough microchemical and physiological study.—WILLIAM CROCKER.

Cercospora beticola.—POOL and MCKAY[27, 28] have made a detailed study of the parasitism of *Cercospora beticola* on the sugar beet (*Beta vulgaris*), paying special attention to climatic factors and the mode of infection. The leaves that show maximum stomatal opening, both in aperture and in duration of opening, showed the greatest susceptibility to infection. Factors that favor

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[25] WERNHAM, H. F., Floral evolution, with particular reference to the sympetalous dicotyledons. New Phytol. 11:373–397. 1912.

[26] GERTZ, OTTO., Über die Schutzmittel einiger Pflanzen gegen schmarotzende *Cuscuta*. Jahrb. Wiss. Bot. 56:123–154. 1915. PFEFFER'S Festschrift.

[27] POOL, V. W., and MCKAY, M. B., Relation of stomatal movement to infection by *Cercospora beticola*. Jour. Agric. Research 5:1011–1038. *pls. 80, 81. figs. 6.* 1916.

[28] ———, Climatic conditions as related to *Cercospora beticola*. Jour. Agric. Research 6:21–60. *pls. 3, 4. figs. 10.* 1916.

stomatal opening, such as temperatures ranging from 70 to 90° F. and high humidity, also chance to favor maximal germination of spores and subsequent growth and therefore lead to severe leaf infection. Such studies as these will do much to lift the fog that shrouds the problem of infection in the field of phytopathology. The term "stomatal movement," of the title, is somewhat unfortunately chosen. The authors have established a relation between infection and stomatal opening rather than between infection and stomatal movement.—GEORGE K. K. LINK.

Addisonia.—This is the title of a new journal issued from the New York Botanical Garden devoted to "colored illustrations and popular descriptions of plants." It is issued quarterly, the first number bearing the date March 1916, and each number will consist of 10 colored plates with accompanying letter press. The subsidy for the journal is furnished by a fund left for this purpose by Judge ADDISON BROWN. This explains the name and also the color of the cover. The annual subscription price is $10. The illustrations and letter press of the initial number are of the highest order, the plants illustrated and described being *Rhododendron carolinianum*, *Cassia polyphylla*, *Robinia Kelseyi*, *Pachyphytum longifolium*, *Begonia Cowellii*, *Echeveria setosa*, *Columnea gloriosa*, *Fouquieria formosa*, *Maxillaria ringens*, and *Nopalea Auberi*.—J. M. C.

Dimery in Brassica.—Cases in which two or more genetic factors produce independently a single somatic character, or modify it in such a manner as not to destroy its identity, are being reported frequently. HALLQVIST[29] gives the results of crossing a form of *Brassica Napus* characterized by undivided leaves, with a form having strongly pinnatifid lobing. The F_2 families showed several grades of lobing in different plants, and in 493 individuals out of 8,296 the recessive unlobed type reappeared, this being almost exactly 1:15. All of the 23 separate F_2 families which make up this total likewise show a very close approximation to the same ratio. The investigation is being continued into the F_3.—GEO. H. SHULL.

Pollen sterility and hybrids.—GATES and GOODSPEED[30] have tested the claim that "bad pollen" is a criterion of hybridity by examining certain geographically isolated Californian plants which have had no opportunity for crossing. *Trillium sessile giganteum*, *Scoliopus Bigelovii*, *Dirca occidentalis*, *Ranunculus californicus*, and *Fritillaria lanceolata floribunda* were selected for examination, and remarkably high percentages of bad pollen were obtained. Their conclusion is that such pollen is not necessarily an indication of

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[29] HALLQVIST, CARL, Ein neuer Fall von Dimerie bei *Brassica Napus*. Bot. Not. 1:39–42. 1916.

[30] GATES, R. R., and GOODSPEED, T. H., Pollen sterility in relation to crossing. Science 43:859–861. 1916.

hybridization, but that pollen sterility is a physiological condition which may be due to a variety of causes, hybridization and mutability being only two of them.—J. M. C.

Codium mucronatum.—Miss HURD[31] has made a study of *Codium mucronatum* from Puget Sound material, to establish the characteristics on the basis of which this species may be distinguished from other species, and to determine whether the division of this species into varieties (*californicum* J. G. Agardh and *novaezelandiae* J. G. Agardh) is justified. The study is a careful one and the plates will prove valuable to students of this genus. She suggests a detailed description for the species and concludes that the splitting of the species into varieties on the basis of the mucoronate tips of the utricles is not justified, since all of the described types are often found on the same plant.—GEORGE B. RIGG.

Röntgen rays.—KOERNICKE[32] finds that a small dosage of Röntgen rays accelerates growth of seeds and seedlings. In larger amounts it has no effect, while in still larger amounts it inhibits growth. In this regard Röntgen rays act like other rays and like toxic materials in general. *Vicia Faba* was especially favorable material for experimentation. Contrary to the claims of SCHWARZ, the effect of a stimulative dose at the seed or seedling stage was lost before maturity was reached. From the results of SCHWARZ, KOERNICKE had thought that Röntgen rays might be applied profitably in practice for increasing yield, but his work shows that this is not the case.—WILLIAM CROCKER.

Trigonocarpus and Ginkgo.—In 1914 SALISBURY[33] published an account of a new species of *Trigonocarpus*, and compared it with the seeds of *Ginkgo*. Later, Miss AFFOURTIT and Miss LA RIVIÈRE[34] investigated the seeds of *Ginkgo* and gave reasons for the belief that such a comparison is not valid. SALISBURY[35] has published a brief note in reply, pointing out more specifically the reasons for his view, and concludes that in the more important features of general organization the ovules of Ginkgoales, Cycadales, and *Trigonocarpus* exhibit a uniformity of construction difficult to explain except on the basis of affinity.—J. M. C.

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[31] HURD, ANNIE M., *Codium mucronatum*. Puget Sound Marine Sta. Publ. **1**: 109–135. *pls. 19–24.* 1916.

[32] KOERNICKE, M., Über die Wirkung verschieden starker Röntgenstrahlen auf Keimung und Wachstum bei den höheren Pflanzen. Jahrb. Wiss. Bot. **56**:416–430. 1915. PFEFFER'S Festschrift.

[33] Review in BOT. GAZ. **57**:440. 1914.

[34] Review in BOT. GAZ. **61**:176. 1916.

[35] SALISBURY, E. J., On the relation between *Trigonocarpus* and *Ginkgo*. Ann. Botany **30**:356. 1916.

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CHEMOTROPISM IN RHIZOPUS NIGRICANS[1]

ARTHUR HARMOUNT GRAVES

(WITH FOUR FIGURES)

Historical

In view of the important relation of the question of chemotropism among the fungi to the problems of fungal parasitism, it is surprising that investigations in this field have been so meager; for while numerous scattered observations may be found here and there in the literature, the works of MIYOSHI (8) and FULTON (5) stand out as the only ones which represent any considerable investigations of the subject. The researches of CLARK (3), although intended primarily to supplement his work on the toxicity of copper compounds, are also of especial importance in this connection.

It is needless to enter here into a detailed historical account of all the researches from which information can be culled regarding chemotropism in the fungi, for an excellent review of these may be found in FULTON'S paper. It is our purpose merely to outline the results obtained by MIYOSHI, CLARK, and FULTON.

MIYOSHI (8) tested a great variety of chemical substances, and maintained that in many cases they exerted a very marked attraction on the fungal hyphae, which then grew toward them (positive chemotropism); others showed a repellent effect, so that the hyphae grew diametrically away from the diffusion center (negative

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[1] From the Department of Plant Physiology and Pathology, Imperial College of Science and Technology, London.

chemotropism); while still others produced no effect. Sugars were especially attractive to the molds.

The most important methods employed by MIYOSHI were as follows: (1) the substances to be tested were injected in solution into leaves, which were then sown with spores; or (2) the substances were incorporated in a gelatin layer, on which was laid a perforated mica plate or membrane, the spores being sown above, either on the free surface of the plate or membrane, or in a superimposed layer of medium lacking the substance to be tested; or (3) fine glass capillary tubes holding the substance to be tested were introduced into a preparation containing germ tubes of the fungus growing in water or in a very dilute nutrient solution.

Where the germ tubes showed a marked turning toward the stomata, perforations, or open ends of the capillary tubes, as the case might be, and thus toward the diffusion centers of the chemical substances, the turnings were interpreted as instances of positive chemotropism. Moreover, there appeared to be an optimum concentration, beyond which such substances exerted a less and less attractive and at length a repellent effect. On the other hand, below the optimum, with gradually decreasing concentrations, the attractive effect also gradually decreased, ultimately to zero. Acids, alkalis, and some salts always produced a repellent effect, however, even in weak solutions.

Perhaps MIYOSHI's strongest point in support of his view was his statement that when no special chemical substances were offered, as, for example, when leaves were injected with pure water only, no turning resulted.

When we survey the investigations of FULTON (5) and CLARK (3), we find that, taken in combination, they completely contradict these results. CLARK injected leaves with various concentrations of copper and cobalt salts, etc., and found that in this case the germ tubes near the stomata curved toward and grew directly into them; but he obtained a similar result with leaves injected with pure water only. In experiments in which he used perforated mica plates, he found that the germ tubes near the perforation always "*grew toward the opening if it communicated with a layer in which no spores had been placed.*" Although he did not follow out this line of work, he advanced the hypothesis that *Rhizopus* is "markedly chemotactic to some secretion of its own mycelium, and this negative chemotropism is much greater than any positive chemotropism it may have for food substances or oxygen."

The work of FULTON showed a decided advance in that, by introducing numerical methods of estimating the direction assumed by the hyphae, he placed on a numerical basis what had heretofore been more or less a matter of personal judgment. As PORODKO (9) has pointed out, in his work on chemotropism in roots, quantitative methods in this field must replace the qualitative ones hitherto used, if definite results are to be obtained. In startling contrast to MIYOSHI's results, FULTON failed to find "the existence of any definite chemotropic sensibility to nutrient substances or other chemical compounds in solution." He believed that if positive chemotropism in the fungi exists, it is not as marked as turning caused by other stimuli. He states: "All of the fungi tested show a tendency to turn from a region in which hyphae of the same kind are growing, toward one destitute of hyphae, or in which the hyphae are less abundant. . . . . This may be regarded as a negative reaction to stimuli from chemical substances which owe their origin in some way to the growing fungus."

These results were so much at variance with MIYOSHI's conclusions that they have been quoted with great reserve by such writers as BARNES (4), PRINGSHEIM (10), and JOST (6); and, on account of their conflicting nature, a restudy of the whole matter seemed imperative. The present work was undertaken, therefore, in the hope of deciding between the views of MIYOSHI on the one hand and those of FULTON and CLARK on the other.

Material and methods

Although the main part of the work was carried on chiefly with *Rhizopus nigricans* Ehrenb., preliminary tests were made also with *Botrytis cinerea* Pers. and a *Penicillium* which, after culture in the requisite media, answered most closely to *Penicillium* no. 24 Thom. Eventually the work was confined entirely to *Rhizopus* because its large spores and hyphae make the microscopic examination of the preparation easier; the comparatively short time necessary for its growth reduces to a minimum the dangers both of contamination and of too extensive a diffusion of the substances tested; and the spores do not cling together in large clumps as in *Penicillium*.

For the experimental work, mica was selected on account of its impermeable nature. Small plates of this were cut from large sheets and perforated with a sharp needle. The area perforated measured 24×24 mm., while at two opposite sides of this square piece small winglike projections were left, in order to rest the preparation on supports. At first, the perforations were made 2 mm. apart, 121 holes thus being punched in each plate; but after experimental chemical tests demonstrated a comparatively rapid diffusion through such plates, the number was changed to 16, the holes then being spaced 6 mm. apart. After each experiment the mica plates were boiled, first in alkali, then in acid, and, after being rinsed and boiled in 2 or 3 changes of distilled water, they were dried finally in a hot air oven.

The spores were grown in thin layers or films of medium approximately 0.5 mm. thick, placed on these mica plates by a method to be described presently. For the medium, agar was used invariably, both because compared with gelatin it afforded less nourishment when used without addition to the substances to be tested, and because in the manipulation of the films, as explained later, it was found impracticable to use gelatin. A 1.5 per cent agar was used for the plain or "non-nutrient" medium; while for the nutrient a double strength solution of the required substance was mixed with an equal volume of 3 per cent agar to make a medium of desired strength containing 1.5 per cent agar.

The method finally adopted for making the films and sowing the spores was as follows. As already indicated, each preparation consisted of a perforated mica plate bearing a thin film of medium on each of its surfaces. The film on one side might contain the spores, while that on the other side would contain the chemical substances to be investigated; but various combinations were tried, as will be shown later.

For various reasons it was found desirable to make all films of approximately uniform area and thickness. An area approximately 24×24 mm., the size of the perforated area in the mica plates, was marked out on a clean slide by means of a brush and a very small quantity of vaseline. Then on a warming table were placed a small beaker in which were water, a thermometer, and a small vial holding the agar for the films. When the agar, previously melted in hot water, had fallen to 40° or slightly below, the requisite number of drops of a suitable suspension of spores in distilled water was added. With a small pipette, marked to indicate the amount of agar solution it should contain for the making of a film, the correct quantity was drawn up, quickly dropped and spread evenly on the area marked out on the glass slide by the vaseline lines. In order to facilitate an even spreading of the agar, the slides were laid on a large glass plate fixed in an exactly horizontal position with the aid of screw supports and a spirit level. It was found that it was very essential to keep the agar agitated during the process of pipetting it from the vial; otherwise the spores would not be distributed evenly. Before the agar on the glass slide had had time to set completely, the mica plate was placed carefully upon it in such a way that the perforated area covered the film, care being taken not to press the mica down and thus force the agar up through the holes. Next, the same amount of agar of the required composition, from another vial, was dropped and spread on the surface of the mica plate up to the boundaries of the perforated area. Transverse grooves in the mica, cut for this purpose, in most cases confined the agar to the perforated area. In case no spores were needed for this upper film, several drops of distilled water had previously been added to the agar, which were equal in volume to that of the spore mixture added to the agar for the lower film. After the upper film was set, the whole preparation, consisting of the mica plate between the two films of agar, was carefully pushed off the slide with the help of a scalpel. It was on account of this last operation that it was found impracticable to use gelatin, since this adhered so closely to the slide that it was impossible to remove it without breaking.

The preparation was now rested in a horizontal position,[2] by means of the extensions at each end, on cork supports in a Petri

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[2] In the earlier experiments, some of the preparations were suspended vertically by means of a thread passed through one of the holes in the mica plate. This vertical position was found to have no influence on the result.

dish, lined on all sides with filter paper moistened in distilled water. In this way the films were situated in an atmosphere practically saturated with water vapor, and each film was under exactly the same conditions as regards access of air. This last was a matter of great importance, since it was found in the earlier experiments that when the preparations were laid on a glass slide, the germination was always poorer in the film next to the glass slide, although normal germination occurred at or near the edges of the film.

To save labor, usually 2 or 3 preparations of the same kind were placed in a single Petri dish. In the preliminary experiments, these dishes had been placed directly in an incubator kept at a uniform temperature of 24–25° C. Even under these circumstances, however, it was realized that the atmospheric condition within the Petri dish might not be uniform throughout, for we know, of course, that Petri dishes so prepared eventually dry out. To obviate this, so far as possible, all the dishes (piled one above the other in a column, which rested on 2 or 3 empty dishes, or on an inverted stender dish) were stood in one of the halves of a larger Petri dish (9 in. diameter) which also was lined with several thicknesses of filter paper and contained a quantity of distilled water. The whole was then covered with a bell-jar, likewise lined throughout with filter paper, and resting in the larger Petri dish so that water was continually being drawn up by the filter paper. In this way the atmosphere within the small Petri dishes was kept as close as possible to a uniform saturation point throughout each dish.

The whole apparatus was now incubated at 24–25° C., and when the germ tubes had grown to the desired length, they were killed by dropping a little 4 per cent formalin on the preparations. They could then be kept for several days if necessary, or until it was convenient to examine them microscopically.[3] For examination under the microscope, the preparation was laid very carefully on a

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[3] The nature of this entire operation, and the amount of time necessary for its completion, made perfect antiseptic precautions impracticable. All that could be done was to have the apparatus scrupulously clean and sterile. As a matter of fact, the short time requisite for the incubation of *Rhizopus*, in few cases more than 10 hours, would allow bacteria little time for development, and no contamination was ever observed in these preparations.

glass slide, and since only the low power was necessary, no cover glass was used, on account of the danger of shifting the film.

In working out the results, it was found essential to get an idea of the amount of growth which had taken place. This was done by estimating the average length of the hyphae in the preparation in question. To obtain this average, only those hyphae near the holes were considered, since in the regions midway between these holes the conditions of nourishment differed from those in the immediate proximity of the holes; especially was this true in the case of germ tubes in films made of plain agar, with a nourishing substance on the other side of the mica plate. A definite area was selected near each hole, and the lengths of all the hyphae in this area noted. In this way 3 or more holes in different parts of the preparation were considered, or until a total of 15 hyphae had been counted and averaged.

Since, as will be shown later, the number of viable spores,[4] as well as the number of hyphae present, was found to have a marked influence on the results, it was desirable for comparative results to have the spore numbers approximately equal. In order to get a suspension of spores which would produce the desired number of spores to each volume of film covered by a square millimeter of surface, the following method was used. Spore-bearing mycelium was allowed to stand in water for a few moments and was then strained through a piece of muslin, which had previously been freed of its starch by thorough boiling and washing. The spore suspension was then tested as to its concentration by the use of a micrometer eyepiece containing accurately ruled squares, and by diluting, or by centrifuging and pouring off some of the water, was brought to the desired strength.

METHOD OF ESTIMATING THE INTENSITY OF THE CHEMOTROPIC REACTION.—In work of this kind, the method of estimating the reaction is all-important, since on it depends the accuracy of the

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[4] It was found that the number of viable spores present bears a definite relation to the percentage of germination. When the spores were excessively numerous no germination at all resulted. The cause of this non-germination was not worked out experimentally, but it is probably referable to the same agencies discussed later in this paper; that is, the excretion of toxic products by the spores, in this case in the pre-germination stages.

results. For this reason it is necessary to describe in some detail the method which was elaborated. First, for delimiting the region about the hole within which the hyphae were to be considered, the following rule was employed. Only those hyphae growing from spores which chanced to be located in a cylinder of medium situated over the hole were taken into consideration. This cylinder was delimited as nearly as possible by the eye, in such a way that the center of its base coincided with the center of the hole, its diameter being 3 times that of the hole. Although the holes varied considerably in diameter (8–45 μ), it was found that the average was about 16 μ, and that, by turning the fine adjustment of the microscope a suitable number of revolutions, a thickness of medium would be traversed approximately equal to one diameter of the hole. In all cases the hyphae coming from spores directly over the hole, that is, in an inner cylinder with the hole as its base, were neglected, owing to the difficulty in many cases of assigning a definite direction to them. Thus the region in which the germ tubes were considered was a cylindrical zone concentric with the hole, and equal in width and height to one diameter of the hole.

It is of course clear that the whole purpose of the adoption of this arbitrary region was to secure uniformity of conditions for collecting the data. For the most exact work, indeed, the portion of the medium chosen for examination should correspond to a hemisphere whose base coincides with the base of the above described cylinder, and thus has a radius equal to 1.5 times the diameter of the hole. However, it is practically impossible to define the outline of such a hemisphere with any accuracy by focusing, and so a cylinder of the dimensions described was chosen as the nearest practical approximation.

The next step was to determine as accurately as possible the direction taken by the hyphae. An imaginary straight line was projected from the center of the hole to the spore itself, in cases where the germ tube was fairly straight (fig. 1); if, however, the germ tube curved markedly, this method was modified slightly, as explained below. This line was supposed to make angles of 45° with two other imaginary lines intersecting one another at right angles at the spore. The 4 regions, of 90° each, thus marked out by these last two lines, were then called *A*, *B*, *C*, and *D*, with orientations as shown in fig. 1. The hypha was then classed as *A*, *B*, *C*, or *D*, according to the quarter in which it grew.

If the hypha curved markedly, only its tip was considered, since the ultimate effect of the reaction was expressed in this portion. In this case, the point of intersection of the two lines at right angles was located at the tip, this having been connected to the center of the hole by a straight line, as before. The hypha was then classified according to the quarter into which the tip pointed (fig. 2). In the few cases where the hyphae curved considerably up or down from their original plane, that is, above or below an optical section of the medium, their direction was taken as that which they would assume if projected on their original plane. Those growing vertically up or down in the medium were neglected.

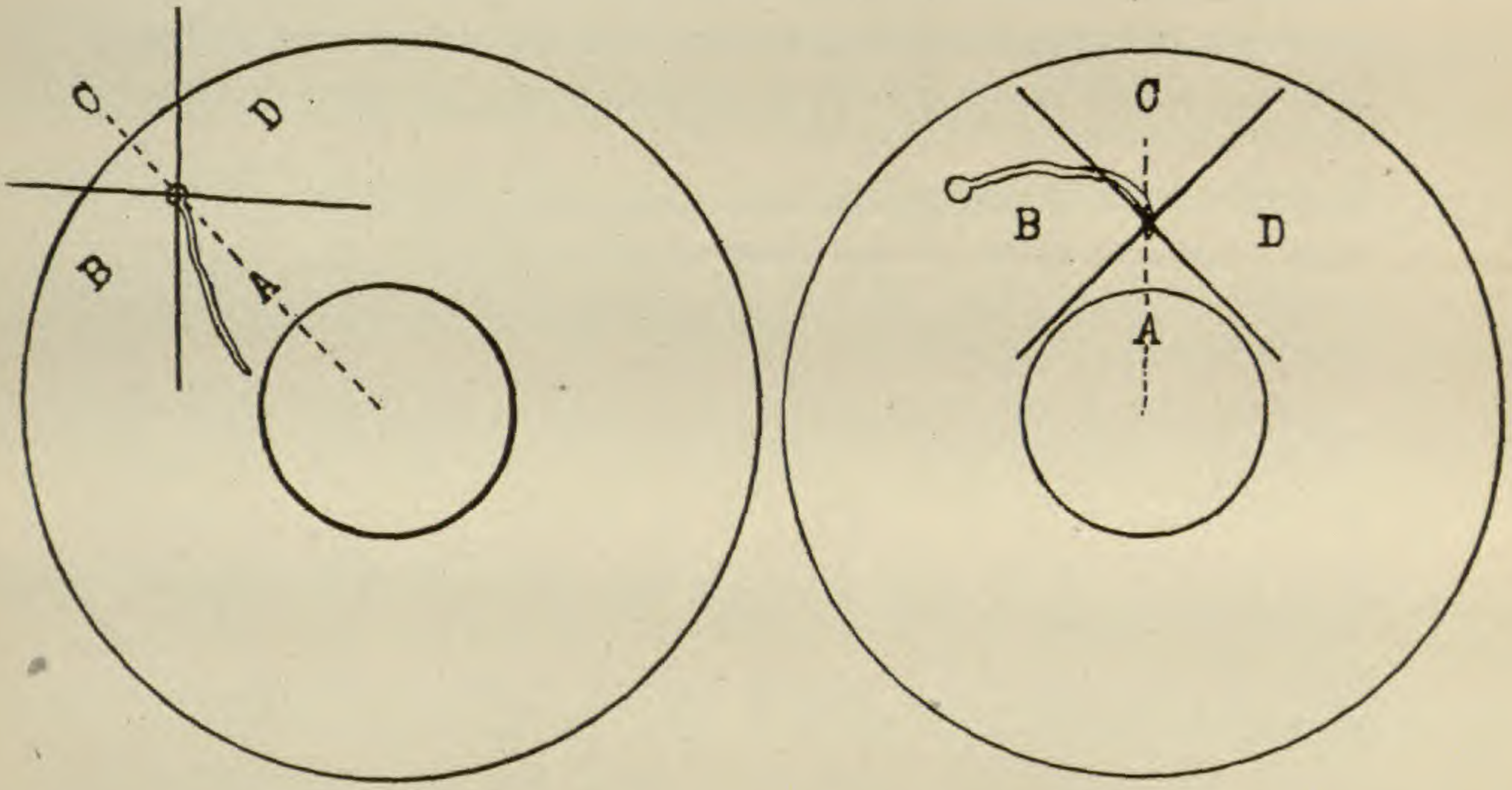

FIG. 1

FIG. 2

FIGS. 1 and 2.—Diagrams showing methods of locating directions of hyphae: fig. 1, where the hypha is fairly straight; fig. 2, where it curves markedly.

The holes in the mica plates were examined in regular succession, one row after the other, in order that no subconscious selection could be made of those that appeared more favorable to the results expected. The data for each hole were noted down separately, and the total was figured up when the preparation was finished. For the most part, for the reasons already stated, 16-holed plates were used, and usually 10 holes in each plate were examined.

The basis of the interpretation of the figures thus obtained lay in the assumption that the average number of hyphae in each of the 4 classes, considering a large number of holes, and supposing that no attractive or repellent forces existed, should, on the doctrine of averages, be approximately the same, that is, 25 per cent of the whole number; or, to put it in general terms, as many hyphae should point in one direction as another. That this assumption is legitimate is shown by the test count described later. Of the whole number, then, 25 per cent would constitute the normal number for each class, and any considerable deviation from this would indicate a reaction to a disturbing force.

Since the *B* and *D* regions are of equal volume and equally subjected to repellent or attractive forces, they may be left out of consideration except in so far as the number occurring in them goes to make up the total for each hole, and thus influences the final percentage of reaction. In order to determine this percentage, then, we are concerned first of all with the *A* and *C* classes. Let us assume that an attractive force exists, derived from a substance on the other side of the mica plate, which is causing the hyphae to a certain extent to grow toward the holes. Since the number of the *A* class will then be greater than the normal number, we must conclude that those in excess of one-fourth have been attracted into this class. If the attractive force is not a very strong one, as indicated by a number not greatly above the normal, we may be fairly certain that the increment has been derived from those which would normally point right or left, that is, those of the *B* and *D* class. The number of *A*'s in excess of the normal, however, does not give us a complete measure of the attraction, for in all probability some hyphae originally in the *C* class have turned into the *B* or *D* direction, and the remaining *C*'s will therefore be fewer than one-fourth. The difference between the remaining *C*'s and the normal number would then represent those which have turned from the *C* direction into *B* or *D*. By adding this number to those in excess of the normal number in *A* we get the total number affected. In order to get the total percentage of reaction, we must divide this number by three-fourths of the total number of hyphae counted in the whole area, since we are dealing with only three-fourths of the hyphae, the one-fourth represented by the normal *A*'s being unaffected, at least visibly.[5]

If we denote the normal number to be expected in each region by *n* (one-fourth of the whole), the number actually in each region by its corresponding letter, and the total number of hyphae in the whole area by *t*, the formula will then read as follows: $\frac{(A-n)+(n-C)}{\frac{3}{4}t}$; or, simplifying, $\frac{A-C}{\frac{3}{4}t}$ = percentage of hyphae reacting. In case a repellent force exists, raising the number of *C*'s above the normal, the final percentage will then be a minus quantity, and it is so expressed in the accompanying tables.

One objection to this method lies in the possibility that some of the normal *C*'s may have been attracted so strongly as to have turned from *C*, *through* the *B* or *D* areas, to *A*; these would then be counted twice over, for they would be included in the gain to *A* as well as in the loss from *C*. It is of course quite impossible to determine for a large number of holes how many hyphae have so acted. The objection, however, is not a serious one; for, as a matter of fact, when the number of *B*'s and *D*'s is fairly large, indicating a force not strong enough to draw them all into the *A* class, we know that the probabilities are much against the force attracting any from the *C* class into the *A* class.

However, when *more* than three-fourths of the total number point in the *A* direction, we can fairly assume that all which were originally *B*'s and *D*'s have now turned to the *A* direction; that is, of this 75 per cent, 25 per cent consist of those originally in *A*, and 50 per cent must have been derived from those nearest the *A* class, namely *B* and *D*; thus any number above 75 per cent must have come from those which were originally growing in the *C*

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[5] Just what individuals among the normal *A*'s have reacted to the stimulus we cannot of course judge, since by their original chance position they are all oriented toward the holes, but the proportion would correspond essentially to that for the remaining 75 per cent of the hyphae.

direction. In other words, chemotropic forces which would cause a turning of slightly more than 90° would bring all the *B*'s and *D*'s to *A;* but if the force were stronger than this, some of the *C* class would turn to the *A* direction. It is clear, therefore, that the number in excess of 75 per cent represents those which have turned from *C* to *A;* and that in this case those remaining in *B* and *D* must have been originally in the *C* class but have not turned strongly enough to become *A*'s.

Accordingly, whenever the total number of hyphae in *A* exceeds 75 per cent of the whole number, a correction must be applied; we must now subtract from our formula the number which have come over from *C* into *A*, to avoid counting them twice. We can ascertain, indirectly, how many of the *C*'s have so turned into *A* by subtracting the number left in *C* from the normal number, and by also subtracting from this result the sum of those left in *B* or *D;* for, as was previously shown, those now in *B* and *D* must also have been originally *C*'s. The remainder, representing the difference between all these and the normal number, will then denote the number which have turned from *C* through the *B* and *D* classes to *A*. In accordance with this, therefore, we must subtract from our first formula $[(n-C)-(B+D)]$. The whole formula would then read: $\frac{(A-n)+(n-C)-[(n-C)-(B+D)]}{\frac{3}{4}t}$; or, simplifying, $\frac{A-n+B+D}{\frac{3}{4}t}$ = percentage of hyphae reacting. As before, should the excess over 75 per cent be in the *C* class, the result of the calculation would be a minus quantity, and would indicate repulsion.

THE EXPERIMENTAL ERROR.—In order to test the accuracy of the method, 10 preparations were made with conditions as regards number and length of the hyphae, and composition of the medium, essentially the same in both upper and lower films. Under these circumstances, in accordance with our hypothesis, there should be no more turning in one direction than in another. Hyphae were examined in the prescribed manner for 10 holes in each preparation, making 100 holes for the entire operation, or, if we count both upper and lower films, in reality 200 lots of hyphae. The results, in the order in which the counts were made, are shown in table I.

It is evident from the results shown in table I that for small numbers, from 100 to 200, the experimental error may vary from 0 to 16 per cent; but by taking a large number, such as 774, the error is reduced to about 10 per cent. Also, apparently owing to the personal equation of the observer, the error is always minus.[6]

TABLE I

RESULTS OF TEST COUNTS TO DETERMINE ACCURACY OF METHOD

Preparation	Class				Total	Percentage of error
	A	*B*	*C*	*D*		
1	50	34	58	38	180	−6
2	57	34	57	43	191	0
3	33	34	52	37	156	−16
4	24	28	39	36	127	−16
5	21	18	32	23	94	−16
					748	
	(Average error for 748 hyphae = −11 per cent)					
6	29	31	35	35	130	−6
7	54	51	73	55	233	−11
8	41	29	49	20	139	−8
9	25	28	36	33	122	−12
10	33	30	48	39	150	−13
					774	
	(Average error for 774 hyphae = −10 per cent)*					

* The same average error of 10–11 per cent was obtained when a recount was made after a few days' interval, so that the method can be trusted to give uniform results.

It is also noteworthy that the large error, up to 16 per cent, occurs only when the hyphae are not markedly turned in any one direction. In the results given later, where a marked turning occurs, the error would be much smaller.

FULTON'S METHOD.—In this connection, the method employed by FULTON (5) may be considered. It is stated as follows:

> Hyphae within a radius of one opening diameter from the margin of each opening were considered, the hyphae within such an area were classed in the counts as those turning toward the openings, those turning away from the

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[6] It is possible that the extra number in the *C* class may be due to some factor hitherto overlooked, which causes a slight repulsion from the holes on both sides of the plate, even when the conditions seem uniform in the two media.

openings, and those apparently indifferent. After an examination of the entire preparation in each case, those holes were selected for the counts which represented the average condition. In calculating the percentages from the counts the difference between those attracted and those repelled was made the dividend, and the total number within the observed area was made the divisor.

This method is open to at least 3 objections. In the first place, no definite method is given for distinguishing between the 3 classes of hyphae, that is, between the indifferent hyphae on the one hand, and those turning away from or toward the openings on the other hand. Without some such definite rule as that used in the method employed in the present paper, the matter of deciding their directions is likely to be influenced to a large degree by the personal equation, on account of the great variety of curves and angles which the hyphae assume. In the second place, apparently *all* the hyphae found in the area at the time of examination were included. It is clear that by such a method one must include some of the hyphae coming from spores located outside the special area; and if we are right in concluding that FULTON's classification of directions is similar to ours, it is evident that in this way a portion at least of the *A* class hyphae belonging to an outer zone would be counted, whereas the *B* and *D* classes would be mostly, and the *C* class hyphae of that zone would be entirely, neglected. Thus, even if there were no turning toward the holes at all, and the number of the *A* class in the prescribed zone were only the normal 25 per cent, by counting in some of the *A* class from the zone outside, the resulting figures would inevitably, though falsely, indicate the existence of a chemotropic reaction. Moreover, this error would be greater as the amount of real turning increased, and it would also increase with the growth in length of the hyphae, since eventually some of those from zones still more remote would reach into the prescribed zone. In the third place, although the area in the plane of the hole was clearly defined by FULTON, no statement was made as to the hyphae found in the parallel planes above the hole. If these were estimated in the same way as those in the plane of the hole, FULTON was really considering the hyphae in a cylinder of medium standing over the hole and reaching to the free surface of the film. If this were the case, he must have counted in hyphae outside of his prescribed 1.5 diameters from the center of the hole, for in all probability his films were thicker than this.

THE RATE OF DIFFUSION THROUGH THE PERFORATED MICA PLATES.—In view of the researches of BROWN and ESCOMBE (2) on diffusion through perforated membranes, it was realized that it was essential to obtain definite data as to the rate of diffusion through the perforated mica plates. Experiments were set up in which the rates of diffusion of cane sugar through 121-holed plates and through 16-holed plates were compared for various periods of time. Without going into the details of the experiments, it may be stated that in either case, although diffusion did not proceed at such a rate as to reduce markedly the difference in concentration in the two films, yet diffusion through the 16-holed plate was naturally slower, and on this account plates so perforated were chosen for the work.

Experimental work

It is not our purpose to present a detailed account of all the experiments. In the following the most important results are outlined, using the data from representative tests. In all cases given, the results have been repeatedly verified. Altogether over 40,000 hyphae were examined and allocated to their 4 classes in the manner described above.

EXPERIMENTS WITH "STALED" SOLUTIONS.—Very early in the work, while various percentages of sugars were being tested for their power of inducing a positive chemotropism, it became quite evident that the hyphae would always turn toward the holes leading to the other agar layer, regardless of whether sugar was present in it or not, provided it contained no germinating spores. It was also significant that the more hyphae there were present in the one film, the stronger was the turning from it toward the film without spores. Furthermore, if the spores were sown in both layers, no turning resulted in either layer, or it was not nearly so pronounced. This was quite in line with the experience of FULTON and CLARK, and according to their hypothesis, previously stated, was probably due to a negative chemotropic reaction to some substance or substances excreted by the hyphae themselves. In accordance with this theory we may thus conceive that the layer in which the hyphae were present would be permeated with their excretions, which in this paper have been termed the "staling substance or substances" (cf. BALLS 1, pp. 559, 576 ff.). Toward the region of the holes the concentration of this staling substance would be less and less, since here it is diffusing through to the other layer. This decreasing gradient of concentration would therefore act as a directive stimulus to the hyphae, causing them to grow toward the regions of less and less concentration, and thus through the holes.

In order to prove this hypothesis, the problem before us was to obtain, if possible, this staling substance free from mycelium, and determine whether, if placed in a sporeless layer, it would prevent the hyphae from growing into that layer. Although cane sugar and glucose were tried in order to obtain vigorous cultures of *Rhizopus* mycelium, and hence a strongly staled solution, the results were unsatisfactory. No medium tried gave anywhere near the vigorous growth that developed in turnip juice, prepared by pressing the juice from autoclaved white turnips.

Several experiments were performed, with unsatisfactory results, as regards a turning away from the medium containing the staled juice, the failure probably being due to the fact that the staling substances were not of the requisite strength. It was eventually found that the desired effect could be produced by allowing the juice to stale for from 3 to 4 weeks, that is, to grow the fungus in it for that length of time. For this purpose sterilized, conical flasks containing 10–15 cc. of sterile turnip juice were inoculated with *Rhizopus* and kept in an incubator at 25° C. In a few days a white weft of mycelium developed, and later sporangia appeared. By the end of 3 weeks the mycelium had taken on a brownish color, and growth had apparently ceased. The liquid remaining was now poured out of the flask. It gave an acid reaction to litmus and had a slightly sour odor of malt. Chemical tests showed the entire absence of oxalic acid or oxalates. The staled solution was now evaporated to one-half its volume, that is, to double strength, at laboratory temperature under reduced pressure. For making up the medium for the films, either one part of 6 per cent agar to 3 parts of the staled solution, or equal volumes of 3 per cent agar and staled solution were used, in order to obtain a 1.5 per cent agar medium.

Table II gives the results of one series of experiments. In preparing the medium for this, care was taken not to heat the staled solution above 40° C.

TABLE II

$$\text{NATURE OF EXPERIMENT}\left\{\text{Mica plate}\rightarrow\frac{\text{Staled turnip juice agar}\atop\text{+non-viable spores*}}{\text{Fresh turnip juice agar}\atop\text{+viable spores}}\right.$$

Number of preparations examined	Period of incubation in hours	Average length of hyphae in μ	Number of spores in lower layer per sq. mm. of film surface	Direction of hyphae				Total number of hyphae counted	Percentage of reaction
				A	*B*	*C*	*D*		
2........	7	67	28	17	37	77	21	152	−53
4........	8.25	157	39	91	87	152	90	420	−19
2........	10	331	29	45	48	91	48	232	−26

* In this case the staled solution was not centrifuged, and this accounts for the presence of spores in this layer. They were probably dead, however, and in any case did not germinate. Their presence may or may not have had its effect on the result, but it is extremely doubtful whether they influenced it to any marked degree. Similar results were obtained in series where the solution had been freed of its spores by centrifuging.

Ten holes in each preparation were counted. As would be expected, the turning away from the holes is most marked in the youngest stage, where the hyphae have not yet produced, by their own activity, a concentration of staling substance in any way comparable to that of the other film.

In preparing these films, equal volumes of the staled turnip juice and 3 per cent distilled water agar had been used. This process diluted the staled juice to one-half strength, or, in reality, to its original strength before evaporation. One would expect, under these conditions, to see an even greater repellent effect than the results show. It is very probable, however, and is also indicated by the following experiment, that the repellent substances are of an unstable or volatile character. Although, in order to avoid any chemical change in the substance, at no time in the making of the preparations was the staled juice agar heated above 40° C., it is possible that even this degree of heat as well as the delay due to evaporation may have led to an alteration of the staling substances.

Table III shows the results of experiments to determine whether raising the staled juice agar to the boiling point altered its repellent effect (cf. figs. 3 and 4).

TABLE III

NATURE OF EXPERIMENT Mica plate → $\frac{\text{Staled turnip juice agar}}{\text{Fresh turnip juice agar + spores}}$

Temperature	Number of preparations examined	Period of incubation in hours	Average length of hyphae in μ	Number of spores per sq. mm. of film surface	Total number of hyphae counted	Percentage of reaction
Staled juice not heated over 40° C.	2........	7.50	69	21	240	−26
	2........	8.75	200	29	310	−28
Staled juice heated to 100° C.	2........	7.00	57	31	168	+18
	2........	8.50	143	23	218	+72

In this series the staled solution used was 3 weeks old, and, in order to dilute it as little as possible, one part of 6 per cent agar was added to 3 parts of the staled juice, a rather difficult operation in the case where there was no heating above 40° C., owing to the fact that at this temperature 6 per cent agar is very close to its gelatinization point.

The results show that the repellent substances of the staled solution are as a whole, or in part, unstable or volatile in character (figs. 3, 4). If they had been entirely destroyed, we should get results similar to those described later for *C* (p. 357), that is, 100 per cent of turning. It is possible, however, that the heating entirely destroyed the repellent substances, and that the smaller reaction as compared with the preparations cited is due to the fact that most of the substances in turnip juice which induce a positive chemotropism have been used up by the fungus which originally produced the staled solution.

In this connection the work of LUTZ (7) is of interest. In the investigation of the effect of used solutions on spore germination and fungous growth, he found that in many cases high temperature

(80–100°) destroyed the effect of these solutions, or at least temporarily altered it. Furthermore, he remarks, "Wenn sich zeigen lässt dass die uns interessierenden Stoffe durch Kochen zerstört werden, so liegt die Annahme nahe dass es sich bei ihnen vielleicht um fermentähnliche Körper handelt" (**7**, p. 106).

The theory advanced by CLARK and FULTON, therefore, that during their growth the hyphae produce some substance or substances to which they are negatively chemotropic, is fully proven by the present work, being supported by 3 lines of evidence: (1) the hyphae turn away from a film without spores if it contains their own staling substances; (2) the hyphae always show a marked turning from their own medium to a second one without hyphae, no matter what the constitution of either medium may be, unless, indeed, the second medium contains substances which exert a negative chemotropic stimulus; (3) when an approximately equal

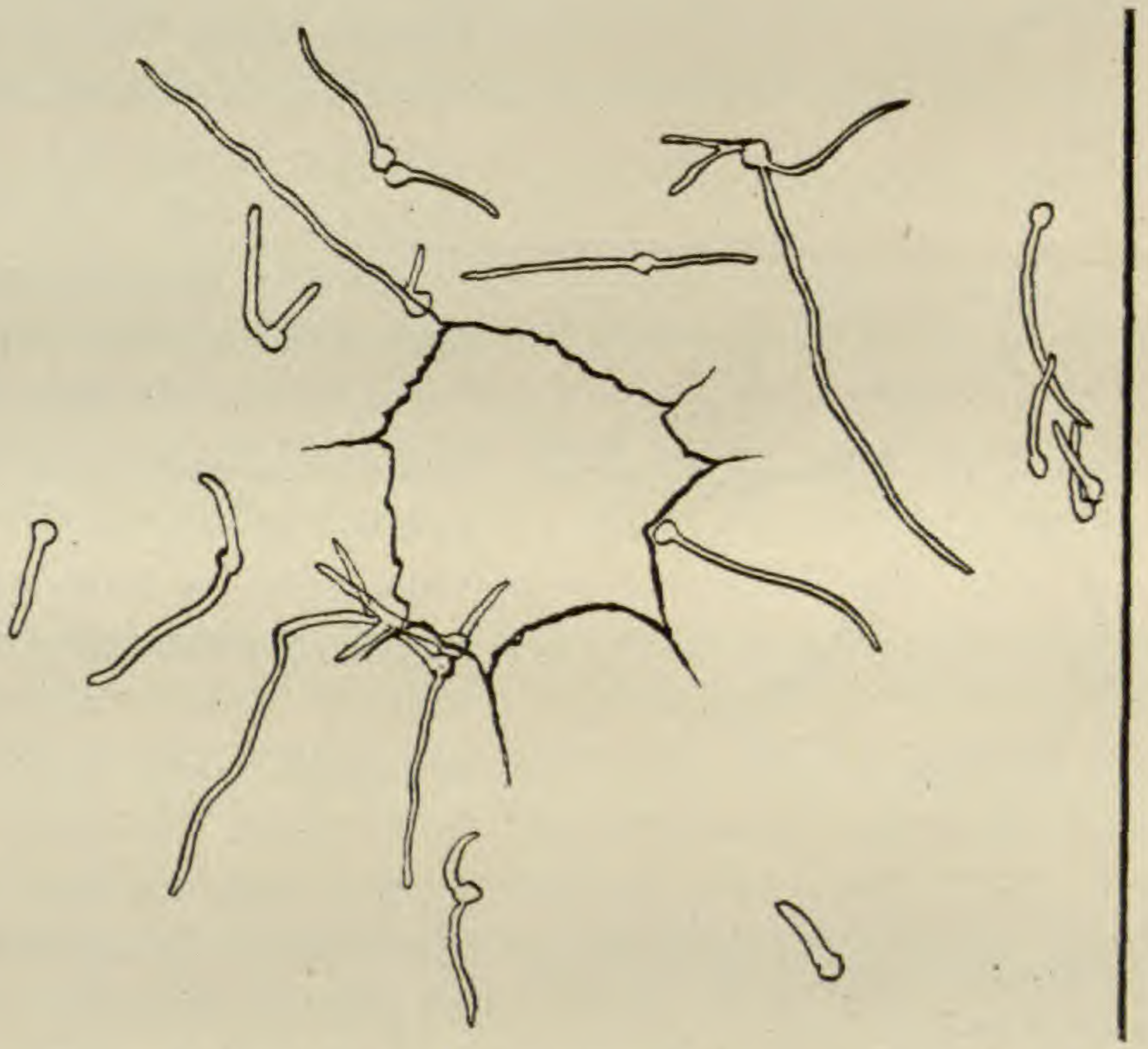

FIG. 3.—Germ tubes of *Rhizopus nigricans* growing away from the hole; film on the other side of mica plate contains strong "staled" turnip juice; hyphae are growing in a medium composed of unstaled turnip juice agar; ×80.

amount of mycelium occurs in 2 films composed of the same medium, no turning results.

EXPERIMENTS WITH UNSTALED TURNIP JUICE.—After the existence of a negative chemotropism of this kind was proved, it became easier to search for a possible positive chemotropism. For, without going into detail, it was clear, from the fact that the hyphae are continually producing a staling substance, that the number of spores and the length of the hyphae must be considered when one is seeking to obtain evidence for positive chemotropism.

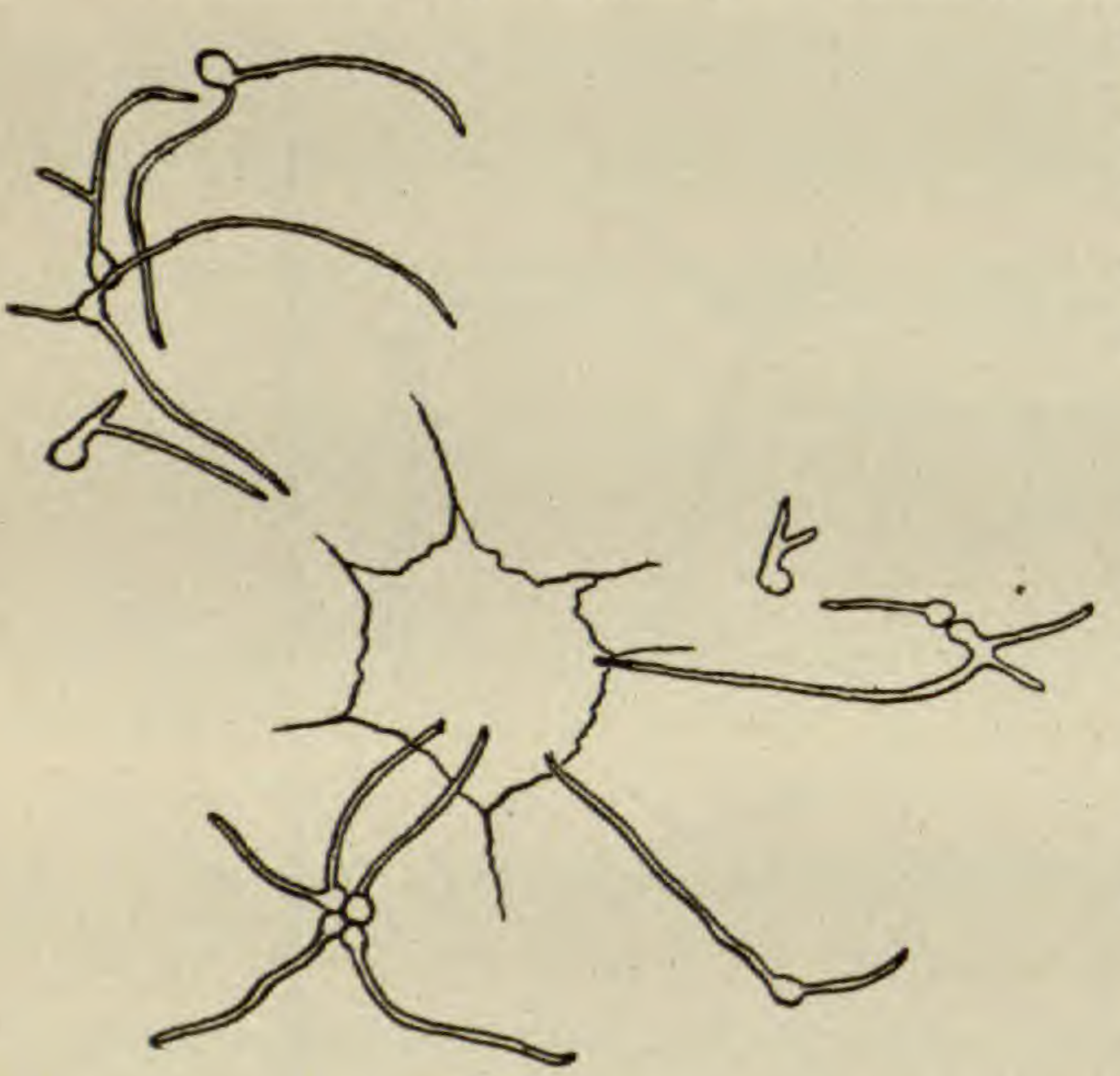

FIG. 4.—Germ tubes growing toward the holes; "staled" turnip juice agar has in this case previously been heated to 100° C.; otherwise conditions are the same as in fig. 3; ×80.

In the experiments with agar made with unstaled turnip juice, the first definite indication of a positive chemotropism (working, it is true, side by side with the negative chemotropism previously demonstrated) was obtained. The hyphae grew much more vigorously in the turnip juice medium than in the sugars. They did not in all cases grow faster; the chief difference consisted in the thickness of the germ tubes, which were twice or three times as thick as those grown in glucose or in cane sugar agar. Probably on account of this healthier development they reacted much better to chemotropic influences than when grown in the sugars. The agar was prepared by mixing equal volumes of turnip juice with 3 per cent distilled water agar, thus forming a medium containing 1.5 per cent agar. Combinations were tried as follows, the amount of reaction in each case being given below:

	A	*B*	*C*
Mica plate →	Plain agar	Turnip juice agar	Turnip juice agar
	Turnip juice agar +spores	Plain agar +spores	Turnip juice agar +spores
Reaction →	+60–90 per cent	+100 per cent	+100 per cent

Since varying conditions of light, heat, and moisture had been eliminated, it is obvious that only chemotropic forces were at work here.

The turning toward the holes in preparations *B* and *C* was very marked, but in *A* it was not nearly so pronounced. The only difference between *B* and *C* lay in the distance from the hole at which the curvature became noticeable in the same period of time (8.5 hours). In *C*, the reaction was apparent at a distance of 3–4 diameters of the hole, counting from its margin; but in *B*, the turning could be observed as far as 10 diameters from the hole. In *B* and *C* the turning in all cases was 100 per cent, while in *A* it varied from 60 to 90 per cent.

In *C*, since the turnip juice was everywhere of practically the same concentration, the only force exerted must be due to the staling substances produced by the hyphae, that is, a negative chemotropic force. In *B*, however, where we have the most marked turning of all, we can fairly assert that the turnip juice in the sporeless layer is the cause of the additional stimulus, and is, therefore, a *positive* chemotropic force. This reasoning was corroborated by the condition in *A*, where in the lower film the positive chemotropic force, due to the turnip juice, would be working *against* the negative chemotropic force due to the staling substance, with the resulting decrease in the amount of turning.

In these experiments the hyphae had been allowed to grow to a considerable length, none being under 300 μ, since it was reasoned that with long hyphae the negative chemotropic stimulus would be much greater than with short ones. On the other hand, the positive chemotropic force should be exerted just as strongly on short hyphae as on long ones. In view of this, younger stages were tried, with results such as are set forth in table IV.

The combinations *A* and *B* correspond to those given previously, but *C* and *D* are new. *D* represents the control, for here conditions were made as much alike as possible in both upper and lower films. The turnip juice being everywhere practically the same in amount, no positive chemotropic force can be acting. If the number and length of the hyphae were also equal in both films, the turning on both sides should be no more pronounced in one direction than in another. It will be seen, however, that in every case the figures fall within the recognized experimental error.

TABLE IV

RESULTS IN RHIZOPUS NIGRICANS WITH TURNIP JUICE AND PLAIN AGAR; YOUNG STAGES OF GROWTH

	Nature of films and location of spores	Number of preparations examined	Period of incubation in hours	Average length of hyphae in μ	Number of spores per sq. mm. of film surface	Total number of hyphae counted	Percentage of reaction
A	Upper film: plain agar Lower film: turnip juice agar+spores	2	6.75	40	37	108	+ 2*
		2	7.25	66	58	168	+18
		2	8.00	111	56	192	+43
B	Upper film: turnip juice agar Lower film: plain agar+spores	2	6.75	75	53	189	+94
		2	7.25	136	49	206	+99
		2	8.00	142	46	198	+94
C	Upper film: turnip juice agar+spores Lower film: plain agar+spores	2	6.75	above 60	20	179	−13
				below 51	15	118	+17
		2	7.25	above 80	23	158	− 8
				below 58	23	155	+33
		2	8.00	above 175	23	192	−30
				below 148	33	223	+46
D	Upper film: turnip juice agar+spores Lower film: turnip juice agar+spores	4	7.00	above 60	21	272	− 8
				below 74	27	292	+0.5
		4	7.75	above 80	23	286	−6.5
				below 101	22	378	−2.0

* + and − signs indicate turning toward or away from the holes, that is, positive and negative reactions respectively.

The most marked turning is shown in *B*, where the hyphae while still very short (75 μ) show 94 per cent of reaction. Here we have both chemotropic forces exerting a stimulus in the same direction. A most interesting contrast to this is shown in *A*, where the turning is toward a plain agar film without spores. The turning is much less marked than in *B*, not in any case even half as great. The hyphae, indeed, are shorter, but this is balanced by the fact that on the whole the spore number, and consequently the number of hyphae, is greater. The result here, therefore, corresponds with the case of *A* among the older preparations described above, and the same observations apply here. Also, as would be expected in the young stage, the effect of the negative chemotropic force is very slight where the hyphae are only 40 μ long. Later, when more staling substance has developed with the growth of the hyphae, the percentage of turning toward the film free from staling substance is much increased; we have seen in the older preparations that when the hyphae attain a considerable length the turning may become nearly 100 per cent.

A consideration of *C* bears out our interpretation of *A* and *B*, for since both films here contain spores, the amount of staling substance is more or less equal throughout the preparation, and the force due to a negative chemotropic stimulus, therefore, is practically eliminated. Any reaction which occurs should be a positive chemotropic one, and, as the tables show, there is a considerable turning from the plain agar to the turnip juice agar. That the extent of this increases with the age of the preparation is not easily explained, though it is no doubt partly due to the fact that some of the hyphae naturally react more slowly than others.[7] On the other hand, the hyphae in the turnip juice agar show an evident repulsion from the plain agar. This repulsion also increases with the age of the preparation, although not so markedly. It may likewise be accounted for by a slower response of some of the hyphae, as well as by the fact that in this film the concentration of the turnip juice decreases, through diffusion, at first only in the immediate vicinity of the holes; later the decreasing concentration extends farther out from the holes and affects more hyphae.

EXPERIMENTS WITH CANE SUGAR.—Strengths of 2.5, 5, and 10 per cent cane sugar were tried, the cane sugar used being the ordinary commercial lump sugar. The following combinations were arranged in the experimentation with each percentage of sugar.

	A	*B*	*C*
Mica plate →	Plain agar	Agar+sugar	Agar+sugar +spores
	Agar+sugar +spores	Plain agar +spores	Plain agar +spores

In 2.5 per cent cane sugar agar, the hyphae appeared very ill nourished, being slender and of slow growth. It seems reasonable

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[7] It may perhaps be caused also by the continual diffusion of the turnip juice farther and farther from the holes into the plain agar, thus acting on more and more hyphae.

to suppose that on this account they reacted weakly to chemotropic stimuli. As would be expected, the greatest turning was shown in *B*, where, when the hyphae were fairly long (200 μ) and sufficiently abundant, the percentage of reaction sometimes reached 40–50 per cent. That this turning was mainly due to a negative chemotropic reaction is apparent from what follows.

The experiments with 5 per cent cane sugar gave the best results, since this proportion seemed to supply the hyphae with a better amount of food, and also exerted a stronger positive chemotropic stimulus. Table V gives the results in condensed form.

TABLE V

RESULTS WITH 5 PER CENT CANE SUGAR

Combinations (as given in diagram above)	Number of preparations examined	Average length of hyphae in μ	Number of germ tubes per sq. mm. of film surface	Total number of hyphae counted	Percentage of reaction
A	3..........	116	17	162	+ 6
	4..........	133	25	282	+ 9
	4..........	155	19	195	+12
	3..........	189	23	185	+32
B	3..........	115	10	99	− 2 – weak grow
	3..........	139	14	150	+37
	3..........	162	12	184	+35
	4..........	197	14	229	+55
C	11..........	above 204	above 18	above 253	above −19
		below 191	below 5	below 280	below −10

Since it was clear from the experiments with turnip juice that the strength of the negative chemotropic stimulus was directly related to the length of the hyphae, the attempt was made to compare preparations containing hyphae of approximately equal length. Thus, the 4 lots of preparations in *A* and *B* roughly correspond. Unfortunately, the number of germ tubes averages much less in *B*, for many of the spores did not germinate, but if it were greater, assuming that it would be accompanied by an increased negative chemotropic stimulus, the final percentages in *B* would even be greater than they now are.

It is evident from the percentages that the hyphae in every stage of growth in *B* except the youngest are subjected to much greater chemotropic stimuli than is the case in *A*. This agrees well with the experiments with turnip juice; in *A* we have the negative chemotropic stimulus working against the positive chemotropic stimulus, while in *B* we have both these forces working together.

The fact that no positive curvature appears in the early stage of *B* may be accounted for by the weak growth of the hyphae in non-nutrient agar. They thus do not react to the sugar diffusing from the upper film, nor have they yet produced sufficient staling substances to cause them to turn to the holes leading to the upper layer. But with the increasing diffusion of sugar they become in time better nourished, develop more of the staling substances, and react to this negative chemotropic stimulus as well as to the positive stimulus exerted by the sugar.

In *C*, although the average number of germ tubes per sq. mm. in the lower layer is given in the table as 5, a result of the poor germination in the plain agar, in this case among the 11 preparations holes were selected which had an approximately equal number of hyphae about them in both films. In order to have germ tubes of fairly equal length in both lower and upper films, the lower films were given a 4 hours' start before the upper films were added. The results, however, are not in line with what we have seen for the same experiment with turnip juice (*C* of table IV), for we would expect to find a positive reaction in the lower layer; but, as already observed, the spores germinate poorly and the hyphae grow slowly in the plain agar. The majority of them germinate only around the holes, where the sugar is diffusing through. With this sugar diffuse also staling substances from the more vigorously growing hyphae above, thus counteracting any stimulus which would otherwise be exerted on the hyphae of the lower layer by their own staling substance. The positive result in the similar case where turnip juice is used is probably due to the greater chemotropic activity of the turnip juice; and possibly also to a more rapid specific diffusion of the active substance of turnip juice as compared with cane sugar, as well as to the more vigorous growth of the lower layer hyphae which are nourished by the diffusing turnip juice.

With 10 per cent sugar, the results confirm the preceding.

Experiments with glucose.—Glucose in strengths of 2.5, 5, and 10 per cent was also tried in the same way as described for cane sugar. The hyphae grew more vigorously in the glucose and reacted better. The results, in general, agree with those given for cane sugar.

Miyoshi's test by direct application of the substances.—Among other tests for chemotropism, Miyoshi (8) sowed spores in a film of 5 per cent gelatin on a glass slide, and when the germ tubes were still quite short, he placed a small amount of glucose at a given point in the gelatin. Diffusion commenced immediately, of course, and spread radially through the gelatin. With *Rhizopus*, he found that although some of the hyphae showed only more copious branching and an increase in thickness, others curved decidedly toward the center of diffusion. With *Penicillium*, however, no effect could be observed. On experimenting with other substances, he concluded that whenever no positive results were obtained, the failure was due to too rapid diffusion of the chemical substances.

Since this method was simple, and appeared practical, it was tried out extensively by the writer, using agar as well as gelatin films, and adding in some cases a small bit of solid glucose, in others, of cane sugar. Small pieces of turnip juice agar were tried also, these being fitted into corresponding cavities in the films. Neither *Rhizopus*, *Penicillium*, nor *Botrytis* gave any positive result, beyond the more copious branching and increased hyphal thickness mentioned by Miyoshi. Even in the case of the turnip juice agar, no turning was evident on inspection with the microscope. Possibly, as Miyoshi suggests, too rapid diffusion is the cause of the failure here. In any case, the mica plate method is far more definite and accurate.

Discussion

The staling substances.—From the foregoing it is clear that changes take place in a medium in which the fungus has grown, so that the medium then acts in a negatively chemotropic way toward the fungus. Whether these changes are due to the excretion of katabolic products by the hyphae, or whether they are the result of chemical changes which these excreted products induce in the medium itself, it is not possible to state with certainty. In any case, vital processes of the fungus are primarily responsible.

Of these two possibilities, however, it is much more probable that the fungal excretions are themselves directly the cause of the negative chemotropic action, chiefly on account of the fact that *all* the media worked with produce a negative chemotropic reaction after the fungus has grown in them for a time. It is unlikely that with these various kinds of media the same repellent substances would invariably be formed by chemical action of the fungal excretions. In support of this view, it is also noteworthy that with *plain agar* the same repellent effect was evidenced as with other media.

That the staling substances are either of a volatile or of a thermolabile nature has already been conclusively demonstrated. BALLS (1) has also shown this, and has ascertained that they exert an inhibitory effect on growth.

RELATIVE VALUES OF POSITIVE AND NEGATIVE CHEMOTROPIC FORCES.—The great difference of various germ tubes in their capacity for reaction was very noticeable in the examination of the preparations. Thus, with two germ tubes at an early stage of growth, and at an equal distance from the hole, one would react markedly, while the other remained indifferent. At a later stage, however, when we may assume that the stimulus was greater, all of the germ tubes within the prescribed region about the hole might react.

Our knowledge of the relation between individual variation in sensitiveness of the germ tubes and the intensity of the acting stimulus is of course very vague,[8] but it seems safe to assume that a larger percentage of turning means a stronger stimulus. Also the rate of diffusion of the stimulating substances would, within limits, affect the number of germ tubes reacting in a given volume

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[8] It is probable that the variation curve of sensitiveness of the germ tubes is of the well known "normal" type, so that the percentage reacting is not directly proportional to the strength of the stimulus. TRÖNDLE (12) has shown that for geotropic sensitiveness the curve is of this type.

of material around the hole; and again, the time during which the stimulus had been acting (since a longer period gives greater opportunities for growth) would also affect the result. Bearing all this in mind, we nevertheless seem justified in assuming that under the conditions of any given experiment the percentage of turning gives some measure, although only a very approximate one, of the intensity of the stimulus or stimuli acting.

By an inspection of the results, especially as shown in tables IV and V, we can thus arrive at some idea of the relative intensity of the negative as compared with the positive chemotropic stimulus. For if we denote by n the percentage effect due to the negatively acting staling substances, and by p the percentage effect produced by the positive stimulus, we have in *A* of table IV, using the percentage of reaction in the oldest preparations, $n-p=43$; whereas in *B* we have $n+p=94$, taking the two oldest preparations here also. Since the hyphae in these two corresponding cases are of approximately equal length, and have therefore probably produced an essentially equal amount of staling substance, a comparison is legitimate. By eliminating the p's, we have $2n=137$, or $n=68.5$; p will then be 25.5. In other words, in the special condition of this experiment, the positive chemotropic stimulus exerted by the turnip juice has an effect of a little more than one-third that of the negative chemotropic stimulus. In a general way these figures are corroborated by the results given for *C*.

In the same way, applying this method to the results given in table V, we may arrange the relative values of the stimuli as shown in table VI.

With the exception of the youngest hyphae, where diffusion of the chemotropic substances has hardly begun, or at least the hyphae have not yet had time to react to it, the figures are surprisingly regular, the percentage of the reaction due to the positive chemotropic stimulus remaining fairly constant, as would be expected. On the other hand, the negative chemotropic stimulus constantly increases with the growth of the hyphae and the excretion of more and more of the negatively chemotropic substances.

In *C* of table V we should expect to find a confirmation of these deductions, as was shown by *C* of the turnip juice experiments

n = % effect due to − [illegible]
+
p =
n − p in exp. A = 43
n + p = 94 in B
eliminate p ∴ 2n = 137
n = 68.5
p = 25.5

(table IV), but we have already seen why there is a discrepancy in the results for the lower film. On the other hand, the −19 per cent in the upper film is not greatly in excess of the amount representing the positive stimulus in *A* and *B*, for since the sugar in this case is in the upper film, the stimulus resulting from this would attract the hyphae away from the holes, and thus cause a negative figure.

TABLE VI

RELATIVE VALUES FOR POSITIVE AND NEGATIVE CHEMOTROPIC STIMULI IN CANE SUGAR EXPERIMENTS

Average length of hyphae	Nature of preparation	Percentage of total reaction	Percentage of reaction due to positive stimulus	Percentage of reaction due to negative stimulus
100–125..........	A B	(+ 6*) (− 2)	(− 4.0)	(+ 2.0)
125–150..........	A B	+ 9 +37	+14.0	+23.0
150–175..........	A B	+12 +35	+11.5	+23.5
175–200..........	A B	+32 +55	+11.5	+43.5
Very long†.......	A B	+49 +71	+11.0	+60.0

* The results in brackets all fall within the experimental error.

† These figures are taken from another series of experiments, not included in table V.

COMPARISON OF THE CHEMOTROPIC STIMULUS CAUSED BY CANE SUGAR AND BY TURNIP JUICE.—The cane sugar medium of 5 per cent strength, which seemed to be the proportion most favorable for growth, exerts, therefore, a comparatively small positive chemotropic force. Since it has been shown that when the turning is not very marked, the counting tends to be about 10 per cent too low, these numbers should probably be increased by about that percentage. But even if this be done, in comparison with it the stronger positive chemotropic force manifested by turnip juice is extremely striking and suggestive in relation to problems of parasitism, since it may indicate that plant juices in general evoke a fairly high positive chemotropic response.

Further work with plant juices is much to be desired. These juices of course contain a large variety of substances, the combined effect of which is evidently (at least in turnip juice) much more powerful than that of cane sugar. This is perhaps one of the reasons why FULTON, working with only simple substances like the sugars and various salts, was unable to demonstrate the existence of the very weak positive chemotropism which these substances may cause. The fact should also be emphasized that the hyphae grew much more vigorously in turnip juice than in the sugars, and probably on that account reacted better as well as much earlier. It is reasonable to associate a stronger reaction with a healthier growth.

THE GRADIENT OF DIFFUSION IN ITS RELATION TO CHEMOTROPISM.—MIYOSHI (8) claimed that when hyphae are placed between two concentrations of a substance, the concentrations being kept constant, no chemotropic reaction occurred unless a definite ratio existed between the two concentrations. Thus a fungus between 0.1 and 0.3 per cent of a sugar solution would not react; but with 0.1 and 1.0 per cent, a positive curvature occurred toward the 1 per cent. Similarly a 0.5 per cent solution must have a 5 per cent solution as an antithesis, if a reaction is to ensue. In other words, the conditions for a sufficient stimulus to produce a turning are in accordance with Weber's law. In our own experiments, in the cases of the hyphae turning toward the holes, it is difficult to conceive how this law can apply. The hyphae are so slender that the difference in concentration of the diffusing substance on opposite sides of a hypha, assuming that the latter is growing tangentially to the hole, could hardly be in the proportion of 10 to 1, given by MIYOSHI as requisite for a reaction. JOST (6) has voiced a similar criticism. As a matter of fact, it is quite evident from our work that the hyphae must react to vastly smaller differences in concentration than this. We must admit, however, that the external factors which bring about chemotropic reaction are still somewhat obscure. Possibly there is a reaction to the direction of the diffusion current.

OSMOTROPISM.—It is possible that osmotropism, as suggested by PORODKO (9), may play some small part in the reactions, but it cannot be an important factor, for if it were, we should get similar results with solutions of equal osmotic pressure. Now, as regards the osmotic pressure of turnip juice, W. BROWN, working at this college, has determined that it is equal to that of a 14 per cent solution of cane sugar. It will be recalled that in the preparation of the turnip juice agar the turnip juice was diluted to half-strength, and its osmotic pressure must therefore be equal to about a 7 per cent solution of cane sugar. But our experiments show that even a 10 per cent solution of cane sugar is far less effective for causing a reaction than the turnip juice. It is clear, therefore, that osmotropism cannot be of great importance.

THE DISTRIBUTION OF A FUNGUS IN ITS HOST.—While the results given in this paper apply only to *Rhizopus nigricans*, there is abundant evidence from similar work carried on with *Penicillium* and *Botrytis* that the same conditions exist there also. It is quite likely that similar chemotropic reactions obtain in the majority of the fungi. If this be the case, it is possible that the distribution of a fungus in its host may depend mainly, not on a positive chemotropic reaction, but on the dominant negative chemotropic stimulus of its own staling products.

Summary

The following results apply to *Rhizopus nigricans* Ehrenb. in particular, but experimental evidence is at hand that the general principles involved apply also to *Botrytis cinerea* Pers. and *Penicillium* no. 24 Thom. Most of the data have been derived from work with two layers of medium separated by a perforated mica plate.

1. The fungus shows a marked negative chemotropic reaction to a medium in which it has been growing for some time.

2. The hypothesis brought forward by CLARK and FULTON, that this negative chemotropism is a reaction of the fungus toward its "staling substances," is conclusively substantiated by the following evidence: (*a*) the hyphae in one layer of medium turn away from another layer without spores if it contains their own staling products; (*b*) the hyphae always show a marked turning from the medium in which they are growing to any medium which is free of hyphae, no matter what the composition of that medium may be, unless the second medium contains the substances which exert a negative chemotropic stimulus; (*c*) when approximately

equal amounts of mycelium occur in two similar layers of medium, no turning of the hyphae from the one to the other results.

3. These staling substances are formed as a result of the vital activities of the fungus itself. They consist probably of excreted products of metabolism.

4. The staling substances appear to be either thermolabile or volatile, for boiling a solution containing them reduces markedly their negative chemotropic influence.

5. Positive chemotropism toward the substances tested (turnip juice, cane sugar, and glucose) also exists; but under ordinary conditions of growth this positive chemotropism is very much weaker than the negative chemotropism previously mentioned.

6. Turnip juice exerts a much stronger positive chemotropic stimulus than the other simple chemical substances tested, much stronger than, for example, 5 per cent cane sugar. This suggests the possibility that plant juices in general evoke a stronger positive chemotropic response than the simple chemical substances heretofore experimented with. The nature of the attractive substance or substances in turnip juice has not yet been determined.

7. It is impossible in the present stage of our knowledge to compare accurately the strength of two chemotropic stimuli; but, using the number of hyphae turning as a test of the relative action of the two stimuli, we certainly may conclude that in comparison with the stimulus exerted by negative chemotropism, that due to positive chemotropism is very much less. For example, in a given preparation with two layers of media separated by a perforated mica plate, and with the hyphae at a certain stage of growth, when 90–100 per cent of them are turning toward the holes from plain agar to turnip juice agar, the stimulus due to the positive chemotropic effect of the turnip juice has, very approximately, one-third of the effect of the stimulus due to the negative chemotropism from the staling products of the fungus itself. In other words, positive chemotropism is responsible at this stage and under these conditions for about one-quarter of the whole reaction. With the sugars the part played by positive chemotropism is very much less and is *easily overlooked*.

8. In contrast to the increase in strength of the negative chemotropic stimulus with the age of the preparation, the

attraction resulting from positive chemotropism remains fairly constant.

9. If the preceding conditions are true of fungi in general, it is probable that the distribution of a fungus in its host is influenced mainly, not by positive chemotropism, but by the dominant negative chemotropism due to its own staling products.

10. The part played by osmotropism in these reactions must, if any, be a very small one.

In conclusion, the writer desires to express his great indebtedness to the invaluable suggestions and hearty cooperation of Professor V. H. BLACKMAN, at whose instance this work was undertaken. The main part of the investigation was carried on under Professor BLACKMAN'S direction at the laboratory of Plant Physiology and Pathology, Imperial College of Science and Technology, London, but the work was finished at the Osborn Botanical Laboratory of Yale University.

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STERILITY AS THE RESULT OF HYBRIDIZATION AND THE CONDITION OF POLLEN IN RUBUS[1]

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(WITH PLATES X–XII)

One of the first well authenticated records of the possibility of crossing species dates from 1694. In that year CAMERARIUS (1) in a letter to VALENTINE mentions the effect of crossing the hemp (*Cannabis*) with the hop (*Humulus*). However, it was not until 1760 that KÖLREUTER (2) made the first plant hybrid which was to be of use to science. He crossed *Nicotiana paniculata* ♂ and *Nicotiana rustica* ♀. In stating his conclusions he recognizes the relative fertility of hybrids from crossed varieties as compared with the relative sterility of hybrids made between distinct species.

As a result of KÖLREUTER'S research much interest was aroused among plant breeders as to the rôle of hybridization and as to the conditions which lead to sterility. Such men as KNIGHT (3) and HERBERT (4) will, perhaps, illustrate the diversity of opinion which sprang up. KNIGHT, on the one hand, held that hybrids from parents of distinct species were sterile, while, on the other hand, the varietal crosses were fertile (an opinion quite like that of KÖLREUTER). HERBERT, on the contrary, found that hybrids between different species are frequently fertile, a fact which he interpreted as signifying that the parents had branched from the same main stock. FOCKE (5) also has pointed out the fact that no peculiarity of hybrids has attracted so much attention as the lessening of the power of reproduction, and that crosses between more distantly related plants are more sterile even up to entire sterility. Other views have been expressed since, some favoring one side and others being equally sure that the contrary is true. However, though this diversity of opinion is prevalent even to the present time, nevertheless it seems clear that sterility is a common characteristic of hybrids.

[1] Contribution from the Laboratories of Plant Morphology of Harvard University.

Sterility may be brought about in several different ways, both physiologically and morphologically. My purpose is not to enter into the subject of physiological sterility, which is of much more interest economically than it is from the evolutionary standpoint; and, accordingly, I shall confine my attention entirely to the morphological side.

One of the most obvious types of morphological sterility is indicated by the sexual organs of the flower. Often as the result of hybridization the anthers and pistils are abnormal, and still more often, even when these are normal, we find a large percentage of sterile ovules present. The pollen shows this type of sterility especially well. I have records as far back as 1832 in which morphologically sterile pollen has been recognized as a criterion for hybridization. In that year DUTROCHET (6) wrote a letter to the Academy in which he attributed the sterility of hybrids in part to the imperfections of pollen. He refers to the flower of a hybrid cherry tree, resulting from the union of *Prunus cerasus* and *P. avium*, whose "stamina" formed a compact mass in which the pollen did not divide into pollinic or fertile dust as in the case of fruitful trees. This appears to be an extreme case, and yet it may rightfully be classed as a type of morphological sterility. GÄRTNER (7), in his well known essay, also refers to pollen sterility as a criterion for hybridization. Again, according to DUCHARTE (8), NAUDIN observed that the degree of fertility of hybrids was *en rapport* with the number of normal pollen grains formed in the hybrid.

Since the discovery of MENDEL'S work (9) a great impetus has been given to plant breeding, and we find many scientists entering into the problems opened up thereby. Most of the work done has been in regard to the inheritance of factors and to the working out of Mendelian laws of heredity. However, several observers have given their attention to the question of the pollen condition. ROSENBERG (10) discovered that in an apparently sterile hybrid sundew (*Drosera longifolia obovata*) the young pollen grains mostly lost their contents, while the embryo sacs usually did not develop beyond the binucleate stage. DETLEFSEN (11), working upon the animal side, has shown that a partial abortion of gametes occurs in the male progeny of a *Cavia* species cross.

Other interesting facts have been brought out from the cytological standpoint. Several investigators have worked at pollen sterility from this side, and though it is not my purpose to go into any prolonged discussion of the matter, yet it may be well to give a brief account of what has been done. JUEL (12), working with a hybrid known as *Syringa chinensis*, noticed that all the pollen was abortive and that abortion did not take place until after the formation of tetrads. TISCHLER (13), through his research upon the same hybrid, and also upon hybrids of *Ribes*, found that pollen abortion seemed to take place usually after the formation of tetrads. He also made a study of hybrids from a *Bryonia* species cross made by CORRENS, and found that the tetrads were formed but that the pollen generally aborted, as did also the embryo sacs. He ascribes the frequent sterility of mutants, accompanied by irregularities similar to those of hybrids during reduction, to the disturbance of the idioplasm. GEERTS (14), in working out the cytology of *Oenothera Lamarckiana* of DEVRIES, determined that one-half of the pollen from each tetrad aborted, and also that one-half of the embryo sacs did not develop. BELLING (15) speaks of this behavior as being unique, since, as he states, "these Oenotheras cannot be considered hybrids." This statement is worthy of note when taken into consideration with views recently expressed, not only about *Oenothera* as a genus, but concerning the Onagraceae as a whole. OSAWA (16) has recently shown that in the case of the Unshu (Satsuma) orange, although this is not known to be a hybrid, yet nearly all the pollen aborts and the abortion takes place only after the formation of tetrads.

As can readily be seen, all these writers emphasize the fact that the abortion of pollen seems generally to take place only after the formation of tetrads. DORSEY (17) not only points out that this seems to be the case with the genus *Vitis*, but he also distinguishes two kinds of abortion. To quote directly:

> In the formation of sterile and fertile pollen of the grape the heterotypic and homotypic divisions and the division of the microspore nucleus take place normally. Sterile pollen in the grape results from degeneration processes in the generative nucleus, or arrested development previous to mitosis in the microspore nucleus. When degeneration begins early after the division of the microspore nucleus, both the generative and vegetative nuclei may remain normal.

Before leaving the general question in regard to the formation of sterile pollen, I should like to call attention to another phase upon which no great amount of work has yet been done. This is in regard to Mendelian research. BRAINERD (30), in one of his articles concerning hybrids of *Viola*, describes violets which he has cultivated and which he knows to be hybrids from their external characters. BATESON (22) makes this statement: "Such an experiment raises the hope that sterility consequent upon crossing, the most obscure of all genetic phenomena, may become one of the possibilities of Mendelian research." JESENKO (19), using a cross between wheat and rye, was able to obtain one hybrid plant with pollen mostly shrunken and sterile. After several artificial crosses he was able to obtain a second generation hybrid which was apparently quite fertile. However, when he repeated the experiment on a larger scale, he obtained several first generation hybrids, but could obtain none of the second generation except through back-crosses. He now thinks that the first second generation plant which he obtained was of such a nature. SUTTON (20) also has done work in crossing varieties and species of cultivated peas, together with a wild pea of Palestine (*Pisum humile*), and his results have been confirmed by BATESON (22). Although he found the first generation hybrid to be either entirely or partially sterile, yet when he obtained second generation plants, he found them to be fertile. This may, perhaps, be explained by the fact that he used plants close in relationship and hence more compatible.

Within a few months a study of pollen sterility has been made by BELLING (15) in which he has attempted to show that certain ratios prevail. His work has been carried on in Florida, chiefly with 3 species of *Stizolobium*. The first, the Florida velvet bean (*Stizolobium deeringianum* Bort.), he states is of unknown origin, but is very commonly known as "a large ornamental tropical climber." The second, the Lyon bean (*Stizolobium niveum* Roxburgh), is commonly grown in the Philippines and elsewhere in South Asia. The Yokohama bean (*Stizolobium hassjoo* Piper and Tracy), which he takes for the third, is common upon the island of Japan. He has worked more especially with these, but some work, though only with the first generation hybrid, has also been done by him with the China bean (*Stizolobium niveum* var. ?), commonly considered as a variety of the Lyon bean. Perhaps it is not well to go into more detail, but rather to allow the reader the privilege of consulting the article for fuller details. His conclusion is that, generally speaking, 50 per cent of the pollen of the F_1 generation loses its contents and becomes sterile. A similar degree of sterility seems also to hold for the embryo sacs. Where the experiment was carried to the F_2 and F_3 generations, it was found that the offspring of the fertile half all bred true to fertility, while those of the sterile half all persisted in having 50 per cent sterile and 50 per cent fertile. The ratio here, as the investigator points out, is 1:1. As to the value of the results obtained, I am not in a position to judge. However, the fact that the investigator is dealing with the haploid generation instead of the usual diploid generation lends a new interest to the matter and opens up a possible new field of research.

Thus far I have attempted to give a general idea of some of the work done heretofore upon sterility in pollen, and its relation to hybridization. The work of DEVRIES and his "Mutationstheorie" resulting therefrom have given a great impetus to a search for the true means of evolution in living matter. The Darwinian idea that species arise through a gradual loss or gain of characters and the survival of the fittest of these has been very seriously attacked. The "sporting" condition which DARWIN recognized as occurring sometimes, but which he considered to be of far less importance than the changes brought about gradually, has been made to play a very much more important part by DEVRIES' mutation idea.

DEVRIES bases his theory primarily upon the much discussed species *Oenothera Lamarckiana*, which he had been growing for several generations in his garden, and of whose origin he is in no way certain. As I have already shown, sterile pollen, though not necessarily present in hybrids, is a common character. Recently JEFFREY (21) has shown that, so far as his examination has extended, morphologically sterile pollen does not occur, except in a percentage small enough to be accounted for by unfavorable conditions, in plants which are either monotypic or are isolated either in a geographical sense or through the time in which the flowers are matured. He has shown, moreover, that below the angiosperms a comparatively small amount of sterility occurs, and that in the gymnosperms it is almost entirely absent. On the other hand, the angiosperms present a very different aspect. Certain families of the last have long been a puzzle to the systematist. BATESON (22) points out that in *Oenothera Lamarckiana* a large percentage of sterile pollen is present. Other geneticists and cytologists, including DEVRIES himself, have noted this, but perhaps BATESON was the first to attribute it to hybridization. JEFFREY (21) has shown that the condition of abortive pollen is characteristic not only of *Oenothera Lamarckiana*, but of the *Oenothera* species in general, and that, moreover, the entire family of the Onagraceae is so characterized. Because of this he considers that DEVRIES' "Mutationstheorie," in so far as it is based upon *Oenothera Lamarckiana*, is lacking in justification. In other words, in order to establish such a theory securely, one must deal with plants and with animals which are species in the fullest sense of the word. Geneticists have long recognized the variability of hybrids, and hence plants with any trace of hybridization should not be used as a basis for a theory of such fundamental importance as that promulgated by DEVRIES. In a recent article DEVRIES (23) has attempted to refute JEFFREY's contention that pollen sterility is a criterion for hybridization. He states that, from such a viewpoint, we might consider the abortion of the 3 mother cells in the formation of the mature egg, which customarily takes place in the angiosperms, as a criterion for their hybrid origin. Again, he cites the case of certain sedges in which ordinarily 3 of the pollen mother cells abort and we find their remnants clinging to the mature pollen grain. His examples appear to me to be chosen from conditions which have no direct relation to the situation found by JEFFREY.

After what I have just said it is fitting, perhaps, to add a few words as to what really helps to make up a true species. In attempting such a discussion I realize that I am treading upon more or less forbidden ground; however, I shall give views which seem to me to be well supported by the observations of experts. There are those who would separate the idea of species entirely from that of a hybrid, but, on the other hand, we find a large number of prominent biologists who hold a different view. In an article by MEEHAN (24) I find the following reference to LINNAEUS: "The great father of botany does not seem to have much, if indeed any, idea of sterility in his comprehension of the term. He believed that a large number of plants which he regarded as true species and to which he gave specific rank and specific names originated as hybrids. Thus we have *Trifolium hybridum*, *Trigonella hybrida*, *Campanula hybrida*, etc., among a large number of similar Linnean names." OLIVER (25) remarks that "natural hybrids have been known for a long time and we have every reason to believe that they have existed when and where conditions were favorable for their production." Recently GATES (26), in an article upon *Oenothera Lamarckiana*, writes as follows: "Crossing must have taken place in the ancestry of *Oenothera Lamarckiana* as well as in other flowers which are open pollinated. Among open pollinated plants (and the same is probably true for animals) there is no such thing as a 'pure' species, but, rather, many interbreeding races, whose combinations vary from generation to generation, make up the population." Upon the animal side views similar to this have been expressed. In an article recently published GEROULD (27) has shown how important a rôle hybridization has shown in forming species, and its relation to mutation.

I might cite other references (such as BATESON **22**, JANCZEWSKI **28**, CASTLE, BACOT, PROUT, etc.) to show how various biologists consider the term species. However, perhaps the foregoing will be sufficient to give some idea of the difference in opinion concerning the matter. Suppose we grant that two such ideas of a true species can exist together, then are the angiosperms, or at least many of them, species in the same sense as those which we find among the gymnosperms? In other words, is not the greater abundance of angiosperms as compared with the number of gymnosperms to be accounted for, to a large extent, through the formation of natural hybrids which breed true and have characters distinct enough to make them good species when viewed from the systematic standpoint? Certain families like the Onagraceae, Violaceae, Solanaceae, Rosaceae, etc., have long presented great difficulties to botanists.

Recently a specialist in *Oenothera* (BARTLETT 29) has published an article in which he makes what he calls "12 elementary species of *Onagra*," 5 of which are made out of our common evening primrose (*Oneothera biennis*). This is surely a perplexing condition. BRAINERD (30), who has made a very careful study of the species of *Viola*, both in the field and under controlled conditions, has met with a very disconcerting situation. He finds a difference in the classes of hybrids. When hybrids are made between nearly related but distinct species, they have characters intermediate between the unlike characters of the parent forms. They are stable, breeding true from generation to generation, and showing a very vigorous growth, but are always more or less sterile. On the other hand, when crosses are made between species which are more or less doubtfully distinct, or between races, or between a species and a variety, there is usually quite a different result. In the first place, the offspring of such crosses have not intermediate characters, but recombinations of the unlike characters of the parent forms. As a result, the individual plants are dissimilar, some reverting to one or the other parent form, and others presenting the "sporting" characteristics. Another very important characteristic difference is that we do not usually find the fertility damaged either in the first or in subsequent generations. He speaks of these as "the so-called Mendelian hybrids, the despair of the systematist but the vantage ground of the breeder of new and useful 'varieties.'" Finally, he cites the instance of several species of *Aquilegia* which he allowed to grow indiscriminately together, and which in a short time gave off 5 hybrids. This case is similar to that of the violets which, owing to the greater amount of the cleared land, can be greatly increased in range and hence have a much greater opportunity for hybridization.

In connection with BRAINERD'S remark concerning his so-called Mendelian hybrids it seems well to quote a few lines from another. CORRENS (31) was one of the first to rediscover MENDEL. He evidently from the first appreciated certain limits to the scope of MENDEL'S laws.

> Inzwischen hat mir ein Aufenthalt in den Alpen Gelegenheit gegeben, eine Anzahl von Bastarden zwischen unzweifelhaften Arten zu untersuchen

(*Cirsium palustre*×*spinosissimum*, *Cirsium heterophyllum*×*spinosissimum*, *Achillea moschata*×*nana*, *Achillea macrophylla*×*moschata*, *Carex echinata*×*foetida*, ferner *Melandryum album*×*rubum*, hier eine vollkomme gleitende Reihe von einer Art zu andern). Dabei ist mir zweifelhaft gebleiben, auch nur bei einzigen dieser Bastarde in einem einzigen Merkmalspaar ein wirkliches Dominiren verkommt, ganz sicher ist, dass in fast allen Punkten, in denen die Eltern differiren, der Bastard die Merkmale beider Eltern zeigt, jedes abgeschwächt, wenn auch in verscheidenem Grade.

Other paragraphs might be cited which bear more or less upon the subject, but I shall cite but the closing line of the article.

> Die Aufdeckung der Mendel'schen Regeln wird also kaum dazu beitragen, dass von jetzt ab Speciesbastarde und Rassenbastarde in einem Topf geworfen werden, und man statt dessen nur von Mono- Di- etc., Polyhybriden sprechen wird, sie wird im Gegentheil wohl der Anfang für eine schärfere Trennung der beiden sein.

The Rosaceae, which is the last of the families mentioned as being a perplexing problem for the systematist, has among its many genera several whose species are very much unsettled. Of these *Crataegus*, *Rosa*, and *Rubus* may be mentioned as distinctive. With these 3 genera there have been great changes wrought within the last few years. Taking *Crataegus*, for example, we find a very interesting account of its present condition by BROWN (32). When GRAY'S *Field, forest, and garden botany* was published in 1857 there were listed in it but 12 species and 2 varieties. Again, in GRAY'S *Manual of botany* of 1867 we find only 10 species and 4 varieties given. It was not until after the publication of CHAPMAN'S *Flora of the southern United States* in 1897, which gave but 15 species within its range, that we find any great increase in the number of species. Since then many species have been described. In fact, since 1896 there have been, according to BROWN, 864 species and 18 varieties described. Where will this species making end? As BROWN states, "Why did not the systematists discover this great number of species before? Have they been recently hybridizing? Undoubtedly a part of the great increase may be explained by a more intensive study upon the part of the systematic botanist, but does this account for it all?" BRAINERD, in reply to BROWN's inquiry, states that "the Rosaceae of all orders is the *most predisposed to hybridize. Rosa, Rubus, Geum, Amelanchier,* and *Malus* are notorious for the forms resulting from interbreeding. By analogy we should expect the same condition of things in *Crataegus*."

Rubus presents a condition of things quite like that of *Crataegus*. Although GRAY'S *Manual* of the last edition contains 38 species all told, yet the authors carefully state that in *Eubatus* we have "a group of great taxonomic difficulty, in which many species have been recently proposed. Of these the better marked have been here freely included, but without entire confidence that the future intensive study may not show them to be intergradient and perhaps in many cases hybrid forms." Because of this great variation and because of the uncertainty as to which are and which are not true species, I have selected this genus in which to make a careful study of the pollen conditions. However, before proceeding to the results of my research, it seems best to give a brief historical account of *Rubus* and the work done upon it up to the present time.

Rubus, like *Crataegus*, has had an almost miraculous growth in number of species within a few years. Only 18 years ago GRAY'S *Manual* recognized but 3 species and 2 varieties for New England, and, as BRAINERD (33) remarks, 2 of these specific names were wrongly applied. In 1898 BAILEY (34) published his *Evolution of native fruits* in which he recognized 13 species of *Rubus* of the *Eubatus* group for New England. Since that time BLANCHARD has published detailed descriptions of 40 or more forms, most of which he has considered as species. Many of these, as I shall show later, appear to be of hybrid origin from a study of the pollen; but from an external study alone he came to the conclusion that they were worthy of specific rank.

In 1911 BICKNELL (35), in an article entitled "Have we enough New England blackberries?" makes the following statement: "*The least unstable species* (*here*) *possess some kind of ready pliancy which answers often with marked emphasis to slightly changed conditions of growth, and further, all the species by some freely practised method of versatility acquire variously in combination with their own proper characters the features of associated members of the group.*"[2] He adds that these facts impart an extraordinary natural variability

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[2] The writer is responsible for the italicizing.

and undoubtedly also *a facility in hybridizing which is, perhaps, not exceeded in any of our flora.* In speaking of BLANCHARD'S species he remarks that "probably 60 per cent of these are synonyms, while the remainder, with possibly a few exceptions, *appear to disclose themselves as scarcely doubtful hybrids.*" In his mind there are about 11 species of *Eubatus* which alone can be considered valid, while the others are hybrids. He also gives a list of their names as he believes they should appear. BRITTON, in the new edition of the *Illustrated flora*, recognizes but 10 species, none of BLANCHARD'S naming being mentioned even as synonyms. More recently RYDBERG in his monograph of *Rubus* has allowed 27 of the *Eubatus* group for the northeastern United States, including 7 of BLANCHARD'S naming and 4 of BAILEY'S. Of the remaining 30 and more of BLANCHARD'S names, 9 are disposed of as synonyms and 24 as hybrids.

BRAINERD (33) in his recent article upon *Rubus*, which he has published after years of study and from which I have drawn many of my historical data, remarks: "No mere herbarium botanist is qualified to disentangle the perplexing intricacies of a group of plants like *Rubus*. The days of random guessing at parents are past or should be." He then states what to him are the proper methods of procedure in determining a true species, and gives a list of 9 "probable good species" in Vermont.

The phenomenon of variability in *Rubus* appears not to be confined to American species, but to be characteristic of the genus everywhere. HOOKER in his *Flora of the British Isles* names 41 forms as species, while GARKE'S *Flora von Deutschland* contains more than 60 species. FOCKE (5) makes the following interesting statement:

> Die Gattung *Rubus* ist ungemein formenreich und zerfällt in eine Anzahl verscheidener wohl charakterisirter Untergattungen. Einige Gruppen bestehen aus aüsserst zahlreichen nahe verwandten Racen, welche die Grenzen zwischen den durch wesentliche Charactere gescheidene Arten oft völlig vermischen. Auch viele sonst gut umgrenzte Arten sehr formenreich natürliche Hybride scheinen innerhalb vieler Artengruppen leicht zu entstehen, wahrscheinlich gehen haufig aus ihnen neue constante Racen hervor.

During the summer of 1915 I was able to obtain from the Harvard Botanical Garden and from the Arnold Arboretum buds of about 40 species of *Rubus*. In every case I picked those buds which were about to open, and in this way I was able to obtain pollen grains which were mature. If these were morphologically good, they were large, round, and very full of protoplasmic substances. Material, on the other hand, in which the pollen had not matured normally showed it to be shrunken and empty, having lost all or most of its protoplasm and having but a vestige of the nucleus present.

During the following winter I made a study of the pollen condition present in these buds, the method in most instances being as follows. Owing to the fact that perfectly formed pollen is very densely filled with protoplasmic substances, no harm resulted from killing directly with 95 per cent alcohol. Other more tedious methods were tried, but no better results were obtained. The buds were then pricked, the air pumped out, and the buds thoroughly dehydrated in absolute alcohol. After this they were placed in a 2 per cent solution of celloidin dissolved in equal parts of synthol and ether. The grades of celloidin were raised 2 per cent every 12 hours, until 16 per cent was reached. Then small pieces of dry celloidin were added at 12- or 24-hour intervals, until a sufficient density was reached. When such a condition was obtained, the buds were removed one at a time and allowed to harden in chloroform for about 12 hours. They were then placed in equal parts of 95 per cent alcohol and glycerine. When completely hardened these were mounted upon blocks in a 6 per cent solution of celloidin and cut upon the microtome. After being cut and stained in Haidenhain's iron alum hematoxylin and counterstained in safranin, they were mounted in balsam. Such a method allows the observer actually to see the pollen as it occurs in the anther, and by so doing it gives one a more accurate idea of the true condition.

The genus *Rubus* in the last edition of GRAY'S *Manual* is divided into 5 groups with FOCKE as an authority for their names. Of the first group, which is called the IDAEOBATIS group, I have 3 different forms (*Rubus strigosus* Michx., *R. neglectus* Peck, and *R. occidentalis* L.). It is here made a very small group, having but 3 well authenticated species. *R. strigosus* Michx. (fig. 1) is given as a synonym for a variety of *R. idaeus* L. I find the pollen condition to be nearly perfect and hence to give every indication that, from a morphological basis at least, the plant should be given a specific rank. *R. neglectus* Peck (fig. 2), on the other hand, shows a large percentage, perhaps 50 per cent, of sterile pollen. This condition goes very well with the remark made in the *Manual* that it probably is a self-perpetuating hybrid. Fig. 3 is an enlarged view of a portion of the field shown in fig. 2. *R. occidentalis* L., which is the well known black raspberry or thimbleberry, is given a specific rank in the *Manual* and the pollen condition indicates that such a position is tenable.

Of the second, or ANAPLOBATUS group, I have 2 members. The first, *R. odoratus* L. (fig. 4), the beautiful flowering raspberry, shows pollen almost universally good. Such a position would be expected from the late period in which it bears its flowers. Even should it be susceptible to crossing with other members of the genus, its lateness in regard to the opening of its buds would make it very hard to do so. *R. parvifolius* Nutt., on the other hand, although it is put here as a good species, contains a large percentage of pollen which is clearly sterile.

I have been unable to obtain any material of the members of the third (CHAMEMORUS) and of the fourth (CYLACTIS) groups, including in the *Manual* but 3 species.

It is the fifth or EUBATUS group which is by far the largest and has given the most trouble. In studying these I have found several whose pollen is nearly perfect, so nearly so in fact that the few shrunken grains which do appear may be accounted for by some unfavorable physiological condition. By far the greater number, however, show a greater or less degree of sterility. For convenience I have divided these last into those members ranging from sterility of about 25 to about 75 per cent; and also those which show a range greater than 75 per cent.

Among those with good pollen appear the following: *R. allegheniensis* Porter, *R. setosus* var., *R. fruticosus* L., and *R. amnicolus* Blanch. *R. allegheniensis* Porter (fig. 5) and *R. setosus* Bigel. (fig. 6) are given specific rank in the *Manual*. *R. fruticosus* L. (fig. 7), which is a European form, is suggested here as perhaps the same as *R. laciniatus* Willd., with the leaves less cut. *R. amnicolus* Blanch. (fig. 8) is placed together with *R. orarius* Blanch. as a synonym for *R. pergratus* Blanch.

Of those with a percentage of over 25 and less than 75 sterile I have *R. frondosus* Bigel., *R. recurvans* Blanch., *R. glandicaulis* Blanch., *R. Jecklyanus* Blanch., *R. orarius* Blanch., *R. arundelanus* Blanch., and *R. amabilis* Blanch. Of these GRAY'S *Manual* cites 4 as probably of specific rank, namely, *R. frondosus* Bigel. (figs. 9 and 10), *R. recurvans* Blanch., *R. glandicaulis* Blanch., and *R. Jecklyanus* Blanch. *R. orarius* Blanch. is placed together with *R. amnicolus* Blanch. as a synonym for *R. pergratus* Blanch. *R. arundelanus* Blanch. is considered as a synonym for *R. recurvans* Blanch. Finally, *R. amabilis* Blanch. appears under the same name as *R. canadensis* L. Comment upon this group is unnecessary, unless to remark upon the apparent perplexity its members have caused systematists. Clearly from the standpoint of sterility much hybridization is now occurring and has already taken place.

Of the last group, those with over 75 per cent of the pollen sterile, I have 4 specimens. These are *R. biformispinus* Blanch. (fig. 11), *R. Andrewsianus* Blanch. (fig. 12), *R. tardatus* Blanch. (fig. 13), and *R. peculiaris* Blanch. These all seem to have distinctive form, and from the standpoint of the systematist may warrant a species rank, at least they are so considered in the *Manual*. However, the pollen condition plainly points not only to hybridization, but to hybridization between species which must have been quite distinct and certainly quite incompatible.

Besides these forms I have 10 or more which are not included with the range of GRAY'S *Manual*. But one of these, *R. deliciosus*, (fig. 14), appears to be a good species when taken from the standpoint of absence of sterile pollen. I have *R. caesus turkestanicus*, *R. corylifolius*, *R. spectabilis*, *R. plicatifolius*, *R. multiformis* (fig. 15), *R. semierectus*, and *R. spectabilis plena* (fig. 16). One of the forms, *R. nobilis* (figs. 17 and 18), which is known as a hybrid, is almost entirely sterile, and, as one would expect, contains but a very few grains which can possibly be considered as viable.

Since my purpose has not been to attempt to place systematically the members of the genus *Rubus*, I have not given any account of the various characters upon which the systematists separate its species. Indeed it appears to be too hard for the systematist himself. Scarcely any two systematists agree as to which are and which are not true species. This is especially well shown by the large number of synonyms which one meets in any systematic work upon the genus.

However, I have found that sterility of pollen is a common characteristic throughout the entire genus. But few of the species indicate a perfect pollen condition and some are almost entirely sterile. In other words, the genus *Rubus* indicates to me a condition which appears to prevail in many angiosperms. JEFFREY (21) has already pointed out how prevalent sterile pollen is throughout the whole family of the Onagraceae, and other groups of plants are now being worked upon in this laboratory which seem to show a similar condition.

In closing, it seems to me that many of the species of the angiosperms are in a very different condition from that of others of the same group and from practically all those of the gymnosperms. They appear often to be natural hybrids which are formed, as BRAINERD has pointed out, as a result of a cross between distinctly separate species. Such crosses do not result in the segregation which Mendelian crosses usually exhibit, but rather in the blending of the characters of the parents. CORRENS (31) in his observations has noted such a situation in regard to certain plants. Such offspring may breed true to their respective characters and thus perpetuate a distinct form which, from a systematic point of view, is entirely of specific rank, but which should be treated by the plant breeder in an entirely different manner. Such forms usually have a large percentage of sterile pollen. Thus *Rubus* hybrids formed by distinct species are very sterile, in fact almost entirely so, while crosses of varieties which are closely related, and which hence are usually more compatible, may give almost no indication of their hybrid origin.

Thus it appears to me that the genus *Rubus* is one of those angiospermic genera in which the term "species" must be used in a different sense from that applied to many other members of the angiosperms, and practically to all of the gymnosperms. It is a group which is a puzzle to the systematist, and whose species will, perhaps, never be definitely established.

Conclusions

1. Sterility of pollen has long been recognized as a criterion of hybridization.

2. Crosses between distinct species have long been known to be more or less sterile and to behave differently from crosses between more closely related forms or varieties.

3. True species when crossed do not, in most cases, follow the laws of MENDEL, but tend rather to blend to form more or less constant types, often systematically recognized as species.

4. Many species of the angiosperms are species in a very different sense from those of the lower plants and of the gymnosperms in particular.

5. In some cases they are natural hybrids which have external characteristics distinct and constant enough to have specific rank from a systematic standpoint.

6. Although these species may be distinct from the systematic standpoint, yet they must be treated in a different manner from the standpoint of the evolutionist and the plant breeder.

7. Finally, the species of the genus *Rubus*, as shown from the pollen condition and also from external characters, clearly hybridize very frequently in nature, giving rise to constant forms often recognized as true species.

In closing I wish to express my sincere thanks to those in charge of the Arnold Arboretum and of the Harvard Botanical Garden for the privilege of collecting. I desire, also, to express my thanks to Miss DAY, librarian of the Gray Herbarium, for assistance in securing literature, and to others who have kindly aided me in carrying out my purpose. This work has been carried on in the Laboratories of Plant Morphology of Harvard University under the direction of Professor E. C. JEFFREY, and to him I am indebted for advice and assistance.

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EXPLANATION OF PLATES X–XII

FIG. 1.—*Rubus strigosus* Michx.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 2.—*R. neglectus* Peck: transverse section of anther showing pollen to be partially sterile; ×125.

FIG. 3.—*R. neglectus* Peck: more highly magnified view of fig. 2; ×300.

FIG. 4.—*R. odoratus* L.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 5.—*R. alleghaniensis* Porter: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 6.—*R. setosus* Bigel.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 7.—*R. fruticosus* L.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 8.—*R. amnicolus* Blanch.: transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 9.—*R. frondosus* Bigel.: transverse section of anther showing pollen to be partially sterile; ×125.

FIG. 10.—*R. frondosus* Bigel.: more highly magnified view of fig. 9; ×300.

FIG. 11.—*R. biformispinus* Blanch.: transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 12.—*R. Andrewsianus* Blanch.: transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 13.—*R. tardatus* Blanch.: transverse section of anther showing large percentage of sterile pollen; ×600.

FIG. 14.—*R. deliciosus:* transverse section of anther showing morphologically perfect pollen; ×125.

FIG. 15.—*R. multiformis:* transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 16.—*R. spectabilis plena:* transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 17.—*R. nobilis:* transverse section of anther showing large percentage of sterile pollen; ×125.

FIG. 18.—*R. nobilis:* more highly magnified view of fig. 17; ×300.

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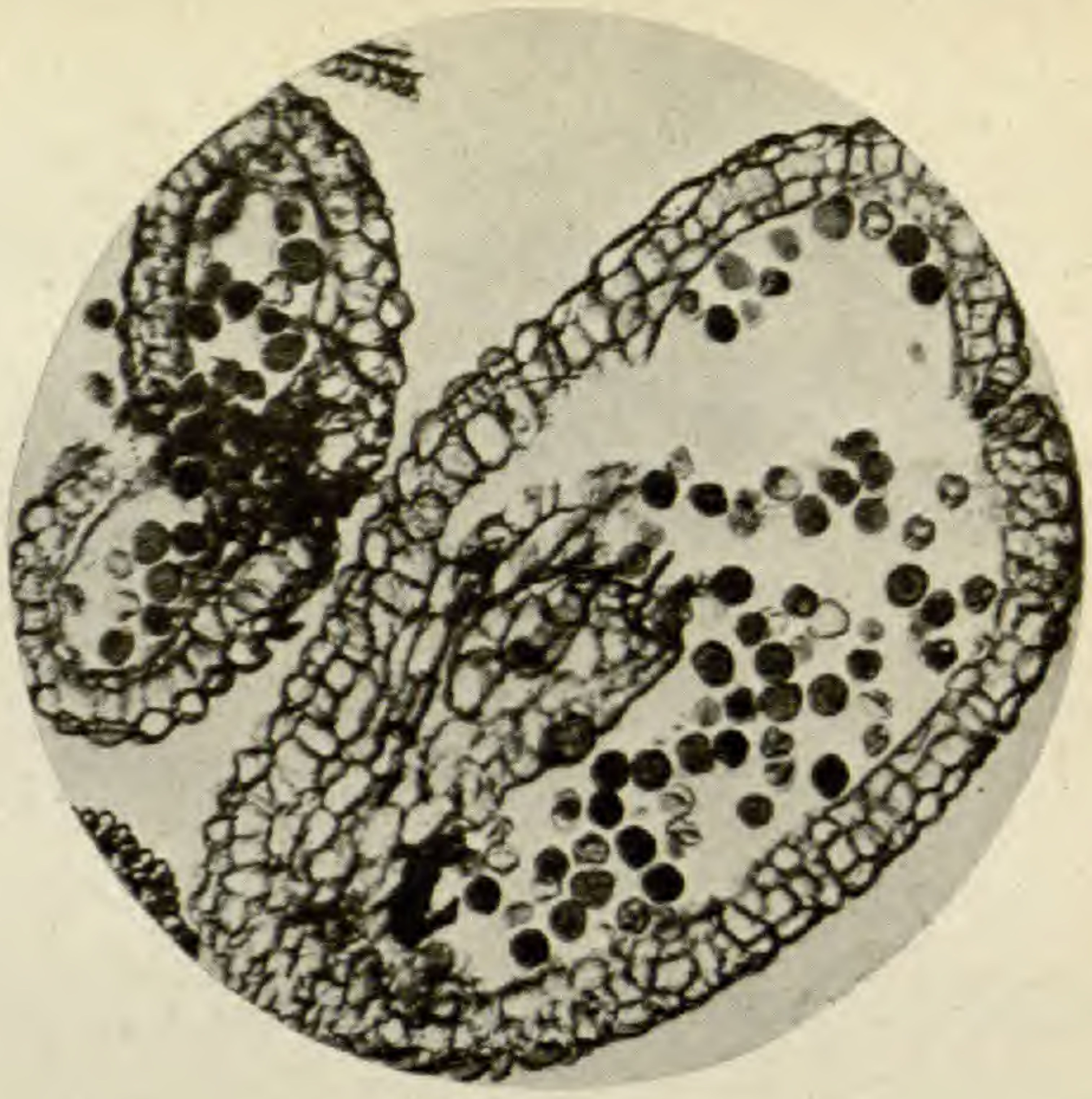

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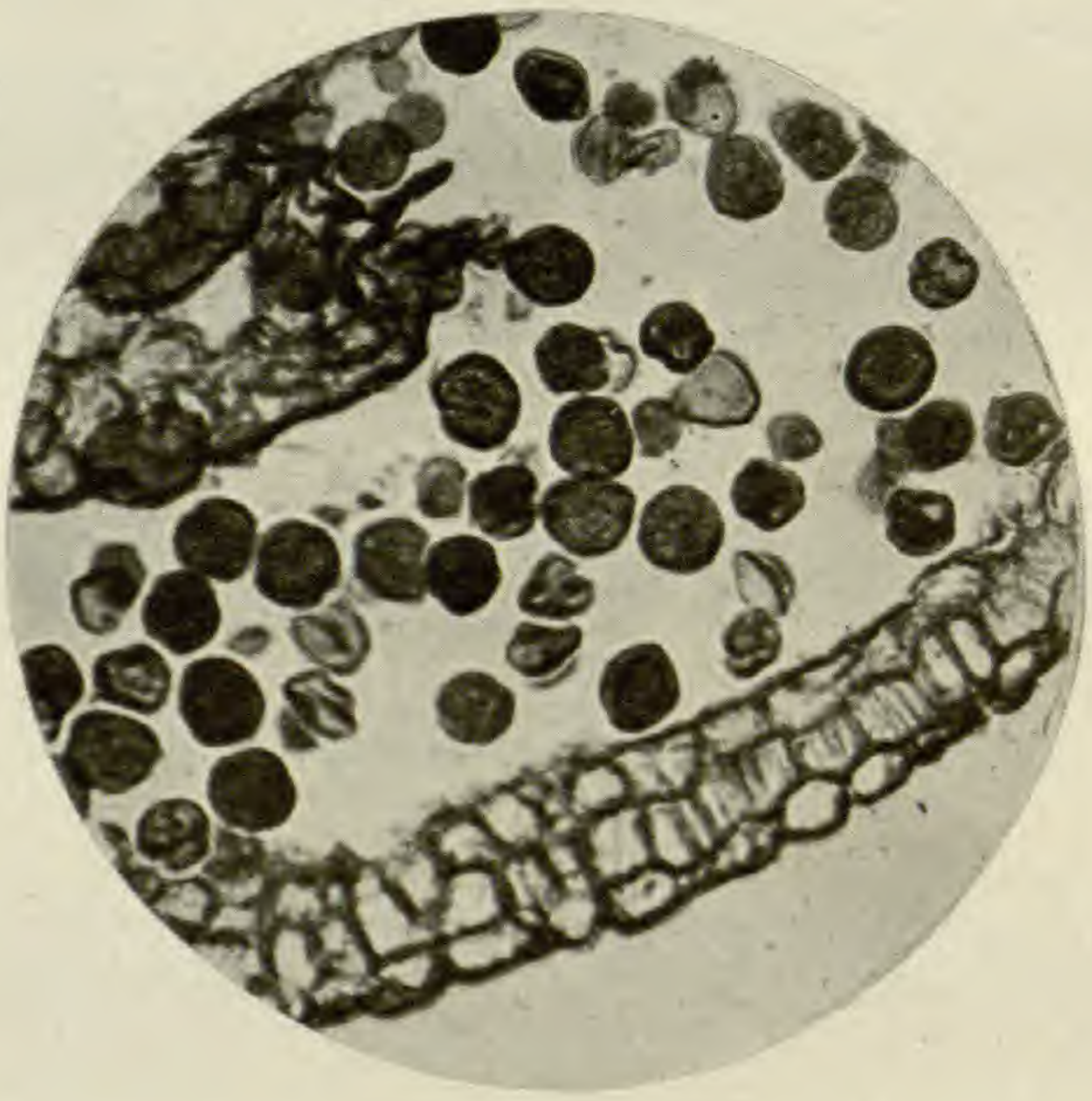

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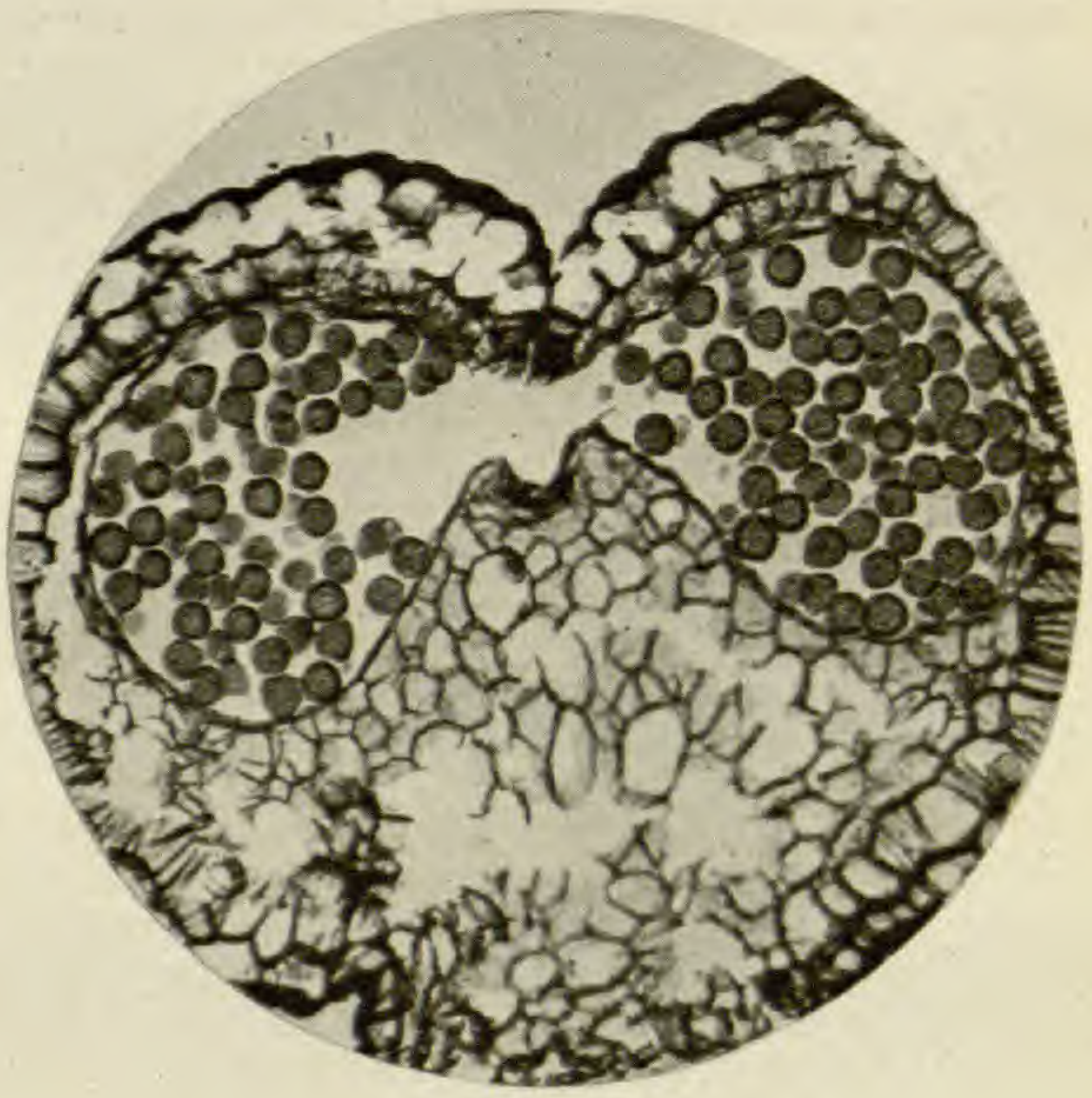

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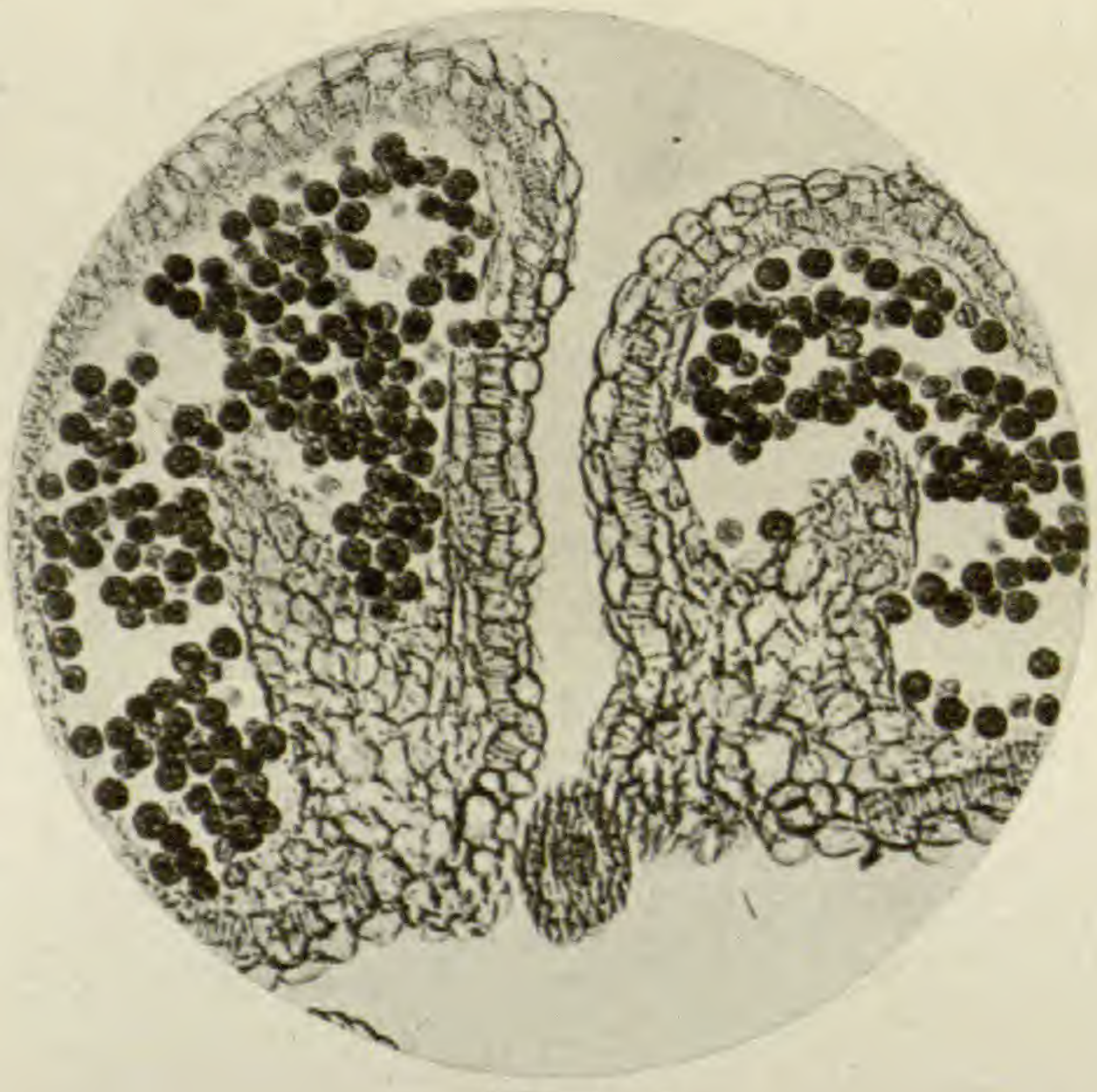

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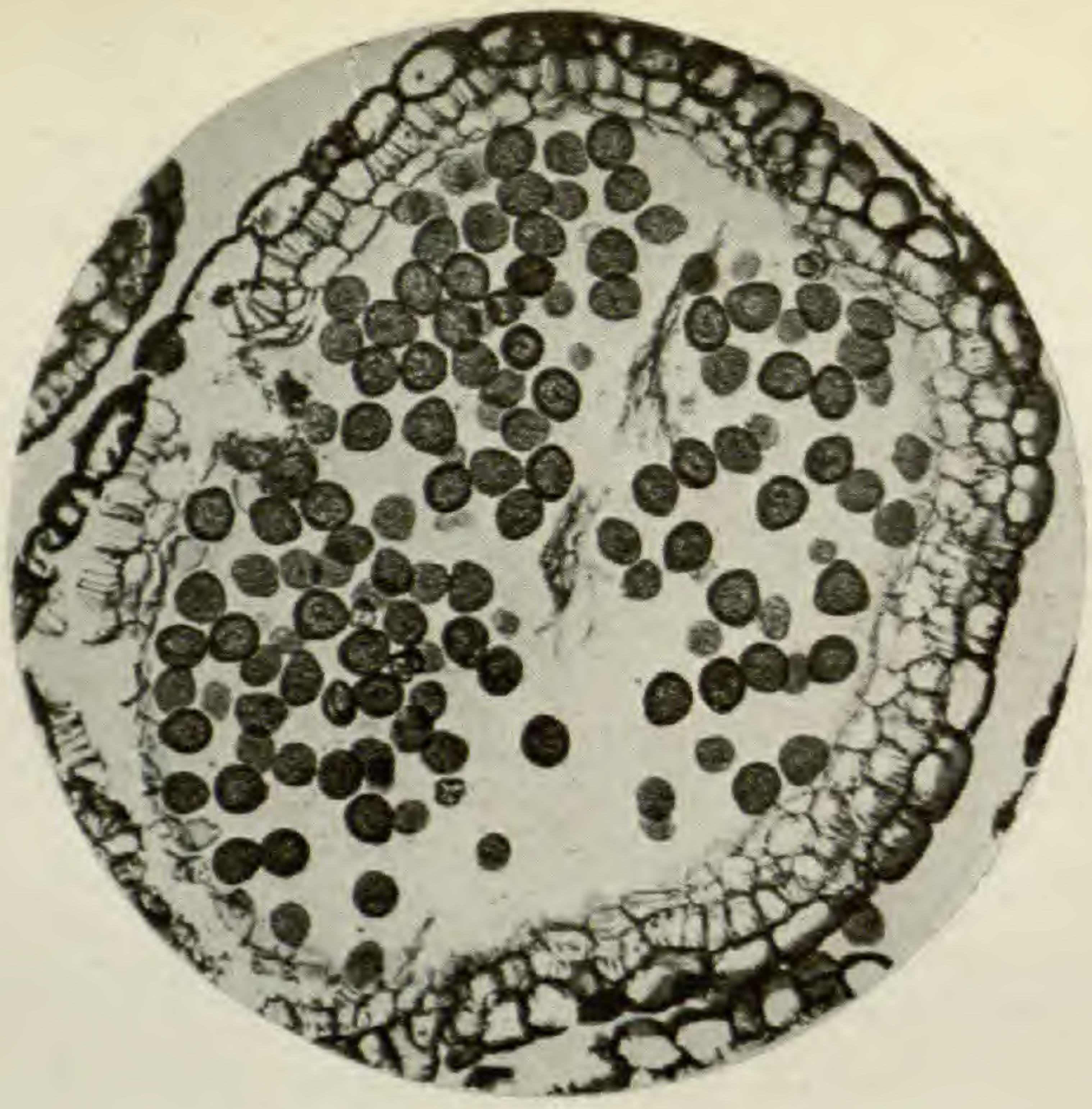
HOAR on RUBUS



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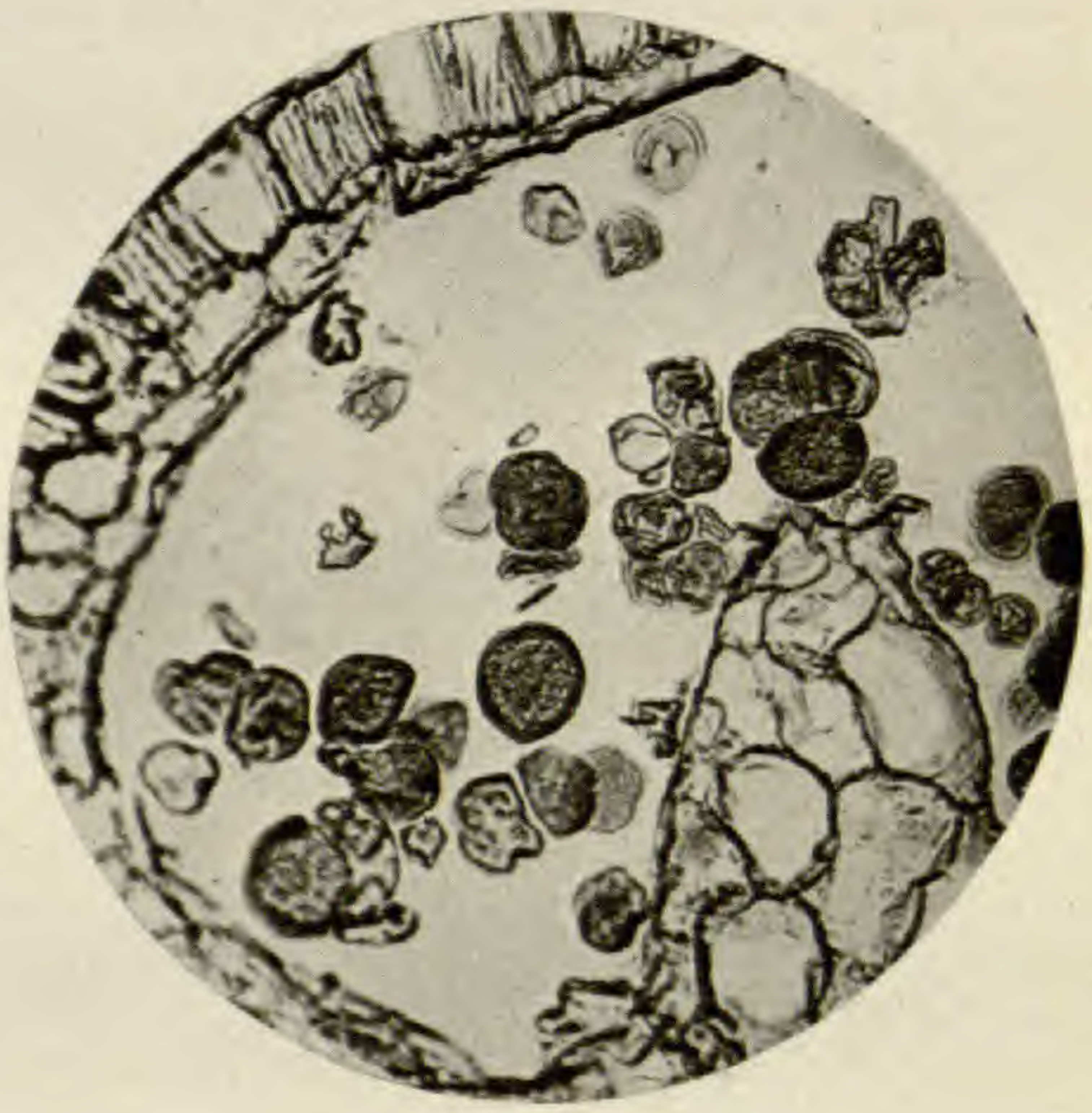

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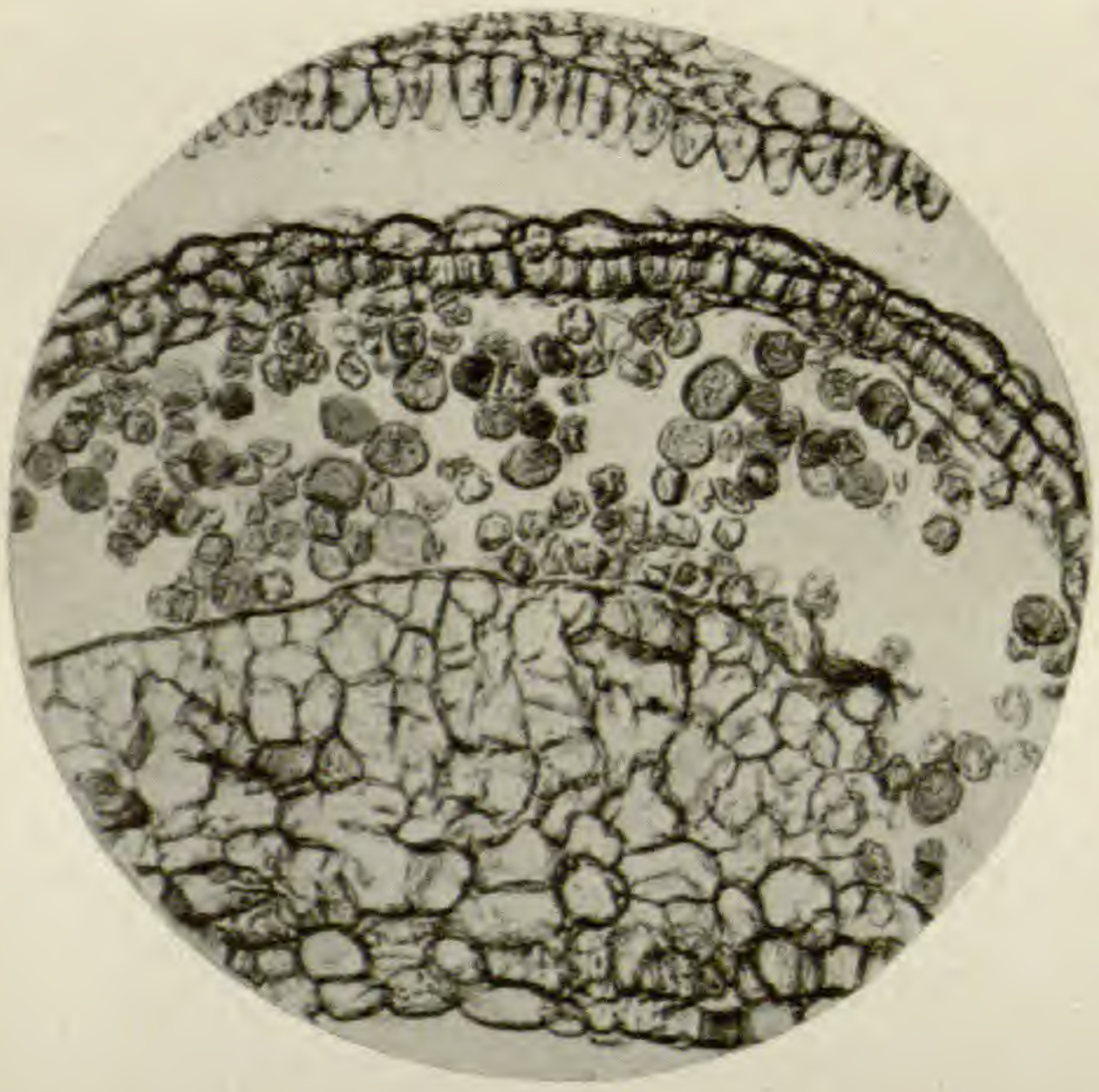

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HOAR on RUBUS

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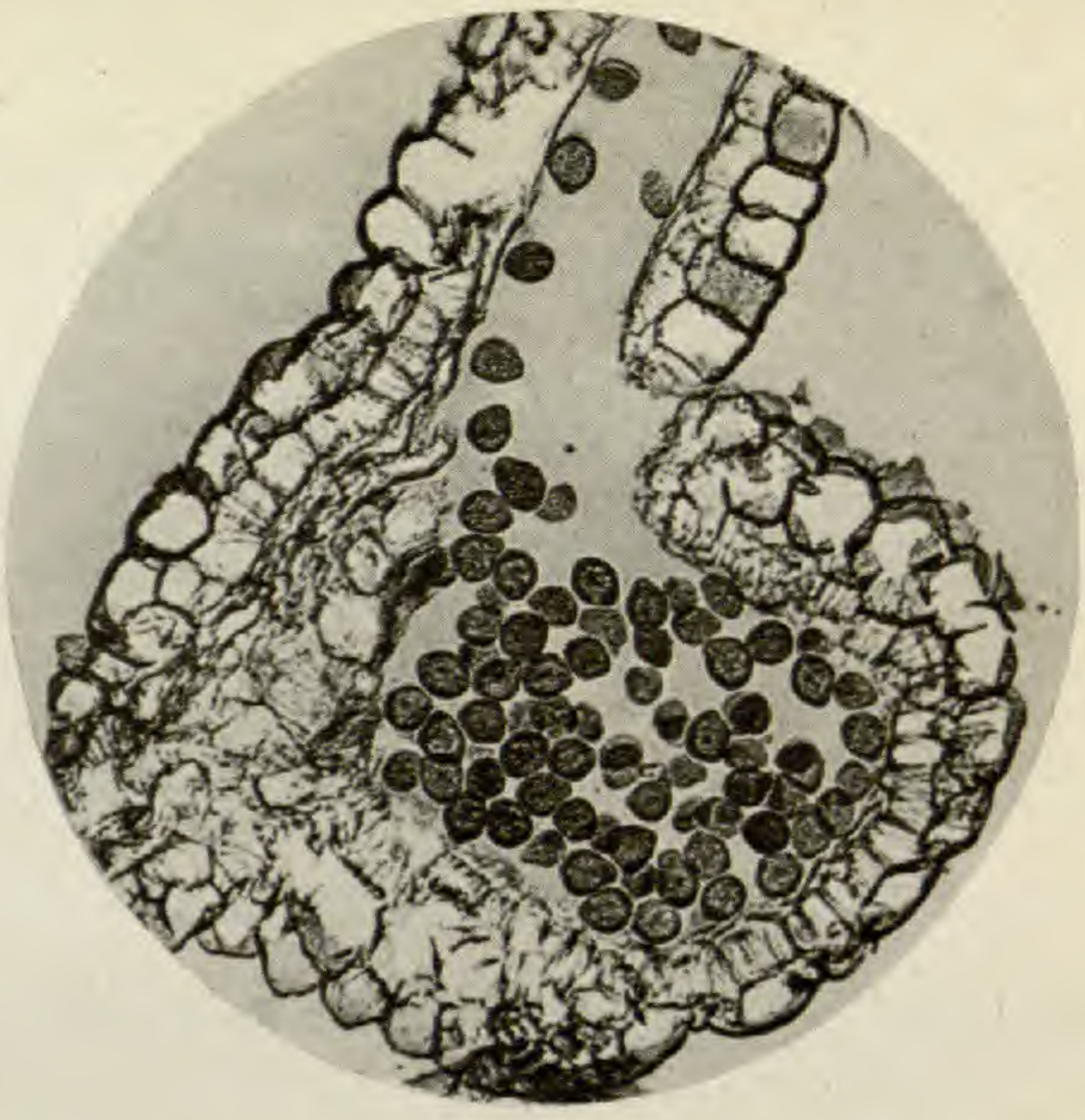

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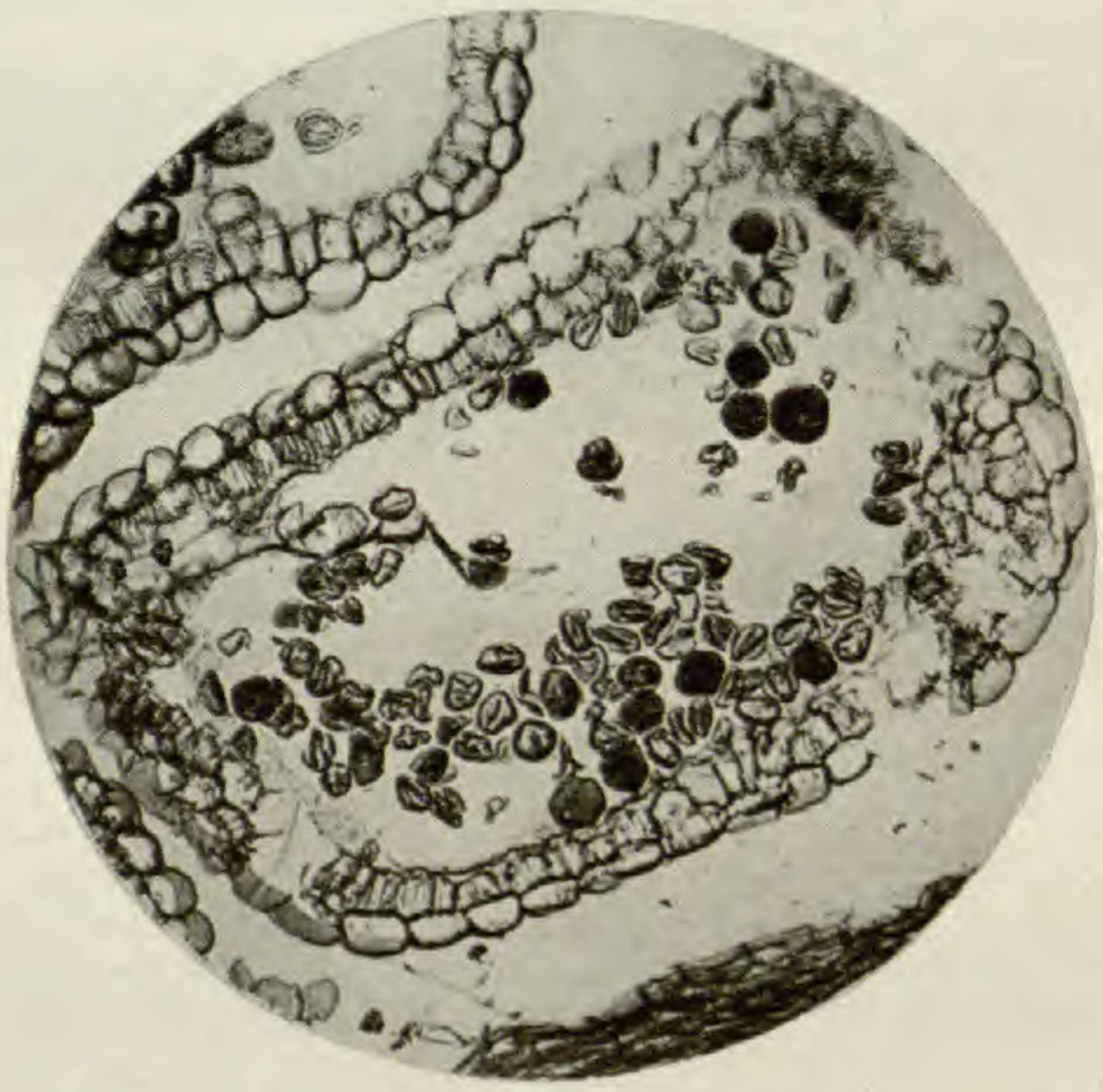

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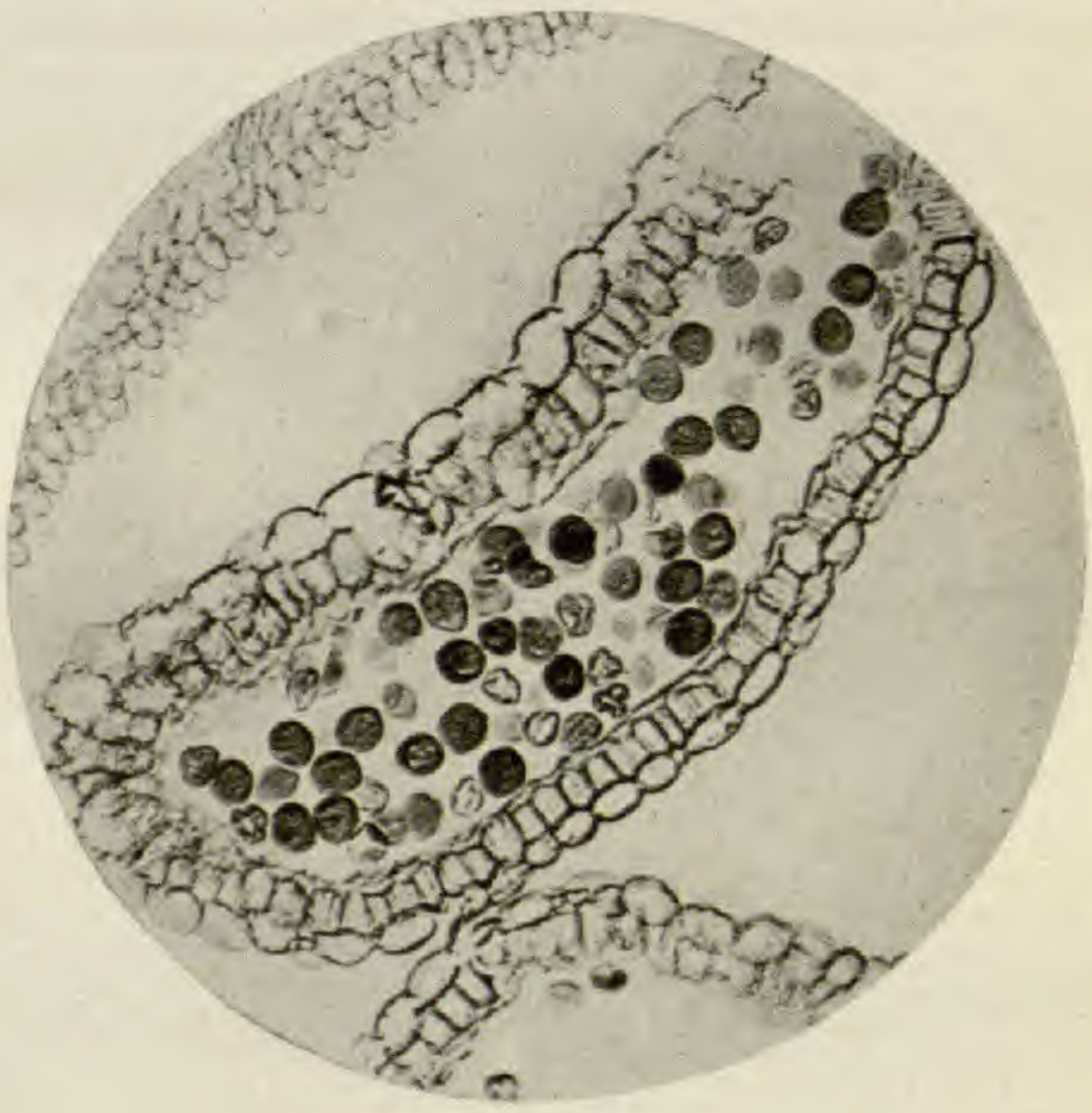

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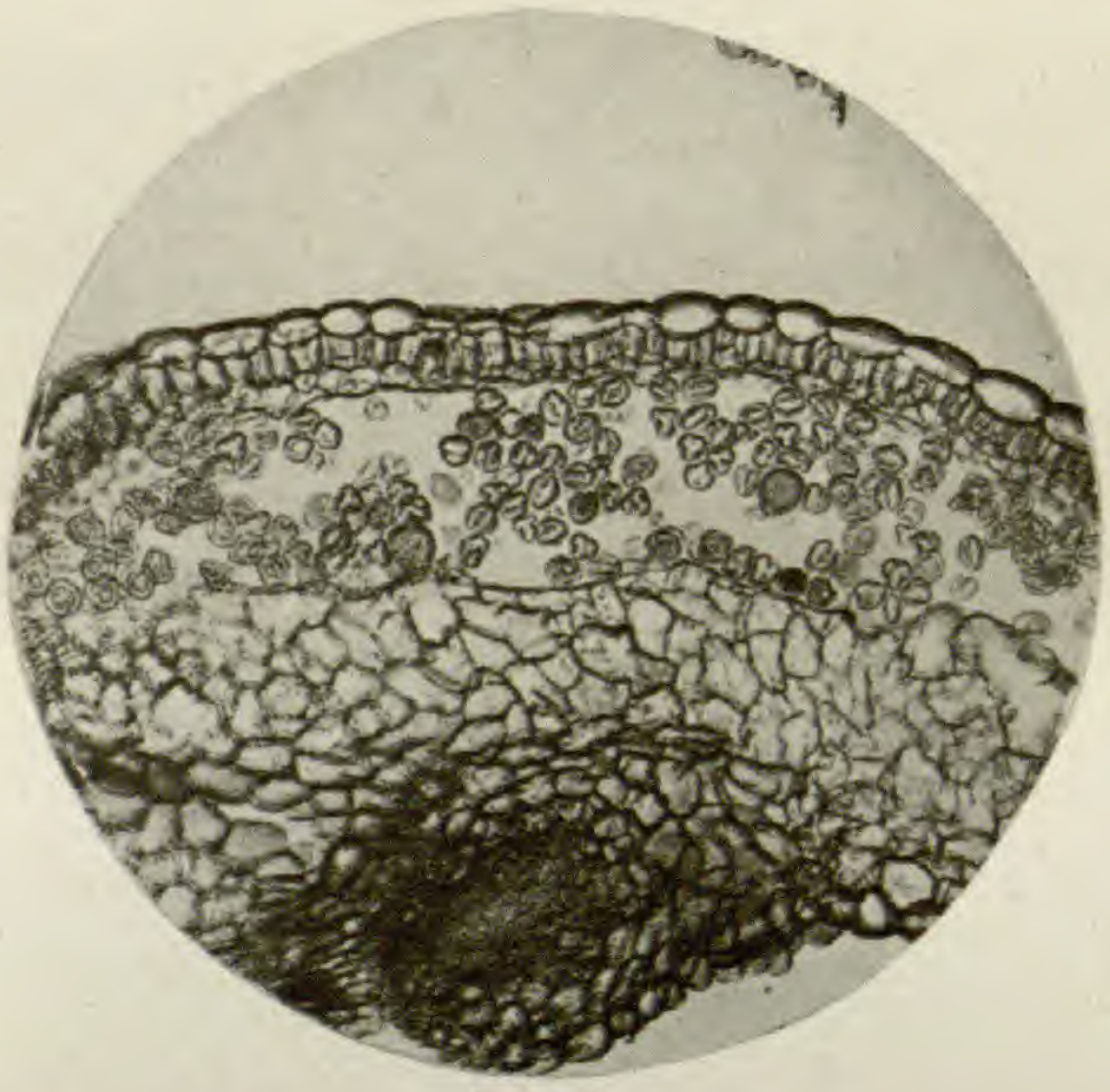

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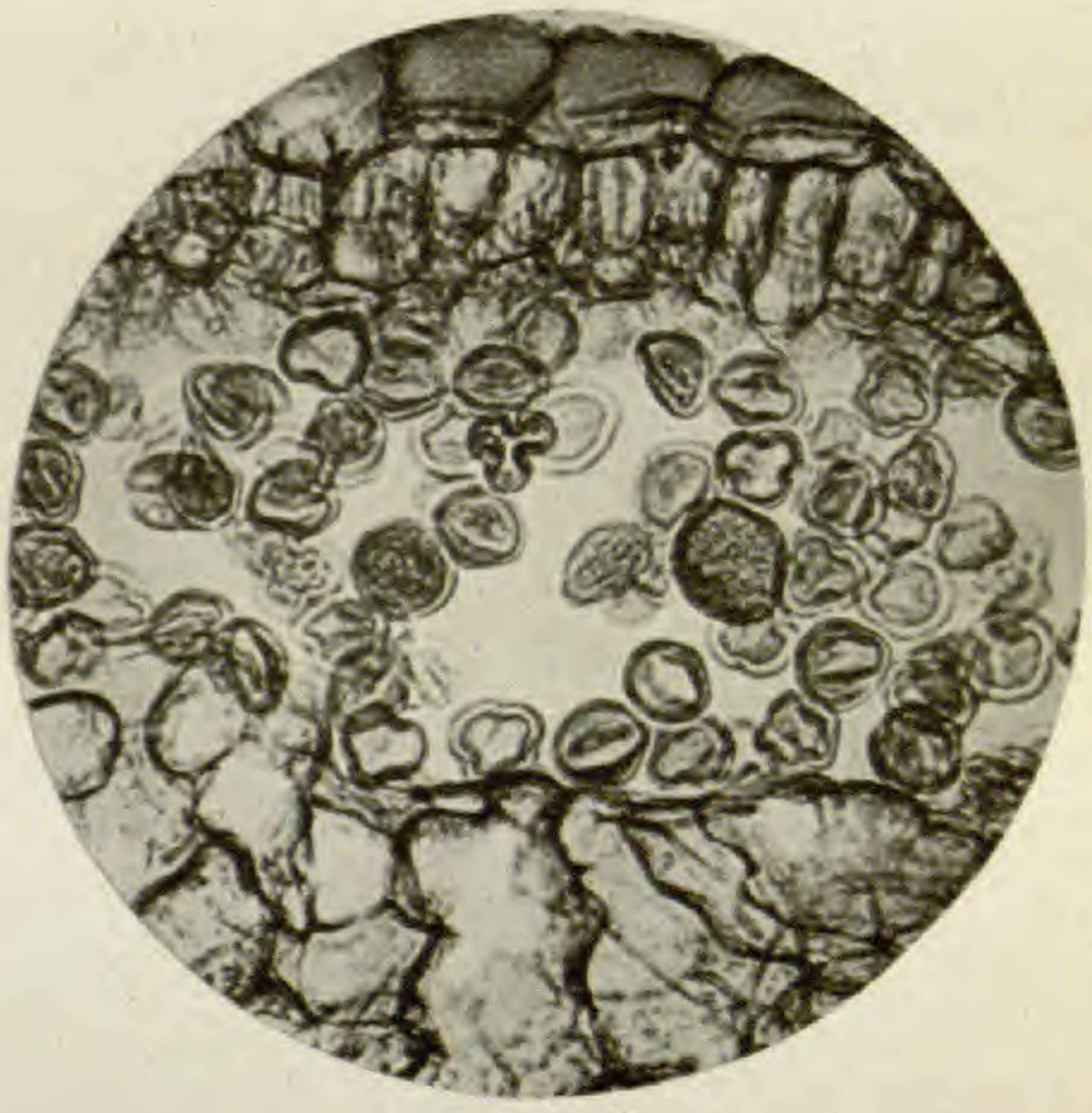

18

HOAR on RUBUS

FURTHER OBSERVATIONS ON CHLORANTHY IN DROSERA INTERMEDIA

MICHAEL LEVINE

(WITH PLATE XIII AND THREE FIGURES)

NAUDIN (15) was among the first to record a new method of propagation other than by seed for the genus *Drosera*. He described the appearance of buds of two young plants on the dorsal surface of a mature leaf of *Drosera intermedia*. These buds appeared between the midvein and the margins of the leaf. KIRSCHLEGER (8) made similar observations in the case of *D. capensis*. He noted buds arising from the epidermal cells at the apex or near the base of the leaf, or on the petiole itself. This species was later studied by WINKLER (19), who confirmed the observations of KIRSCHLEGER. NITSCHKE (14) described vegetative budding for *D. rotundifolia* from material collected in the field. The buds are described as appearing on the dorsal surface of the leaf, and were most commonly found in the early fall. Others have confirmed and extended the observations of NITSCHKE and the earlier data to which I have referred. GROUT (7) ascribes the appearance of these buds to excessive moisture conditions. GRAVES (6) in 1896 collected and placed plants of *D. rotundifolia* in an artificial bog. He found that a number of small plants appeared on the upper surface of old leaves, and in some cases these plants appeared from the ventral surface and edges of the leaf as in *Bryophyllum*. LEAVITT (9, 12) was able to propagate *D. filiformis*, *D. binata*, and *D. dichotoma* from cut leaves. He noticed that after placing leaves of the species named in sphagnum for a period of about 3 weeks new plants appeared from adventitious buds, which finally grew to maturity. He likewise observed that leaves, first formed from such buds in plants of *D. binata*, were orbicular like those of *D. rotundifolia;* while leaves coming from buds on *D. filiformis* were like those of *D. intermedia*. These observations, together with other studies on reversion (LEAVITT 10), led him to the conclusion that the leaf of *D. rotundifolia* is the original type of leaf from which those of other species have arisen. AMES (1) showed the possibility of propagating *Drosera* species from old leaves cut from mature plants. He worked with *D. filiformis*, *D. intermedia americana*, *D. rotundifolia*, and *D. binata*. He noticed that when mature leaves fell on the sand in which these plants were growing, adventitious buds appeared. The condition most favorable to the development of these buds in his opinion is a low temperature.

DIXON (2), like AMES and LEAVITT, was able to obtain adventitious buds on leaves of *D. rotundifolia*. He differs with GROUT in that he asserts that adventitious buds appear only when the parent plant is allowed to dry out. He found that under these conditions the leaves of *D. rotundifolia* produce adventitious buds on the dorsal surfaces after 2 months. He also found that new plants may arise in the axils of leaves and between the petiole and main axis of the inflorescence as axillary buds. GOEBEL (4) showed further that a portion of a leaf of *D. binata* could produce adventitious buds. He cut an arm of a leaf of *D. binata* and placed it on a moist substratum. After a time he observed the appearance of adventitious buds whose leaves were rounded and similar to those of *D. rotundifolia*, and he states that these leaves agree in shape with those of the young plants of *D. binata*.

Miss ROBINSON (17) repeated the work of LEAVITT, AMES, and GOEBEL, with similar results. She describes the appearance of roots on the young adventitious buds which appeared on leaves of *D. rotundifolia* placed in sphagnum. She also found that leaves placed with the dorsal surface down developed buds on the upper or ventral surface. Miss ROBINSON holds that these adventitious buds are in no way connected with the vascular system of the parent plant.

SALISBURY (18) found the appearance of adventitious buds in *D. rotundifolia* and *D. intermedia* Hayne. Signs of these buds appear on the leaves of these 2 plants in the late spring of the year following their removal to the greenhouse. The age of the leaf which bears these buds may vary. The first leaf rudiment appears as a small protuberance on the dorsal surface of the leaf. This is followed by another leaf rudiment opposite the first, and the next leaves follow in succession, forming a rosette-like structure in the axils of the leaves first formed. As against Miss ROBINSON, KIRSCHLEGER and WINKLER, SALISBURY maintains that sections through these buds show that they are connected with the fibrovascular bundle of the leaf on which they appear. Salisbury believes that these buds form a definite reproductive mechanism in these species of plants.

GOEBEL (5) reports on *D. pygmaea* the appearance of a highly specialized type of brood body resembling in shape the gemmae of *Marchantia*. These bodies appear in rosettes and are dorsiventral. The under surface of each body is smooth, while the upper surface is horseshoe-shaped. Stomata and vascular bundles are present. GOEBEL believes that each brood body arises from a leaf *anlage*.

PLANCHON (16) described an anomaly in the flowers of *D. intermedia*. He asserts that the ovary becomes elongated and the carpels bear both tentacles and ovules. All gradations between an ovule and a tentacle were found. The petals of these flowers were likewise modified, being leaflike in nature and bearing tentacles. PLANCHON asserts that all organs become chloranthic except the calyx. FERNALD (3), while collecting bog plants, found a form of *D. rotundifolia* in which the inflorescence was subcapitate and consisted of a few flowers. The petals and sepals were greenish to crimson in color, and the ovary instead of producing a normal capsule formed a rosette of glandular foliage leaves. This tendency was noted in other floral organs. LEAVITT (11) studied these flowers and states that they are quite sterile. FERNALD makes no mention of stamens. It seems that they are generally absent, and this constitutes the main difference between this form and the one described later. The constancy of this abnormality in *D. rotundifolia* led FERNALD to make a new variety which he called *D. rotundifolia comosa*. Neither FERNALD nor LEAVITT gives figures of these modified flowers. FERNALD regards them as a means of vegetative propagation.

In studying the development of pollen my attention was attracted by the appearance of a number of plants of *D. intermedia*.

These were growing in the water, and at the apex of the flower stalk a head of tentaculiferous flowers was borne. Again, in August 1915 I found in the same locality a number of these plants. They were growing in the water of a shallow arm of Horicon Lake, Lakehurst, New Jersey. The plants were of the long-stemmed type such as has been figured by NITSCHKE (14) for *D. rotundifolia*, and extended about 4–5 in. above the surface of the water. Associated with them was a *Castalia* species, and the banks of the bog were covered with sphagnum in which *D. rotundifolia* and *D. filiformis* grew in great abundance. Normal plants of *D. intermedia* were present also. These abnormal inflorescences resemble in all respects those described by FERNALD (3) for *D. rotundifolia*. The plants were not so numerous; in fact, the monstrosity appeared in comparatively few plants. As the case is quite a striking one, I have thought it worth while to figure and describe the abnormal flowers in some detail. Herbarium specimens have been deposited in the New York Botanical Garden.

The plants bearing the abnormal flowers were in all other respects like the normal *D. intermedia*. Under the conditions in which they were growing the exposed leaves and their tentacles were reddish. The long stem and delicate roots were submerged and buried in the mud. The apical portion of the stem bears an imperfect rosette of leaves. Here also appears the short, thick flower stalk on the summit of which the abnormal heads of greenish flowers are found. On superficial examination (text figs. 1–3), the small headlike inflorescences seem to be mere clusters of diminutive leaves. More careful study shows that the cluster is really a group of chloranthic flowers. The term "chloranthy," as used here, indicates that all or nearly all of the organs of the flower assume the appearance and form of leaves (13). Each flower is commonly subtended by 3 bracts. These bracts are narrow, lanceolate, leaflike structures which may have small sessile glands. All the parts of the flower except the stamens have become leaflike. This is a more extreme case of chloranthy than that described by PLANCHON (16). The chloranthic flowers of an inflorescence are in different stages of development, the size of the flower generally indicating its relative age. The largest I found were 6–8 mm. in diameter, and the smallest were 1–2 mm. in diameter. There is no correspondence between the degree of development of the flower and its position in the inflorescence. They seem to be promiscuously scattered over the slightly thickened end of the flower stalk.

There are 5 sepals as in the normal flowers. They are slightly green and have a number of tentacles as shown in fig. 2. The marginal tentacles are generally larger and as a rule vary in number from 2 to 4. The surface tentacles have smaller stalks and are very often lacking. In one case I found 2 adjacent fused sepals as shown in fig. 1, which represents the ventral surface of the calyx showing the attachment of the sepals. The tentaculiferous petals alternate with the sepals. While in the normal flower they are usually white or pinkish in color, in the chloranthic flowers they are pale green, and, as noted, covered with a varying number of normal tentacles (fig. 3). The shape of these petals is like that of the foliage leaf with the characteristic spatulate blade and a rather long, narrow, and flattened petiole. The tentacles are scattered quite regularly along the margin and over the surface

FIG. 1

of the blade. The marginal ones are larger. Inside this whorl of petals there are 5 stamens. These appear to be in every respect normal and compare favorably in size and shape with the stamens in normal flowers. Normal tetrad pollen grains have been found in their anthers (fig. 4). In the case (fig. 5) of one flower which is mentioned later, the filaments were broader and the anthers in some cases consisted of a single locule each, yet all intermediate stages between unilocular and bilocular anthers were present. In these anthers no pollen was found. Unlike PLANCHON'S (16) chloranthic flowers of *D. intermedia*, these had no carpels or any indication of carpels (figs. 6, 13). The center of the flower was occupied by a cluster of leaf rudiments in various stages of development, as described by FERNALD (3) and LEAVITT (11) for *D. rotundifolia* leaf. The outer leaf rudiments are best developed, while the ones toward the center are progressively smaller (fig. 6). In older flowers these rudiments have become fully developed spatulate blades resembling those of the normal vegetative leaf. In a single capitate inflorescence all stages in the development of the central leaf rudiments can be found. Fig. 7 represents a small chloranthic flower in such a head. The sepals are fully developed, while the petals, although they are fully expanded, have not all extended their tentacles. The unfolding leaflike petals show the characteristic incurved form common to the vegetative leaves. The stamens in this flower (fig. 7) are normal and are few in number. The carpels as such are entirely lacking, but are replaced by a small number of very minute leaf rudiments, of which one is considerably larger than the others. Figs. 8 and 9 represent larger chloranthic flowers. The sepals shown in fig. 8 are larger and much broader than the average I observed. In the center of the flower there is a large rosette of young leaves entirely replacing the carpels (fig. 8). The outer leaf rudiments are large and almost ready to unfold. Fig. 9 represents a chloranthic flower in which the leaves are slightly farther advanced. Fig. 10 represents a chloranthic flower in which the central leaves have attained the greatest development I found. The flower measured approximately 6 mm. in diameter. Only one petal and sepal are figured, but the usual number 5 was present here also. The outer whorl of the apical leaf group has fully developed into 4 comparatively large spatulate leaves covered with numerous tentacles and having a well developed vascular system (compare modified sepals, petals, and leaves in fig. 10). The central rosette of young leaves is large and in all appearances resembles the normal winter buds common to *D. intermedia.*

The cases figured represent what appears to be the ordinary structure of the chloranthic flowers in this species of *Drosera*, but variations from this type can be found. Fig. 11 represents a flower in which some of the leaves in the petal series have few tentacles. Fig. 12 represents one of a cluster of 6 flowers in a capitate inflorescence in which an abnormality appeared. In this flower the number of sepals is reduced to 2; they have tentacles and are like those previously described. The number of petals is likewise reduced. The stamens are 5 in number and are characterized by having broad filaments (fig. 5) with anthers, as just noted, consisting of 1 or 2 locules in which no pollen grains were found. The leaf rudiments resemble those in the ordinary chloranthic flowers. Although I have not observed their further development, there can be no doubt that these chloranthic flowers serve as a

FIG. 2

means of vegetative propagation. The central rosette of leaf rudiments is comparable to a diminutive form of winter bud, such as is common to this and other species of the *Drosera*. The appearance of tentacles and especially chlorophyll on the petals of the chloranthic flowers may be an adaptive feature to enable the flower to maintain itself until rooted in the substratum.

FERNALD'S (3) monstrous flowers in *D. rotundifolia comosa* appear to be the most marked case of chloranthy described for the genus. The organs of the flowers of this species may all become modified and assume the appearance of leaflike structures. It seems that vegetative propagation is absolutely essential to the perpetuation of this form. PLANCHON'S case of chloranthy in *D. intermedia* shows flowers with organs least modified. The calyx in these flowers never becomes modified and the normal carpels may also be present. The stamens and the petals are invariably modified. In the case observed all the organs except the stamens are modified to assume the form of tentaculiferous leaves. The carpels are replaced by a rosette of leaves resembling

FIG. 3

a winter bud. The degree of chloranthy is more developed than in the case described by PLANCHON, and appears also to be more constant, although abnormalities among the monstrosities occur. It appears from these cases that the degree of chloranthy may vary. It may be argued that a case like the one described in the present paper is less abnormal than the other cases mentioned. It appears that normal pollen may be formed, and under favorable conditions cross-pollination may take place between these forms and the normal species of *Drosera;* that is, sexual reproduction has not entirely disappeared. At the same time, these plants appear to be capable of propagating themselves vegetatively by the chloranthic flowers which may function as buds. The case is perhaps parallel to the adventitious buds on the leaves.

The conditions which favor the development of these flowers are not evident. It may be, as suggested by GROUT for adventitious buds, that an abundance of moisture is very essential. It seems from FERNALD'S observations and my own that chloranthic flowers in *Drosera* species are formed only in plants which grow in water. The bearing of such cases of reversion of flowers to vegetative buds on our general conception as to the relation of sexual and asexual reproduction is not very clear. It appears, however, that under unfavorable conditions vegetative reproduction replaces in part, if not altogether, the sexual method. This is contrary to our general conception of reproduction, since it is commonly conceded that under favorable conditions vegetative reproduction prevails almost entirely to the exclusion of sexual reproduction.

COLUMBIA UNIVERSITY

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EXPLANATION OF PLATE XIII

All figures were drawn with the aid of a Leitz hand lens ocular 10.

CHLORANTHIC FLOWERS OF DROSERA INTERMEDIA HAYNE

FIG. 1.—Ventral surface of a calyx of a chloranthic flower in which 2 sepals have fused.

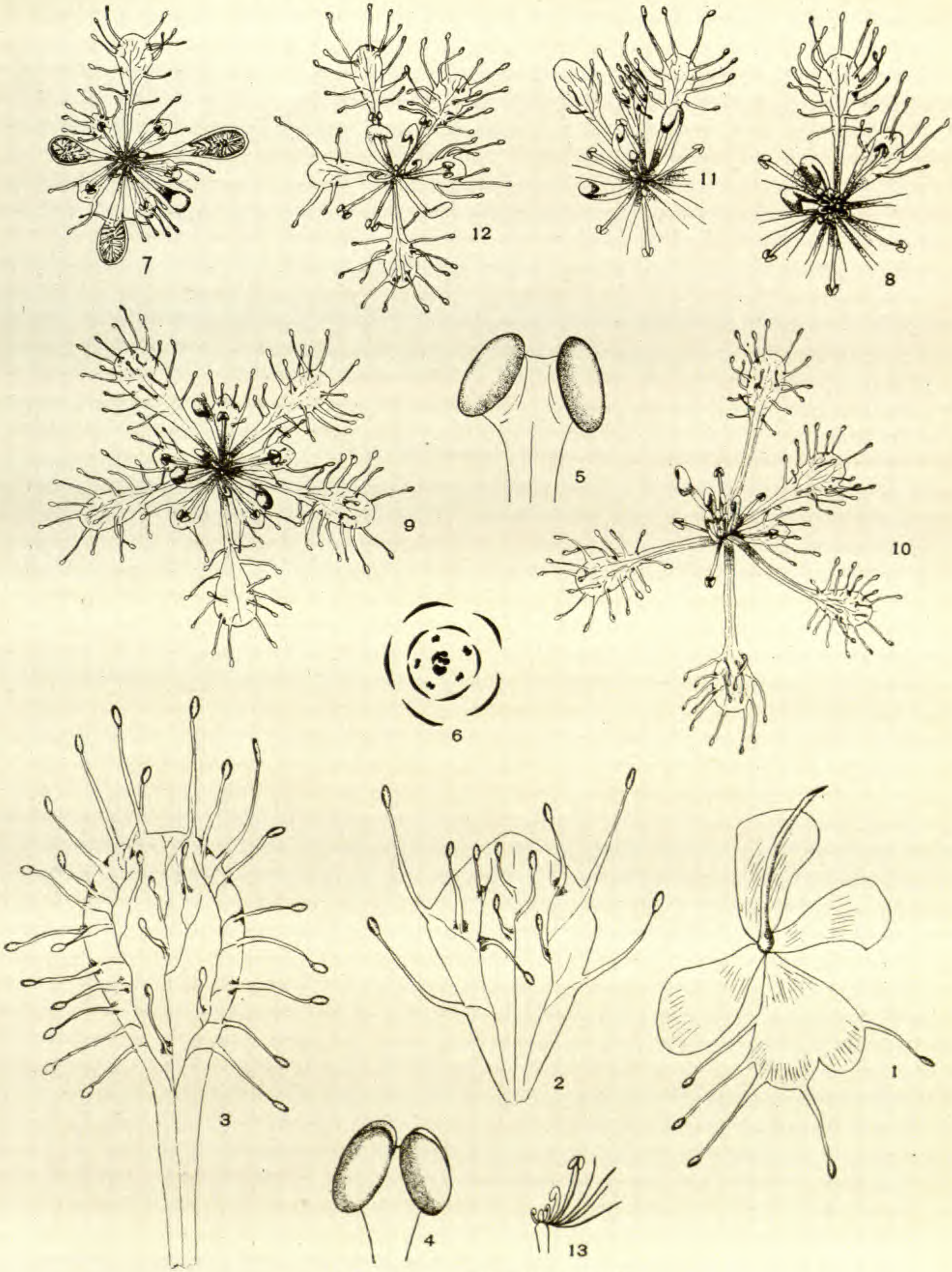
FIG. 2.—Dorsal surface of a sepal of a chloranthic flower covered with tentacles.

FIG. 3.—Dorsal surface of a petal of a chloranthic flower showing numerous tentacles.

FIG. 4.—Normal stamen of a chloranthic flower in which normal pollen is borne.

FIG. 5.—Abnormal stamen in which no pollen is found.

FIG. 6.—Schematized cross-section of chloranthic flower.



LEVINE on CHLORANTHY

FIG. 7.—Young chloranthic flower in which the leaflike petals are not yet fully developed; the carpel is replaced by a number of leaf rudiments.

FIG. 8.—A slightly older stage; sepals very large.

FIG. 9.—Another slightly older stage.

FIG. 10.—An older stage in which outer whorl of leaf rudiments are developed into leaflike organs resembling normal spatulate leaf of *D. intermedia*.

FIG. 11.—Variation of a chloranthic flower in which the number of tentacles on the petals is relatively small.

FIG. 12.—Another abnormal chloranthic flower in which the number of sepals and petals is reduced to 2 and 3 respectively and the stamens are abnormal, as in fig. 5.

FIG. 13.—Schematized longitudinal section of a chloranthic flower.

A CONTRIBUTION TO OUR KNOWLEDGE OF SPLACHNIDIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 220

MABEL LEWIS ROE

(WITH PLATES XIV–XVIII)

Introduction

Splachnidium rugosum Grev. is a monotypic genus of more than ordinary interest because investigators have placed it in one group or another according as some particular feature seemed more worthy of emphasis. The earliest mention of this plant was by LINNAEUS (9) as *Ulva rugosa;* a few years later SUHR (18) described it as *Dumontia rugosa;* and in 1830 GREVILLE (3) founded the genus *Splachnidium*, retaining the specific name already given.

Most authors have placed it with the Fucaceae, but in 1892 MITCHELL and WHITTING (10) published an account which was incorporated in 1895 by MURRAY (11) in his book. This account includes the morphology of both vegetative and reproductive tissues. As a result of their investigations, these authors felt justified in establishing a new family, making the following statement (p. 9): "The sum of the characters of *Splachnidium* so expressly excludes it from any existing natural order that there is no other course open to us than to establish one for its reception under the name of Splachnidiaceae." KJELLMAN'S account (6) restored it to the Fucaceae; and in 1904 OLTMANNS (13) placed it with the Ectocarpaceae under the subgroup Encoelieae.

The material for this investigation was secured by Professor CHARLES J. CHAMBERLAIN at Glen Cairn, near Cape Town, South Africa, on February 28, 1912. A second collection was made at the same place on August 30, 1914, by Miss EDITH STEPHENS of South African College, Cape Town, and sent to Professor CHAMBERLAIN. The work was undertaken with the hope of determining (1) the nature of the contents of the reproductive sacs, whether zoospores or gametes; and (2) the origin of the so-called "apical cell," a strikingly characteristic feature of *Splachnidium*. The material proved inadequate for the study of reproduction, but furnished a good series for the development and fate of the "apical cell." No mitosis was observed, and hence no chromosome count was possible. It is hoped that some one in the region where *Splachnidium* grows may be interested enough to investigate material secured at the exact time when cell division occurs, probably at night, or to observe the behavior of the living bodies after discharge from the reproductive sac.

Origin of the initial row

The first feature which tends to set the initial cells apart from adjacent border tissue is the taking on of a heavy coat of mucilage (fig. 1). In this and subsequent early stages the heavy mucilage coat is the only character which distinguishes the initial from young vegetative hairs abundant in the apical region (figs. 2–5). A second feature marking this unique structure is the beginning of resorption of cross-walls and loss of original definite nuclear structure (figs. 4, 6, 13, 15). Gradually a complete linear row of cells, reaching entirely through the thallus, is differentiated from the surrounding tissue as an initial row (figs. 5, 7, 8, 13, 15).

Sometimes there is no evident differentiation of tissue at an early stage, but portions of old apical hairs may assume the heavy mucilage coat (figs. 9–11), and there follows an involving of the entire linear row of which the hair is a terminal portion. Part of the old hair may be cast off, eventually leaving only the lower portion (fig. 11).

By the time an entire linear row is so differentiated, the characteristic filiform appendage, which has been described (**10**) as "growing between the cells of the thallus toward the center of the branch," begins to be evident (figs. 15, 16). Pressure of abutting tissue has narrowed the basal portion into a tail-like process as resorption of old walls and cell contents has gone on, whereas freedom from pressure above has allowed expansion into a pear-shaped oval. At this stage all of the original contents left in the filiform portion consist of granular patches of cytoplasm, and in the oval portion there is a similar granular substance with the addition of darkly staining bodies; there is no trace left of nuclear structure.

As active growth continues on each side of the initial row, rapid division occurs in the external layers of the thallus, whereas the inner cells, lying on the border of the central cavity, are pulled apart, not dividing to keep pace with the active outer tissue. This separation leaves the filiform basal process swinging loose, as it were, in the central cavity (figs. 17–20). The entire structure lies at the base of a slight depression, the result of arrested growth of the initial row followed by activity of abutting tissue.

Such, then, is the history of the initial row. *It is the result of more or less disintegration of an entire linear row of the thallus*, with perhaps the addition of portions of a terminal hair. *The exact amount of tissue originally involved*, whether including a terminal hair in part or in entirety, *determines the length and size of the mature structure*. For a long time the writer was puzzled by the great variation in length displayed by these bodies, and the explanation was apparent only when their origin was completely worked out.

The structure described in the foregoing is found in connection with any activity of the plant, as growth at the main apex, or production of lateral branches, or inception of conceptacles.

The conceptacle

In the formation of the conceptacle, the cavity in which the initial row lies becomes deepened as more and more of the abutting border tissue is involved. *Rapid radial division of these border cells contributes new tissue to the conceptacle at the same rate and in the same way as ordinary thallus tissue is developed.* In the early stages of the conceptacle, except for the initial row, there is no essential difference between adjacent tissue of the thallus and that of the actual cavity of the conceptacle (fig. 19). Presently, however, the lining cells of the conceptacle become elongated, and subsequently, by transverse division at the basal end, develop into septate hairs (figs. 20, 21). This behavior is exactly as noted by the writer in a recent investigation of *Fucus* (14) as the stage in the history of the conceptacle known as the "hair pit."

Some of the hairs, as in *Fucus*, are shed later and the lining cells put forth new papillate growths which elongate into sacs (fig. 22) which are ultimately filled with spherical bodies (fig. 24), variously described as zoospores (**10, 11**) and gametes. AGARDH (**1**), HARVEY (**4**), HOOKER (**5**), and LAING (**8**) all describe *Splachnidium* as dioecious, although no one of these authors reports having actually observed antheridia. LAING speaks of oogonia "obscurely pedicelled," developed from the lining of the conceptacle, each oogonium giving rise "to a large number of oospheres, thus differing from all other Fucaceae that have hitherto been described. Each oosphere is very small, compared with the oospheres of other Fucaceae." In another paragraph he states that "as antheridia have never been observed it is just possible that these hairs [apical hairs] may be antheridial in function." The writer's feeling is that the reproductive sacs contain isogamous gametes. However that may be, the conceptacles, at the time the reproductive bodies are ready for discharge, still contain the "initial" as a conspicuous feature (fig. 23).

Discussion

MURRAY (**11, 12**) and also MITCHELL and WHITTING (**10**) speak of the "modified cell" of *Splachnidium* as homologous with the "initial" of *Fucus;* and yet these same authors make this "unique cell" one of the reasons for separating *Splachnidium* from other families. The similarity holds true only in so far as the conceptacle is concerned, where, as previously shown by the writer (**14**), the conceptacle in each genus is the result of arrested development and partial breaking down of a portion of the external layer of the thallus followed by great activity of abutting tissue. The initial row is in no way comparable to the true apical cell of *Fucus*, segments of which are the basis of all tissue of the thallus.

The fact that the initial row of Splachnidium occurs wherever there is great activity is additional evidence for the simple origin of the conceptacle. Splachnidium is a particularly favorable plant for study along this line, since the same structure accompanies ordinary vegetative growth as well as reproductive activity.

In establishing a new family MITCHELL and WHITTING (10) summarize their reasons in the following statement (p. 8):

> While, then, the characteristics of *Splachnidium rugosum* are such that it can be placed in no existing natural order of the Phaeophyceae, its nearest allies appear to be the Fucaceae on the one hand and the Laminariaceae on the other. In its vegetative structure, in the nature of its thallus, and in the existence of conceptacles, *Splachnidium* bears a resemblance to the Fucaceae; it differs from them, however, in its mode of growth, the former increasing by means of an apical meristem, while the growth of the latter is due to division of an apical cell. In the presence of an apical meristem *Splachnidium* approaches the Laminariaceae, but at the same time there is no plant in this order which has a cell corresponding to the remarkable cell found at the apex of the main axis and lateral branches of *Splachnidium*. In its reproduction it is allied to the Laminariaceae, and the production of sporangia within conceptacles might seem to point to a narrower limitation of the fertile sorus of plants of this order, recalling the difference between perithecium and apothecium in the fungi.

As to vegetative growth, the tissue of the thallus of *Splachnidium* bears an equal resemblance to the Fucaceae and the Laminariaceae, the only essential difference being in origin. *Splachnidium* and the Laminariaceae are products of a meristem, the former apical, the latter intercalary, according to the evidence of most authorities, and not apical as described by MITCHELL and WHITTING in the passage just quoted. The Fucaceae, too, are characterized by apical growth of the thallus, but all tissue is the actual result of segmentation of a definite apical cell. In vegetative growth, then, *Splachnidium* is intermediate between the two groups.

The presence of conceptacles certainly seems a definite connecting link with the Fucaceae; and yet some of the Laminariaceae show a tendency to approach this method of reproduction. SMITH and WHITTING (17) describe deep furrows in *Macrocystis* and *Postelsia*, which closely resemble conceptacles in form and origin. In this feature, too, *Splachnidium* is intermediate between the Fucaceae and the Laminariaceae.

MURRAY (11, 12) and MITCHELL and WHITTING (10) conclude that the reproductive sacs of *Splachnidium* are neither oogonia nor antheridia homologous with those of the Fucaceae, but are sporangia homologous with those of the Laminariaceae, because of the size of the organ, its unilocular nature, its single wall, the number of spores contained, the size of the spore, and the persistent empty case. On the basis of these features there is no reason why *Splachnidium* may not equally well be considered as having unilocular gametangia containing isogamous gametes. This would give to *Splachnidium* a primitive position among the Fucaceae, but would offer for the Phaeophyceae a link in the phylogenetic sequence comparable to the position occupied in the Chlorophyceae by such plants as *Ulothrix*.

Moreover, in the Fucaceae the oogonia and antheridia are unilocular, no permanent walls separating the gametes. Also DREW (**2**) has reported conjugation of isogamous gametes in *Laminaria digitata* and *L. saccharina*. "The resulting zygospore divides and gives rise to a chain of cells which may represent the $2x$ generation, and this in turn gives rise to the *Laminaria* plant, which represents the gametophyte, or x generation." His observations have as yet neither been verified nor disproved.

Recently SAUVAGEAU (**15, 16**) has concluded from his investigations that *Sacchorhiza* (and probably all Laminarias) presents a heterogamous sexuality with alternation of generations. The large plant is the sporophyte bearing uniform sporangia, and each sporangium develops like zoospores "which after transformation into embryospores become male gametophytes or female gametophytes of microscopic size and independent for life. The oosphere expelled from the female gametophyte, then fertilized, germinates at once and develops the plant of *Laminaria*."

In the contents of the reproductive sacs, therefore, as well as in form and origin of the conceptacles and in vegetative growth, *Splachnidium* is intermediate between the Fucaceae and the Laminariaceae. There seems to be no justification for establishing a separate family (**10**) with its main character "reproduction by spores contained in sporangia which are borne within conceptacles."

Further investigation will doubtless show a much more intimate connection between the Laminariaceae and Fucaceae than is at present recognized. *Splachnidium* may be placed with either group, since it has features common to both. Perhaps the presence of definite and conspicuous conceptacles, even though scattered indefinitely over the entire thallus, places it more closely with the Fucaceae. There seems no good reason for placing *Splachnidium* (13) with the Ectocarpaceae.

Summary

1. The initial row of *Splachnidium* is similar in origin and development to the "initial" of the Fucaceae, except that in *Splachnidium* it involves an entire linear row of thallus tissue; it may or may not include a terminal hair; and it accompanies vegetative as well as reproductive activity.

2. It seems unwise to place *Splachnidium* under a separate family; rather is it preferable to retain it under the Fucaceae, regarding it as a primitive member of that group for the following reasons: (*a*) it closely resembles the Fucaceae in the structure of the thallus, but has an apical meristem in place of a segmenting apical cell with consequent dichotomy; (*b*) true conceptacles are present which in origin and development are of the same general type as those of the Fucaceae, but are scattered indefinitely over the entire plant body; (*c*) the reproductive sacs may prove to contain isogamous gametes.

Acknowledgment is due to Professors JOHN M. COULTER and CHARLES J. CHAMBERLAIN for their suggestions and criticisms.

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EXPLANATION OF PLATES XIV–XVIII

All the drawings were made with the aid of the Abbe camera and reduced one-half in reproduction. For figs. 1–21, original magnification was 1580; for fig. 22, 790; and for figs. 23, 24, 1050.

Fig. 1.—Modified superficial cell, showing heavy coat of mucilage which distinguishes initial row from other border cells.

Fig. 2.—Two-celled stage of initial row.

Fig. 3.—Three-celled stage of same.

Fig. 4.—Two stages in development of initial rows; a two-celled stage at right and an older stage at left in which some of the cross-walls have been resorbed.

Fig. 5.—A linear row of the thallus, showing mucilage coat characteristic of an initial row.

Fig. 6.—An initial row in which cross-walls have been almost completely resorbed; traces of old walls still distinguishable.

Fig. 7.—A cut showing how initial row is usually terminated by sister cells, one of which usually fails to mature but sometimes initiates a young hair, as in fig. 8.

Fig. 8.—An initial row together with a young hair.

Fig. 9.—Basal portion of a branch of an apical hair which has become an "initial."

Fig. 10.—Lateral branch and basal portion of apical hairs which have become "initials."

Fig. 11.—Basal portion of an old apical hair in which cross-walls have been resorbed; the upper portion (unshaded) will be shed later.

Fig. 12.—An ordinary vegetative hair characteristic of apical region.

FIG. 13.—A completely involved linear row of the thallus with outer portion showing resorption of cross-walls and loss of nuclear detail.

FIG. 14.—Two initial rows which are modified young hairs.

FIG. 15.—An initial row including a linear row of the thallus together with a terminal hair; entire structure shows more or less disintegration and loss of cross-walls.

FIG. 16.—A stage which begins to show characteristic "filiform appendage" at basal end of initial row; almost all the cross-walls have been resorbed and original cell contents have disintegrated into granular patches.

FIGS. 17, 18.—Showing how interior cells of thallus pull away and release the "filiform appendage" from pressure.

FIG. 19.—A young conceptacle with young septate hairs.

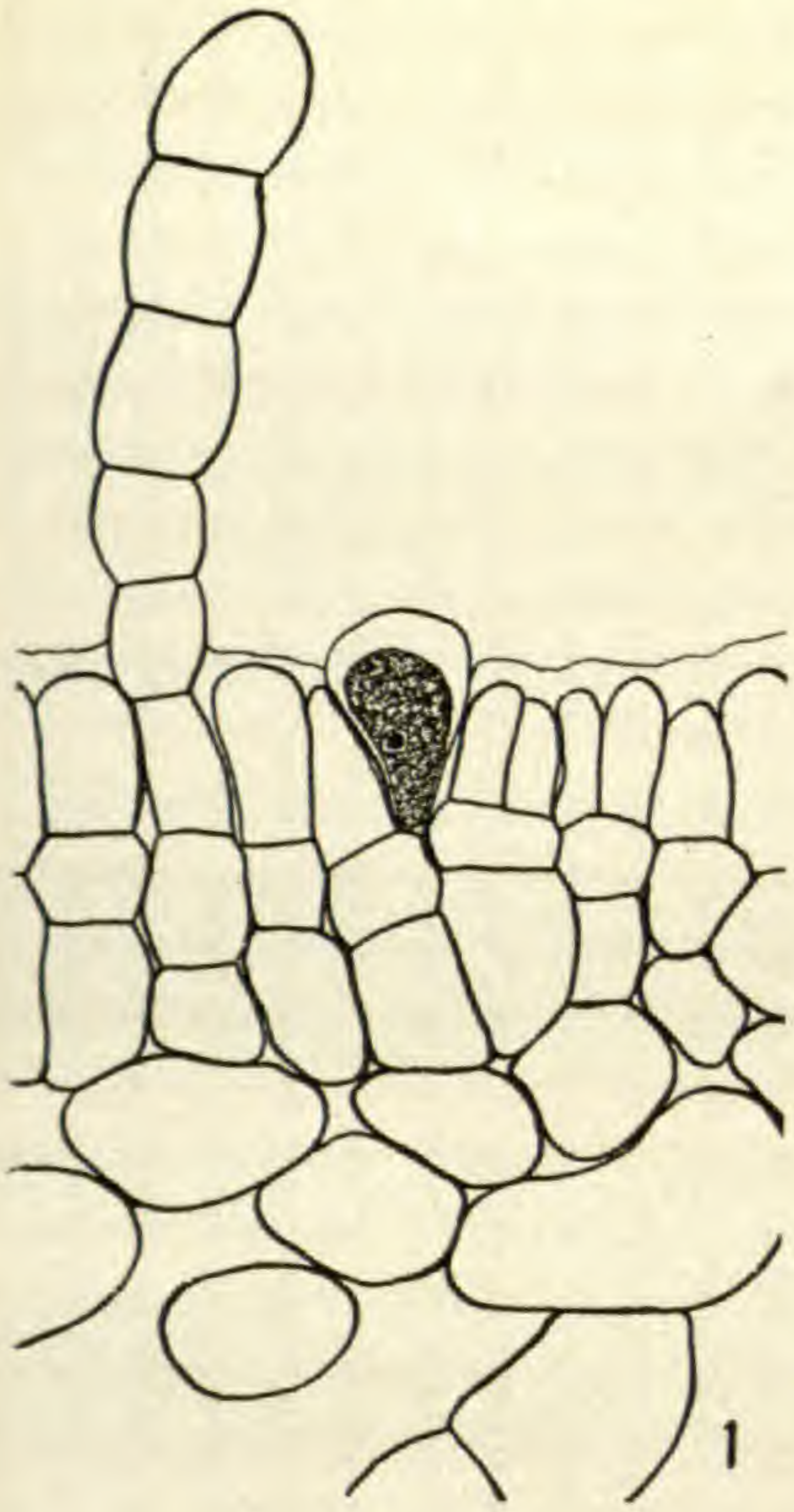
FIG. 20.—A later stage of a conceptacle with development of septate hairs.

FIG. 21.—The hair pit stage of the conceptacle.

FIG. 22.—A mature conceptacle, showing various stages of reproductive sacs (gametangia ?) and a few fragile hairs; old hairs have been shed.

FIG. 23.—The initial row as it appears in a conceptacle at stage shown in fig. 22.

FIG. 24.—Gametangium (?) just before discharge of contents.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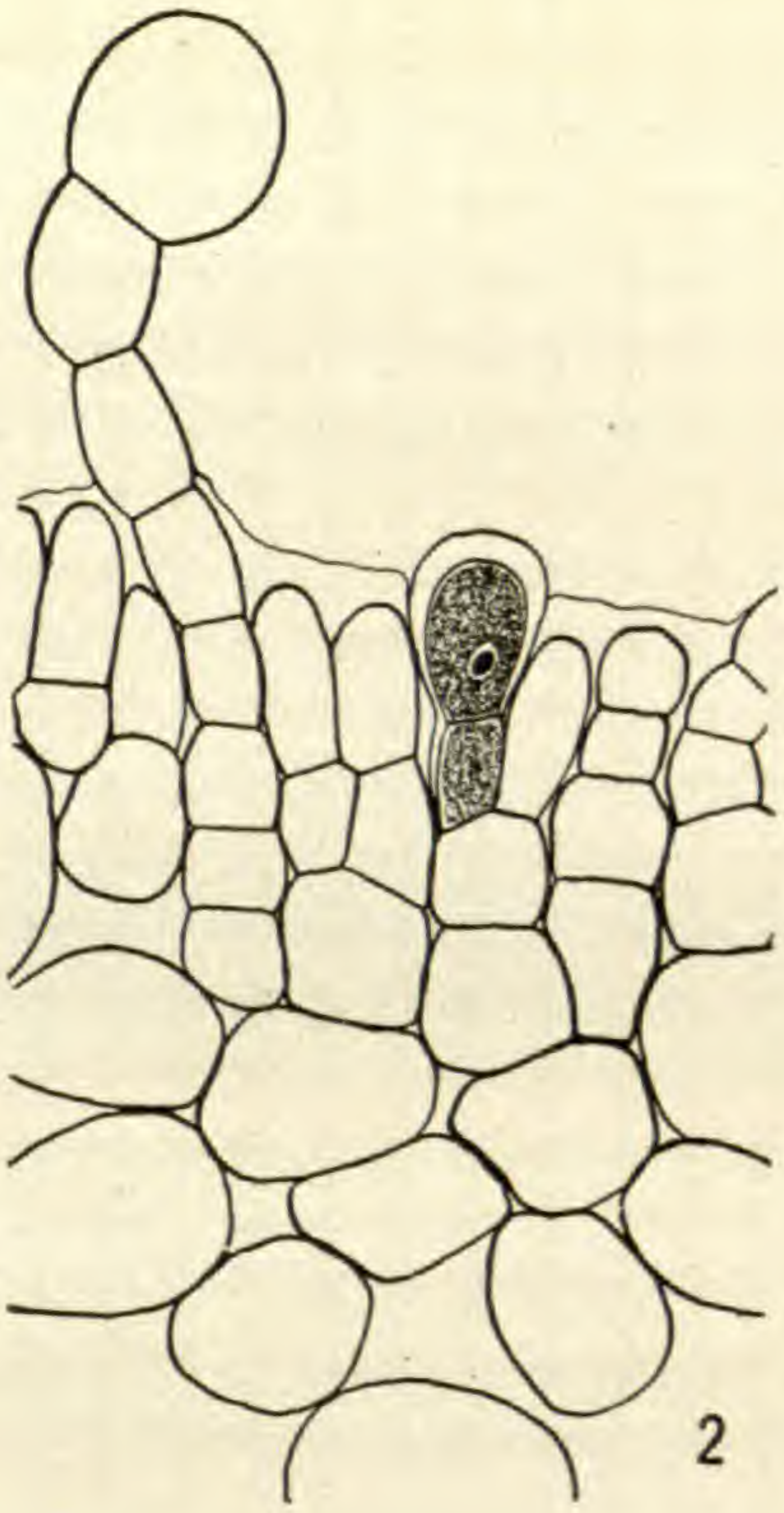
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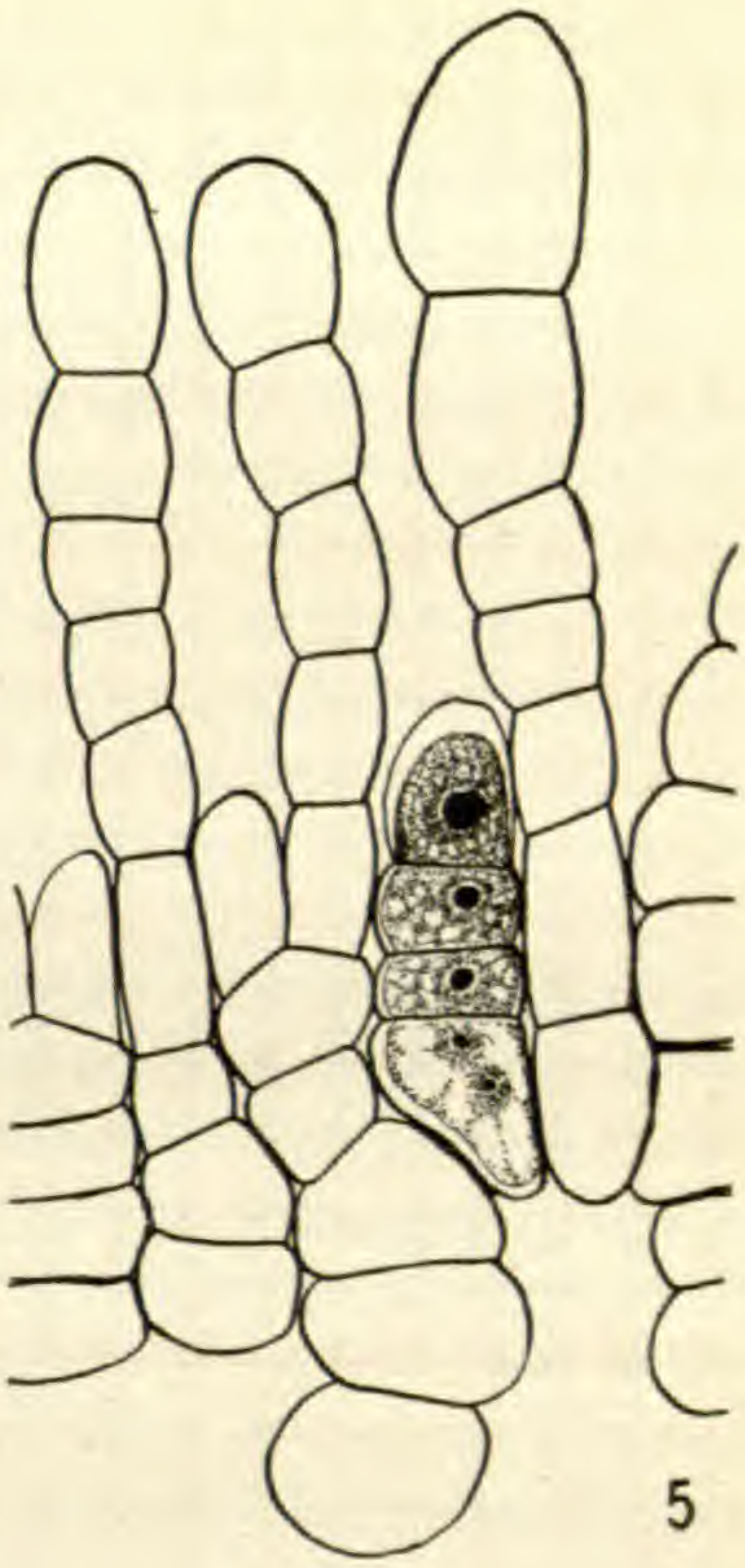
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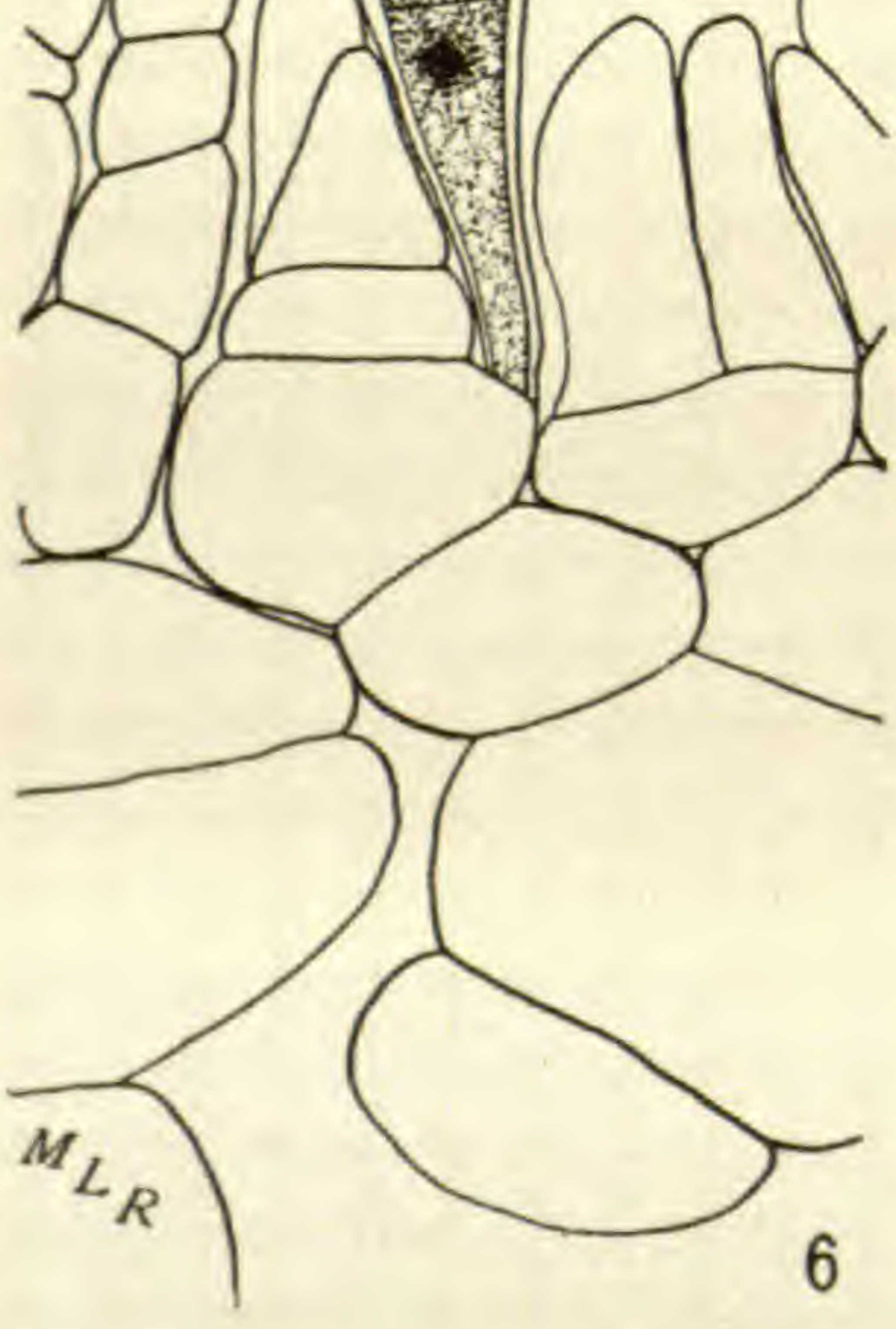

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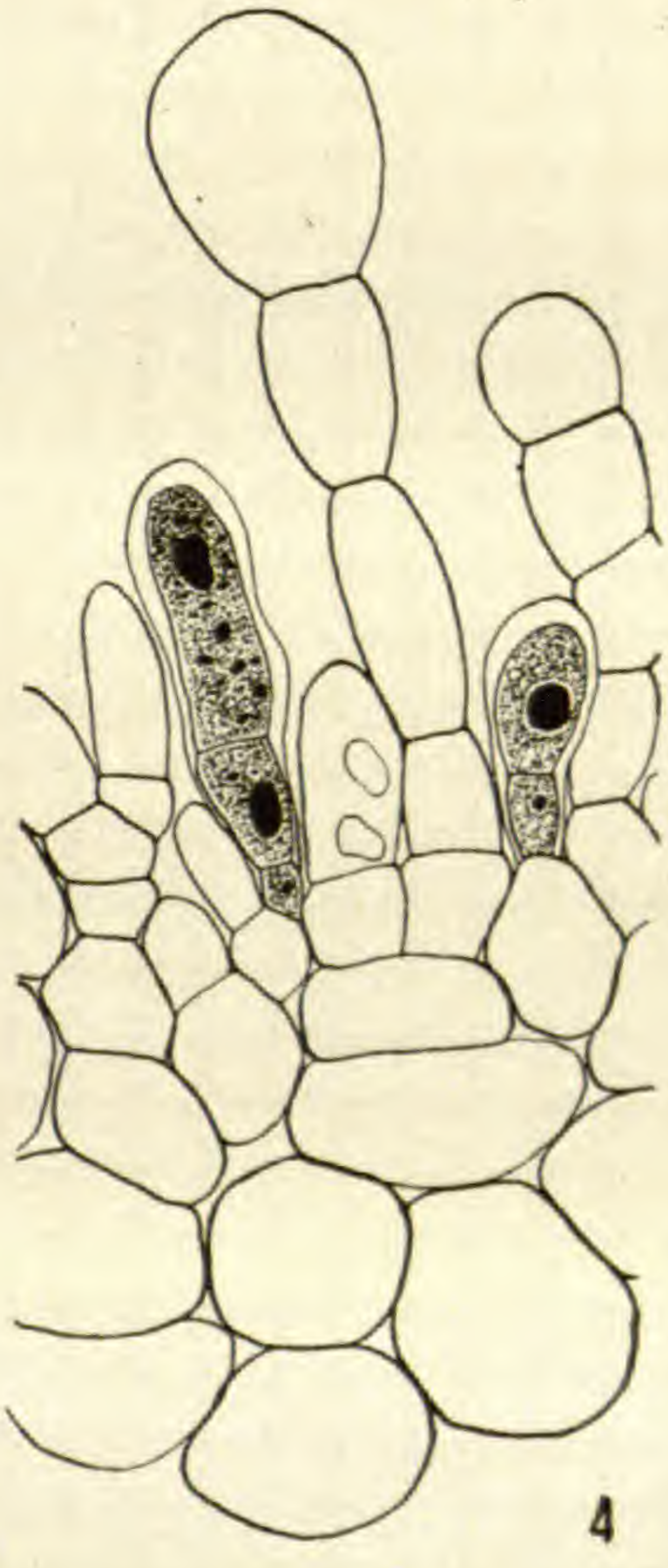
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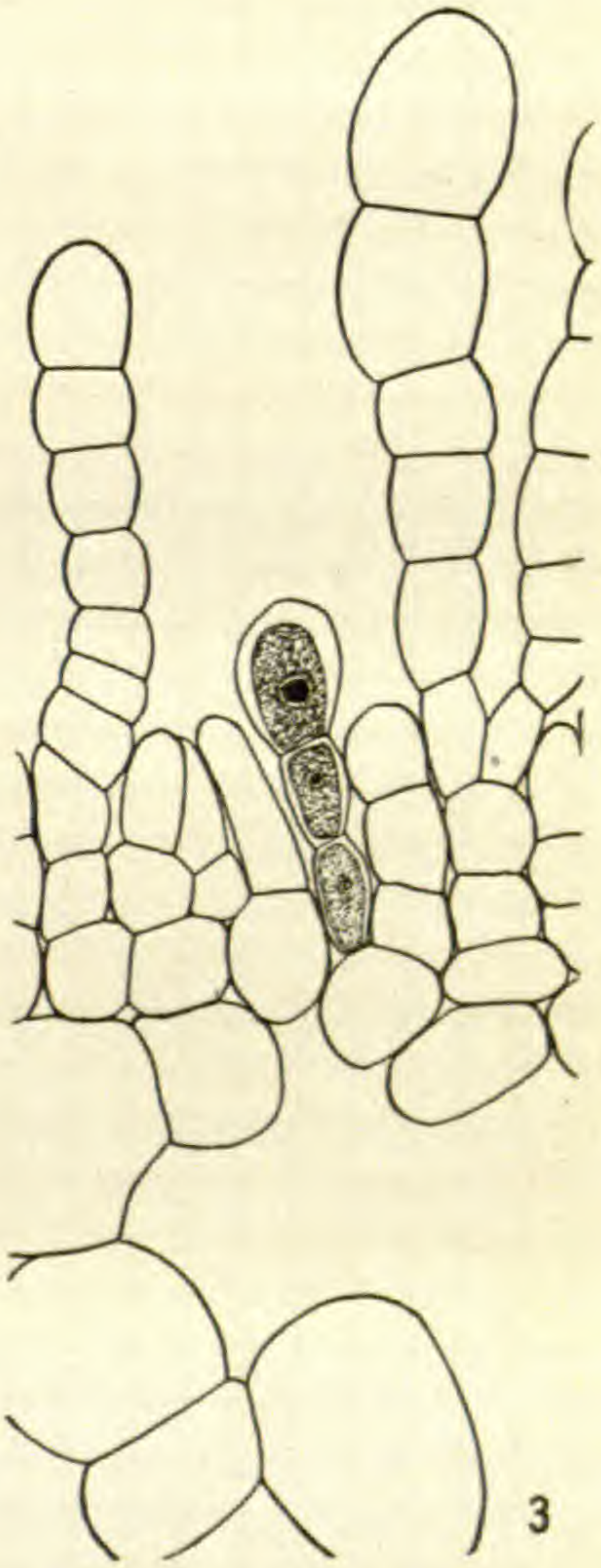
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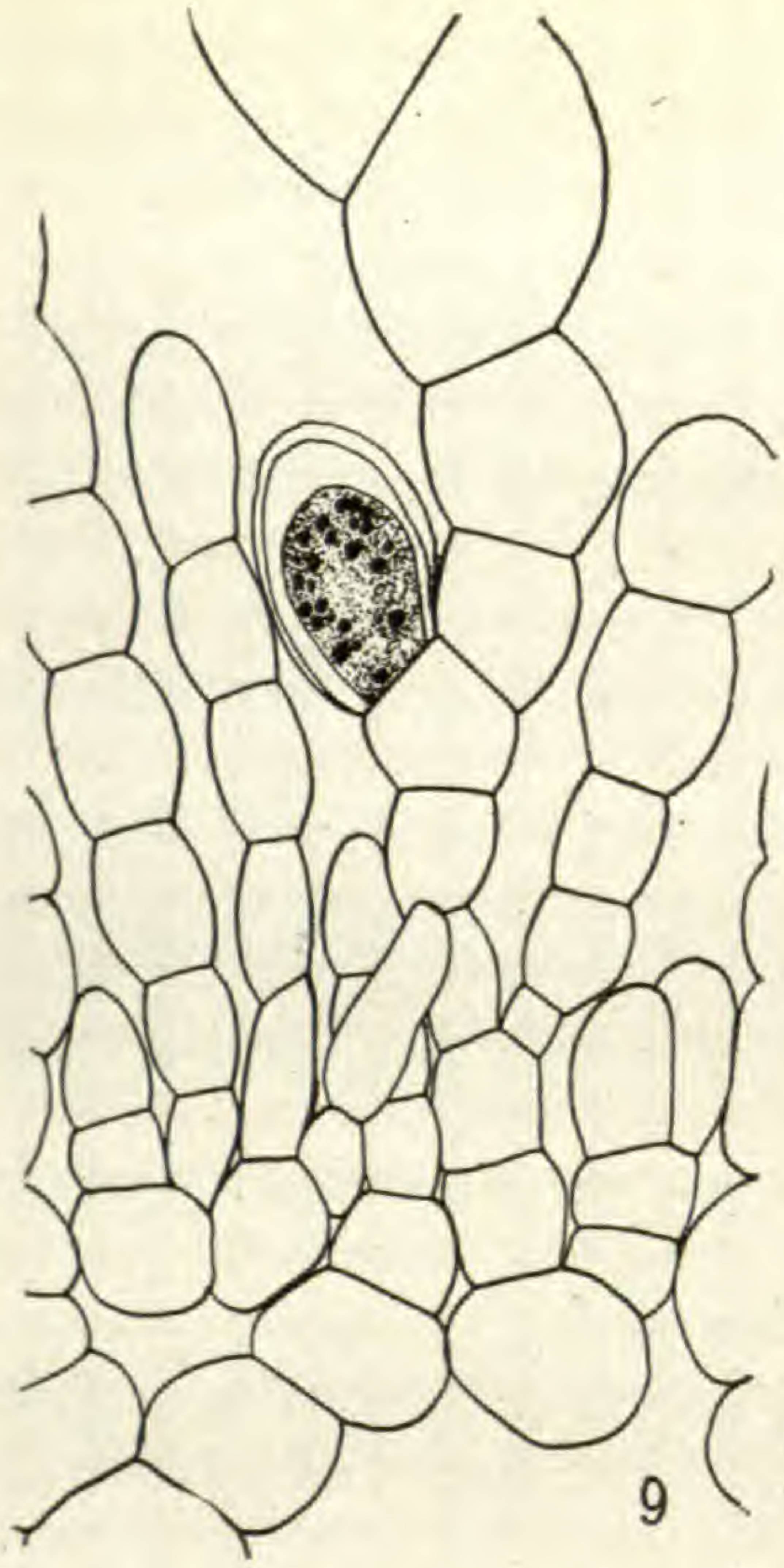
ROE on SPLACHNIDIUM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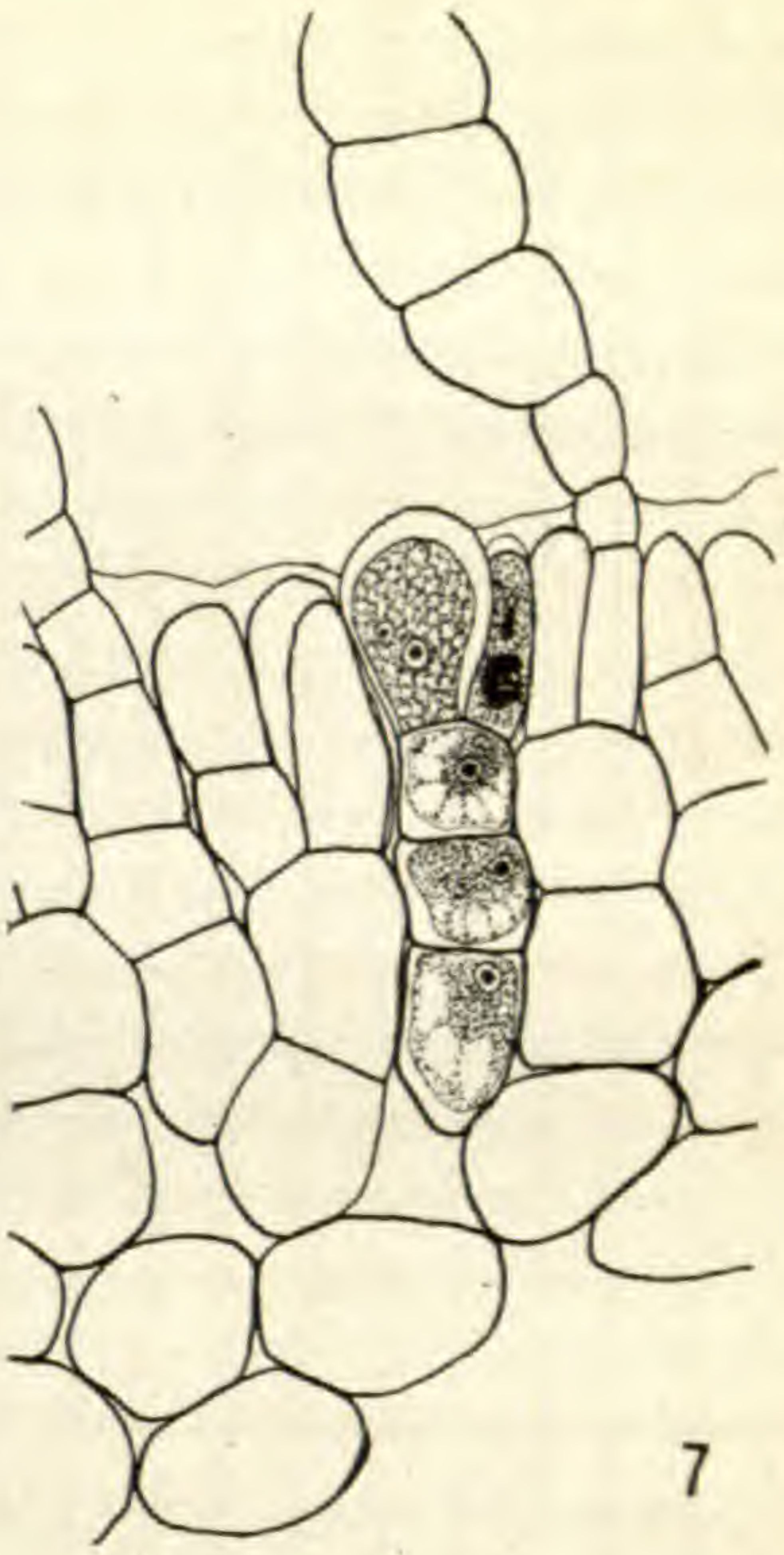
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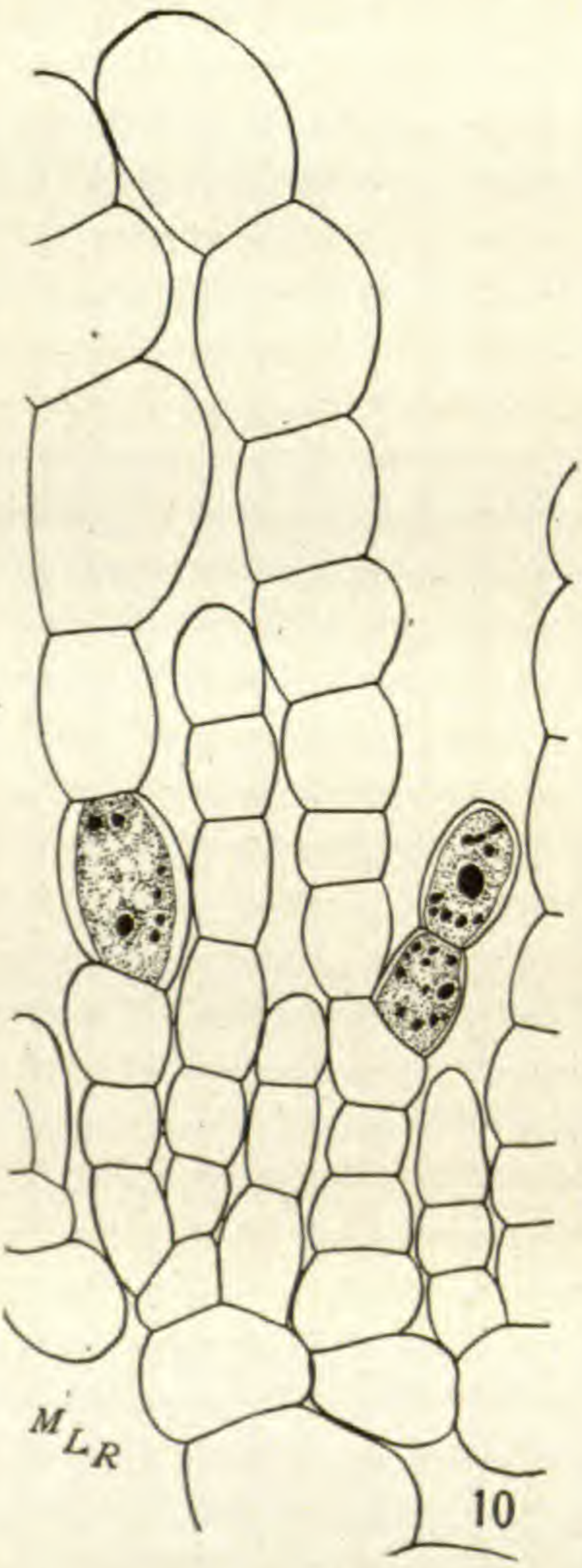

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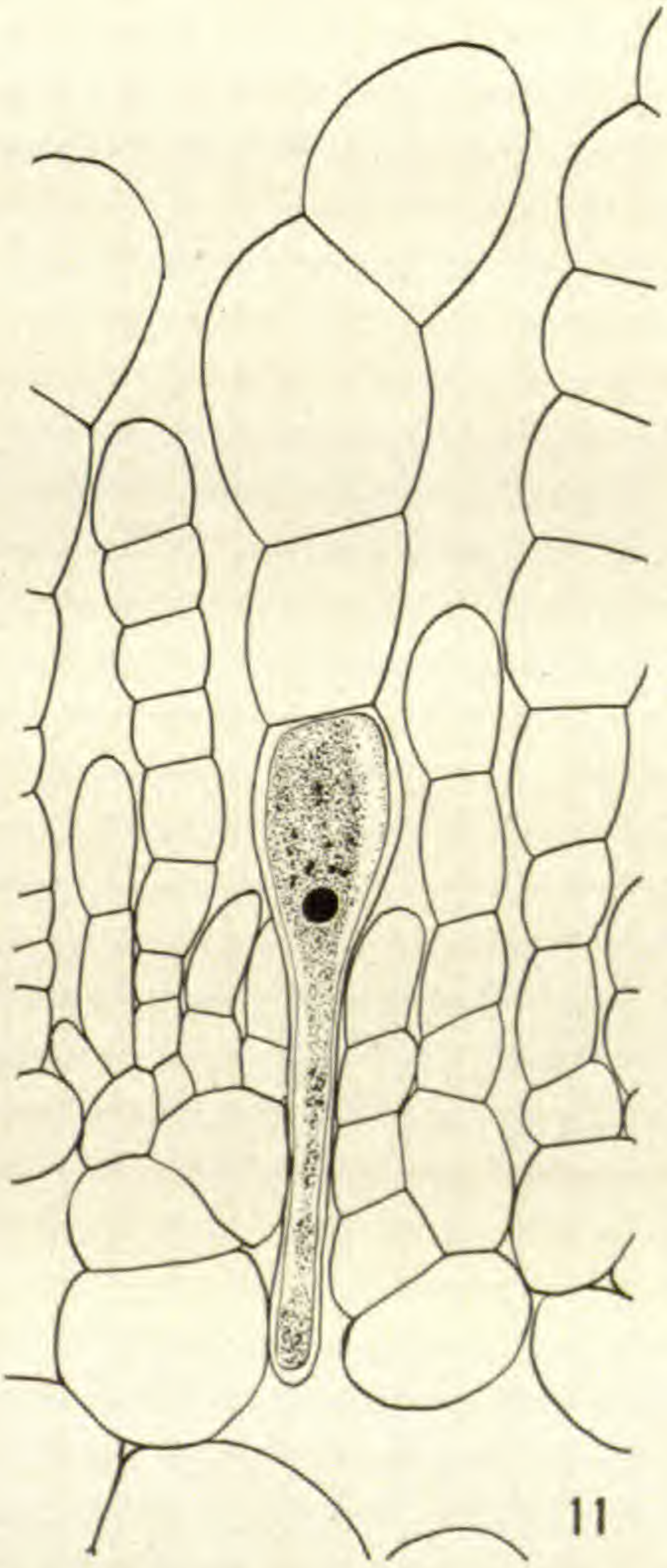
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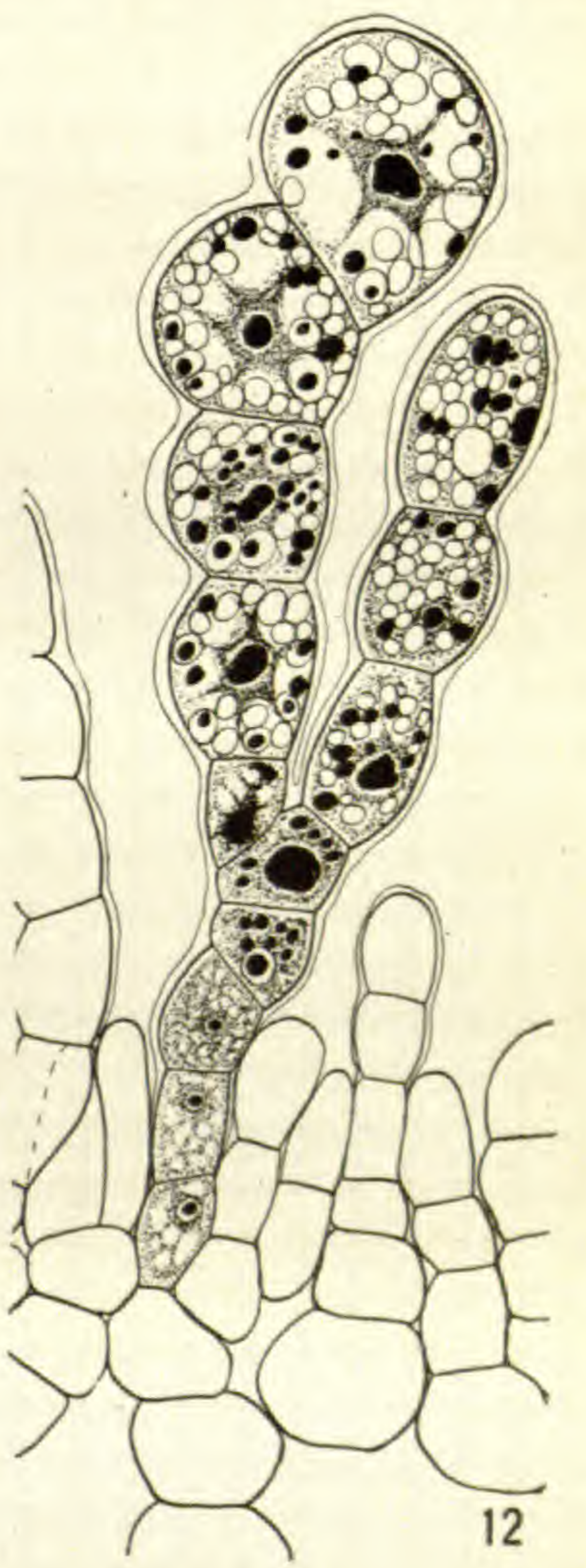
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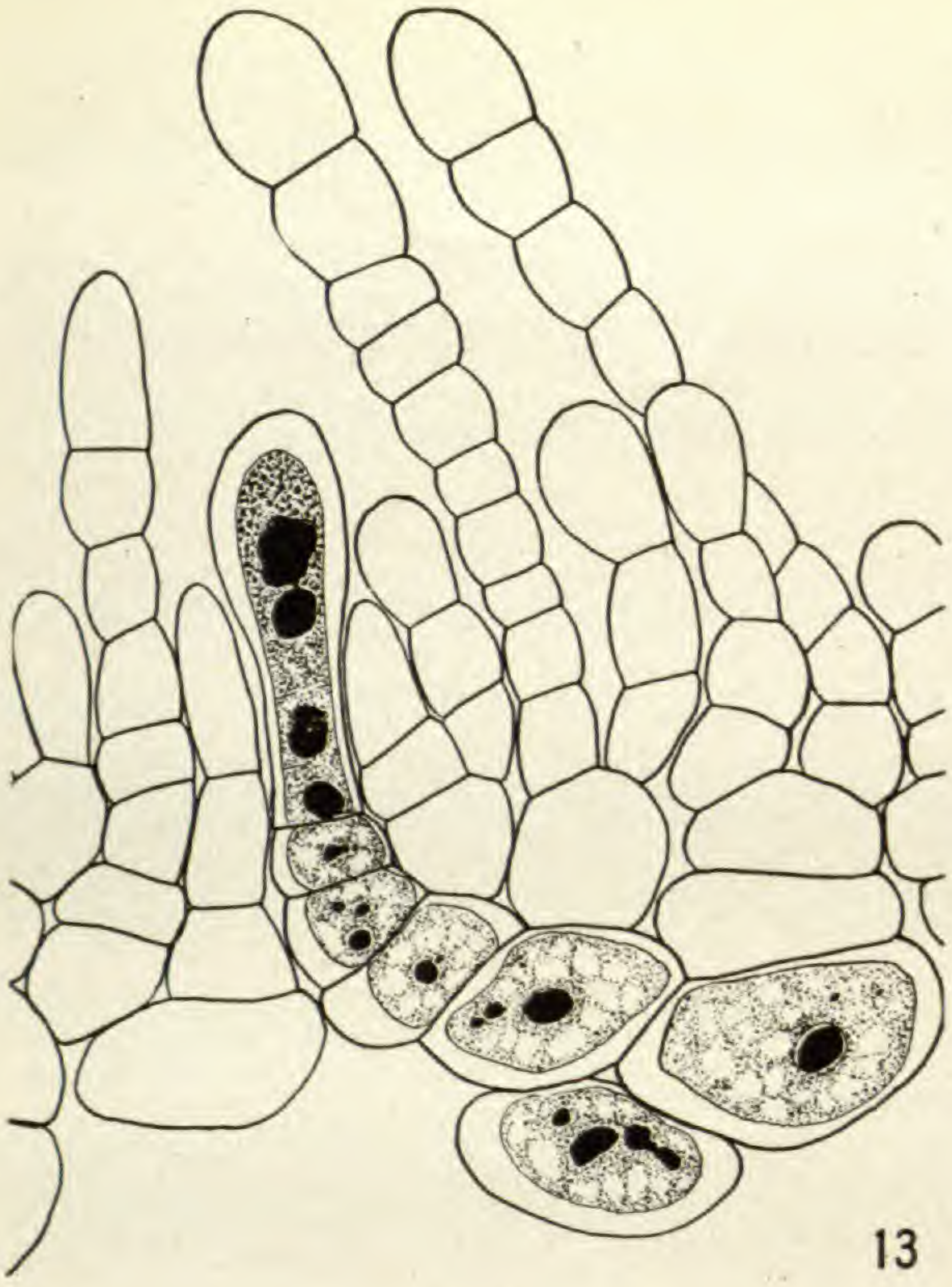

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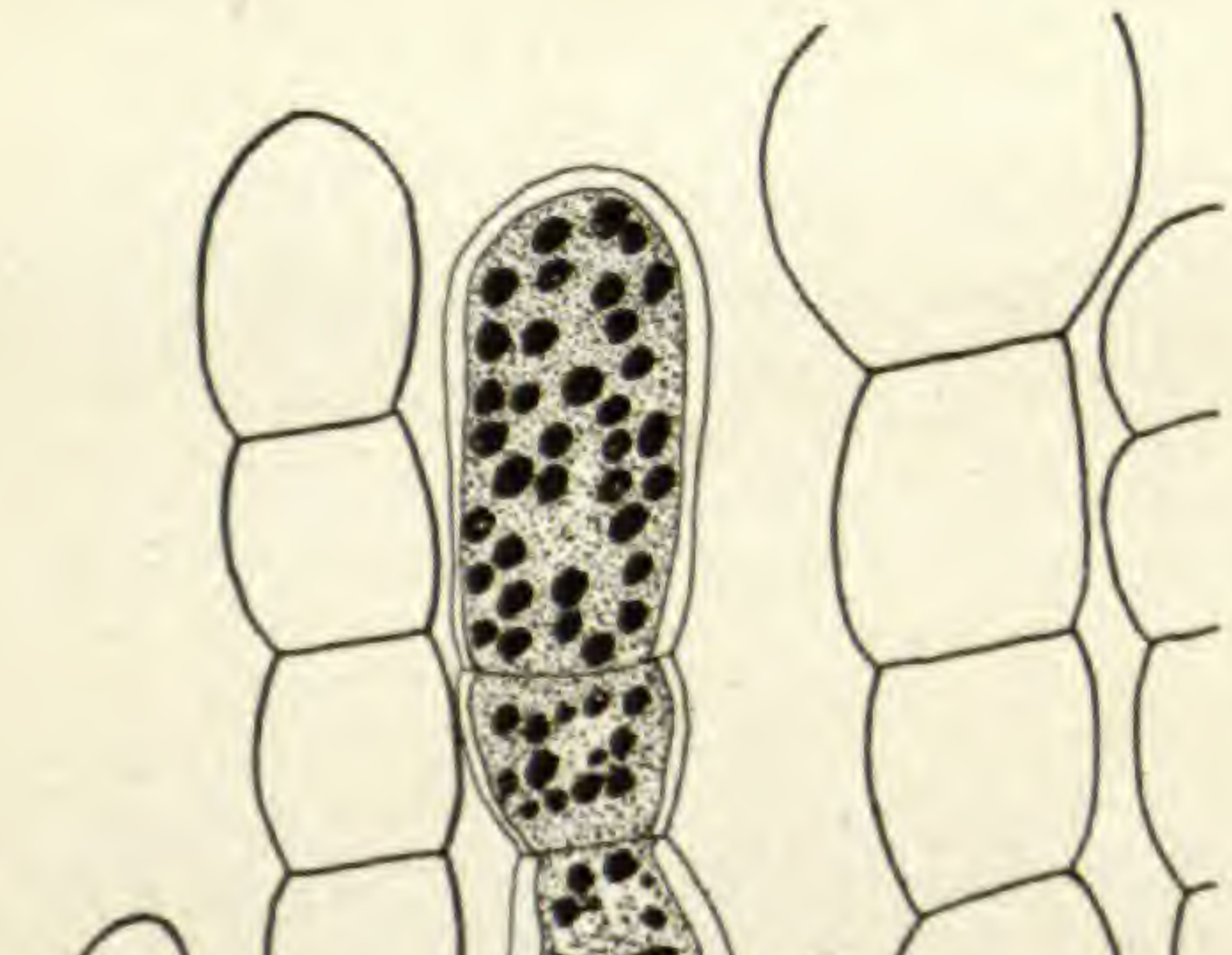

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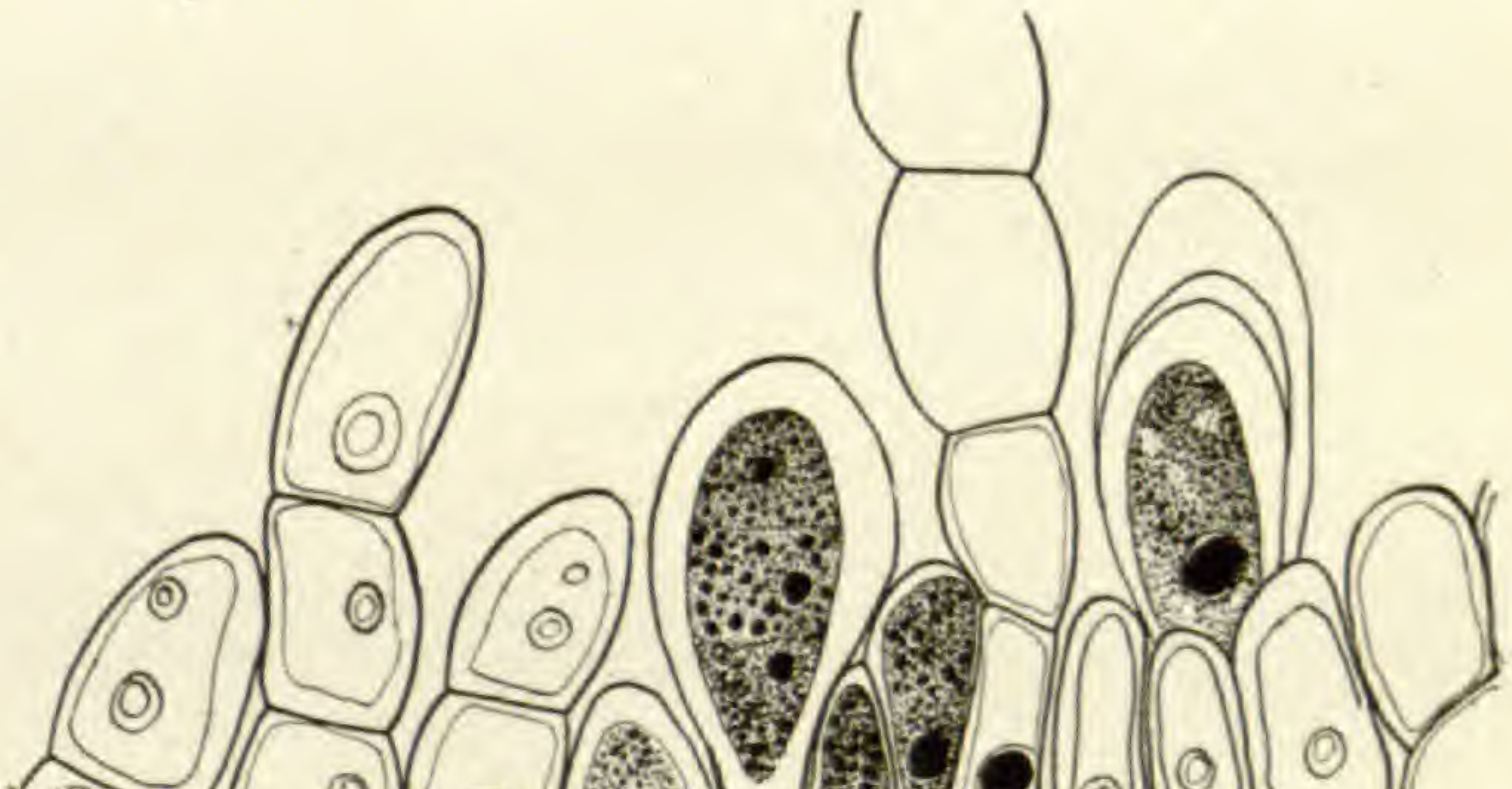

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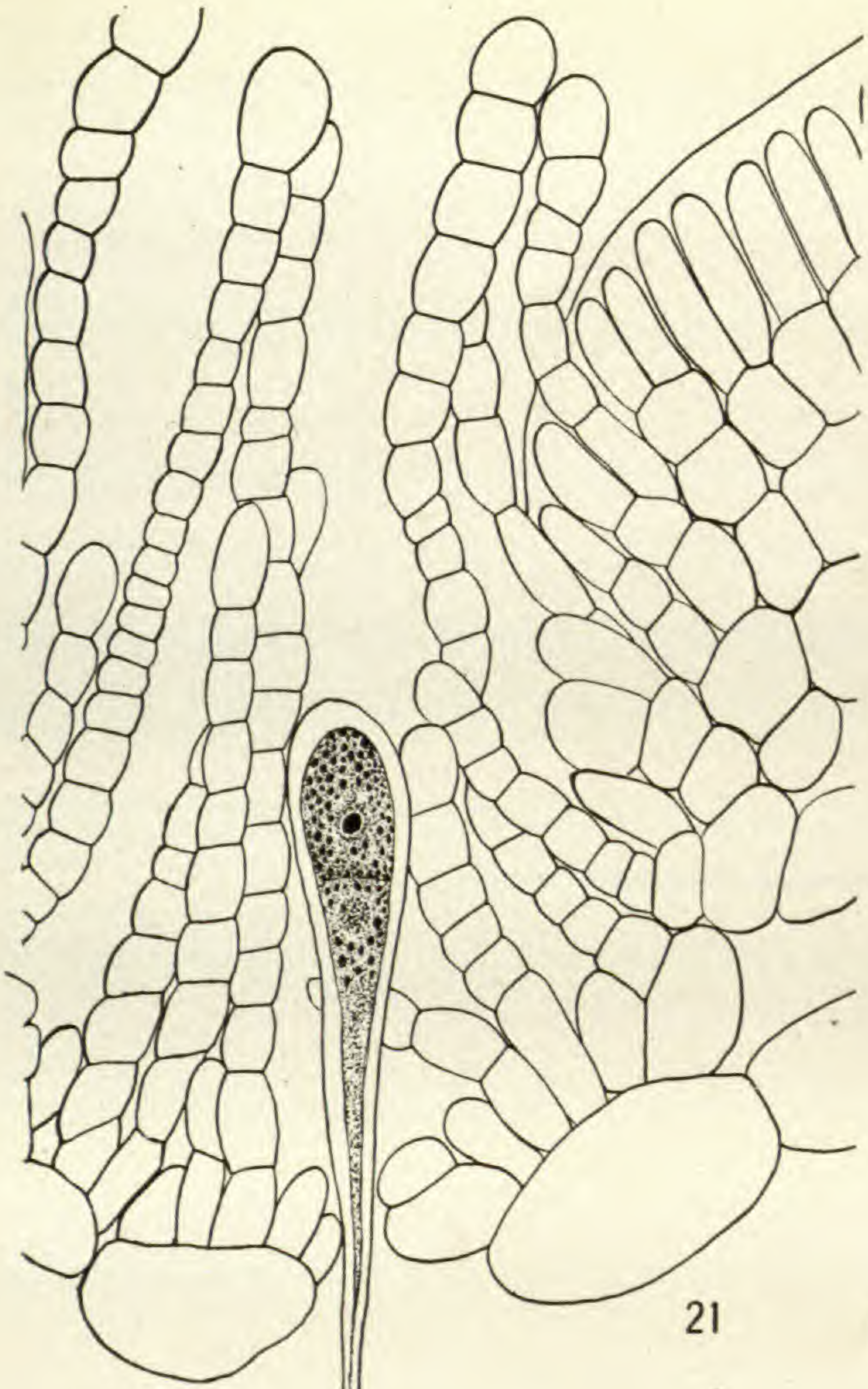
ROE on SPLACHNIDIUM

ROE on SPLACHNIDIUM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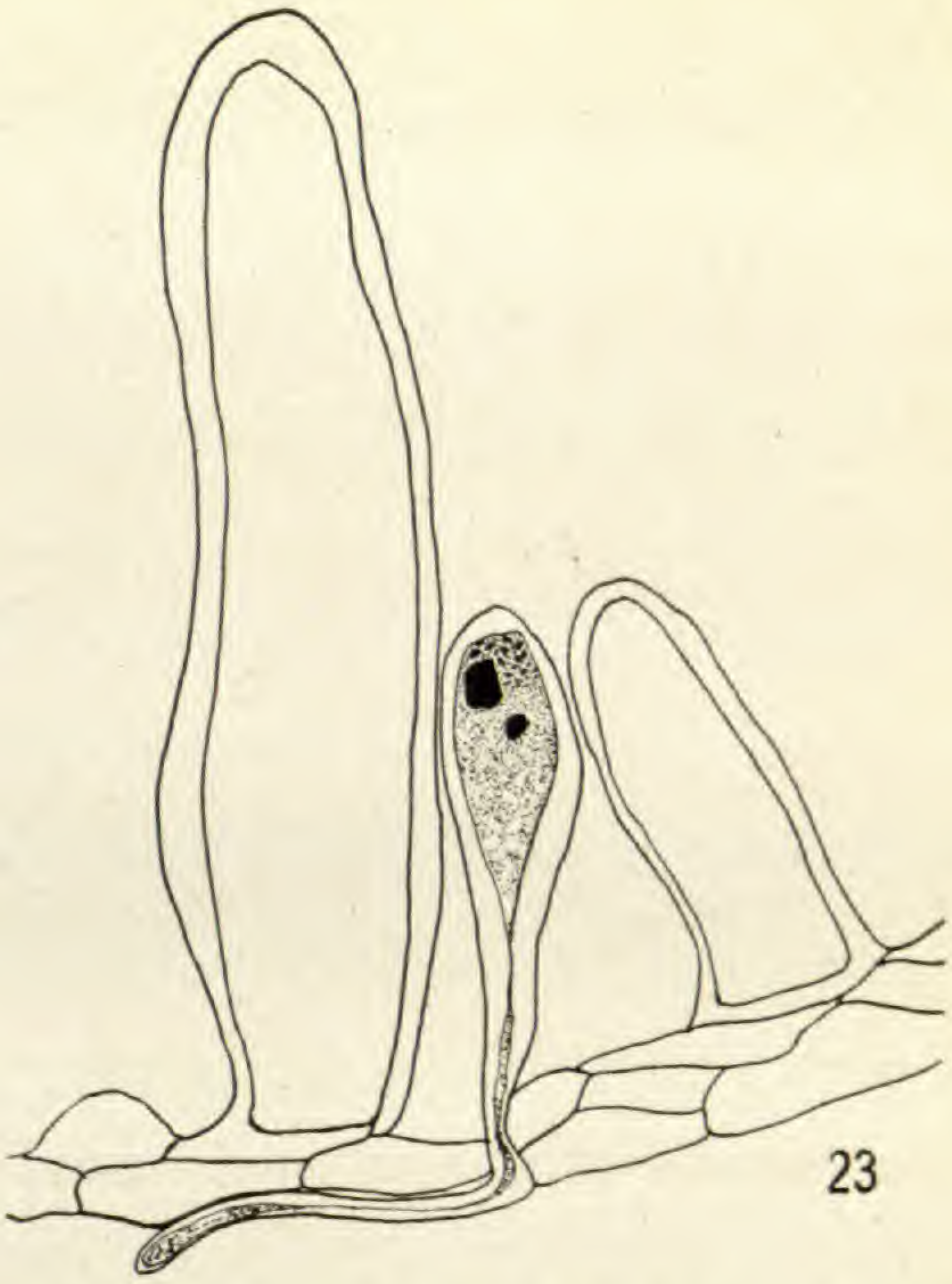
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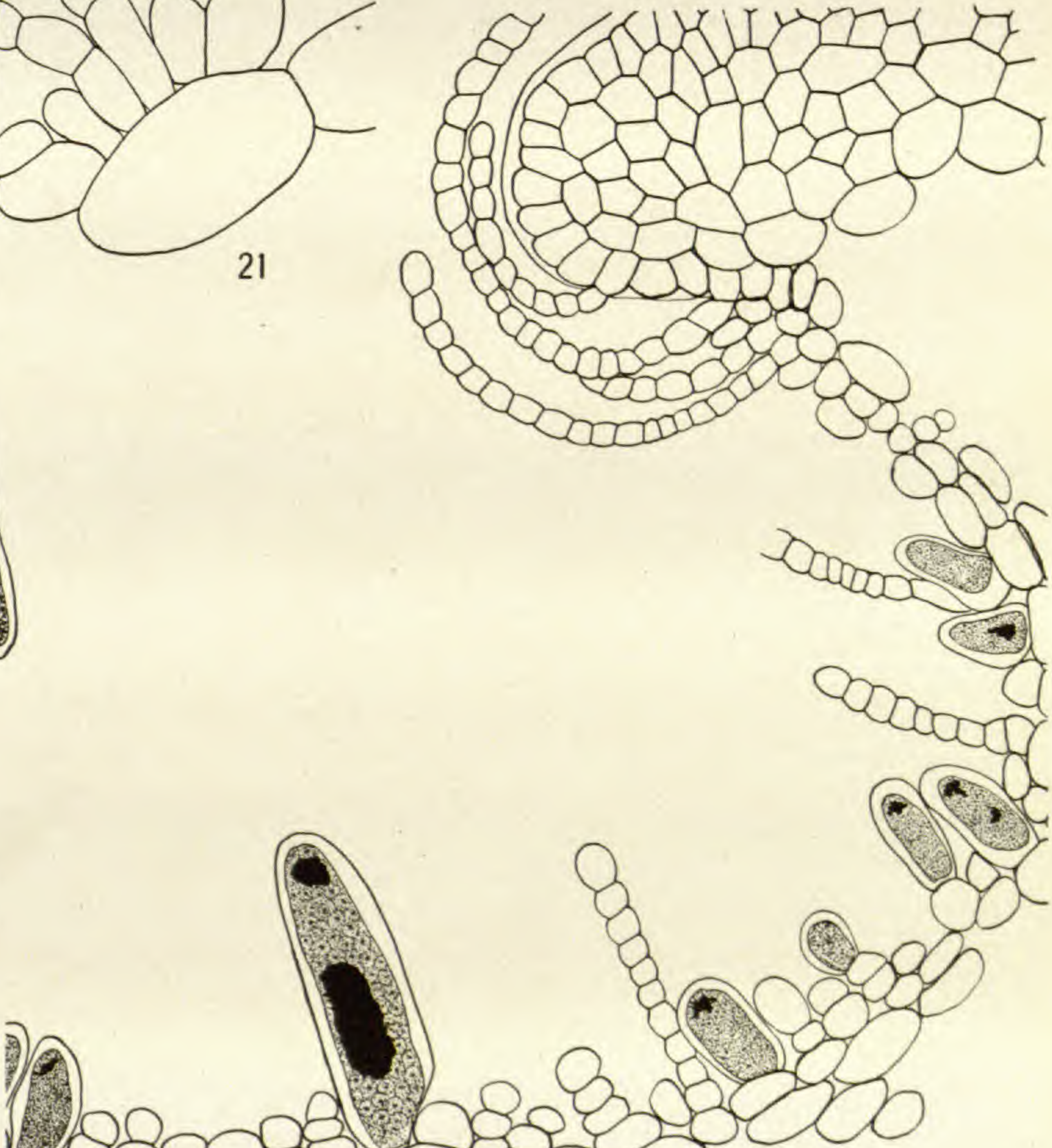
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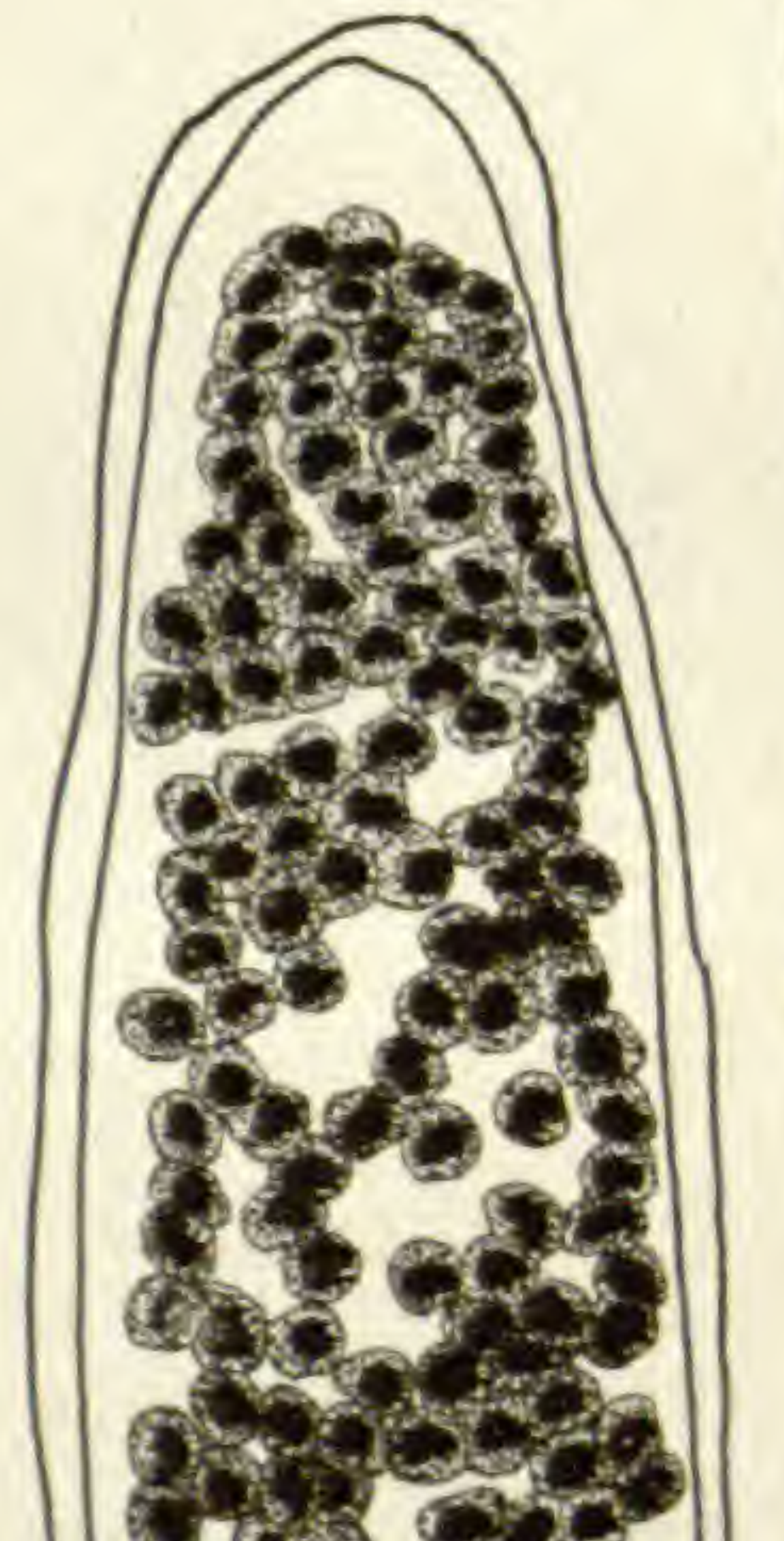
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ROE on SPLACHNIDIUM

THE RELATION BETWEEN OXIDASE AND CATALASE IN PLANT TISSUES

G. B. REED

(WITH ONE FIGURE)

In a previous paper[1] the writer has pointed out that although substances which act as oxidases or peroxidases usually decompose hydrogen peroxide, and although many authors think that there is a causal connection between the two processes, yet in the case of colloidal platinum they are quite independent. The question then arises, to what extent does this apply to the living cell? This is of particular interest in view of the fact that some investigators suppose that oxidase action depends in some way upon the activity of a catalase.

A few instances are on record in which, by some special treatment, plant oxidases have been prepared so as to give no catalase action. LIEBERMANN[2] found that by shaking an extract of malt with mercuric oxide and magnesia, or by heating the extract to 80° C., its catalase activity was destroyed but it still exhibited some peroxidase action. LOEW[3] reports that after treating an aqueous extract of fresh tobacco with one-fifth its volume of absolute alcohol it had no action on hydrogen peroxide, but still activated directly the oxidation of gum guaiac. Finally, KASANSKI[4] has shown that the catalase action of certain plant and animal extracts may be destroyed by the addition of strong solutions of pyrogallol or sugar, without completely inhibiting their peroxidase action.

More conclusive evidence that peroxidase action is independent of any ability to decompose hydrogen peroxide was obtained by the writer from a study of pineapple extracts. Pineapple juice always contains very active peroxidases; catalase reactions were

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[1] REED, G. B., BOT. GAZ. 62:233–238. 1916.

[2] LIEBERMANN, P., and L., Pflüger's Archiv. 108:489–495. 1905.

[3] LOEW, O., Report no. 68. U.S. Dept. Agric., p. 47. 1901.

[4] KASANSKI, Biochem. Zeit. 39:64–72. 1911.

only obtained, however, under certain conditions which are concerned with the stage of development of the fruit.

After some preliminary experimentation, 3 fruits of the same variety were selected. One was quite ripe and soft; another, although it had turned yellow, was still rather hard; the third had only partly lost its green color. The juice of each of these fruits was pressed out separately in a mortar, filtered, and the catalase activity determined by mixing 2 cc. of the juice in each case with 100 cc. of 0.05 M hydrogen peroxide and measuring the pressure of the oxygen evolved. For this purpose the hydrogen peroxide was placed in a 300 cc. bottle which was provided with a 3-hole stopper carrying a small separatory funnel in which the juice was placed, a paraffin manometer and a tube, closed by a stopcock, for the equalization of the initial pressure. The whole apparatus was submerged in a tank of water with a glass wall, the temperature of which was kept constant. After all the solutions had reached the same temperature the stopcock of the funnel was open and the juice allowed to run into the peroxide. On closing the stopcock of the funnel, as well as that of the pressure tube which had previously been open, the reaction was started

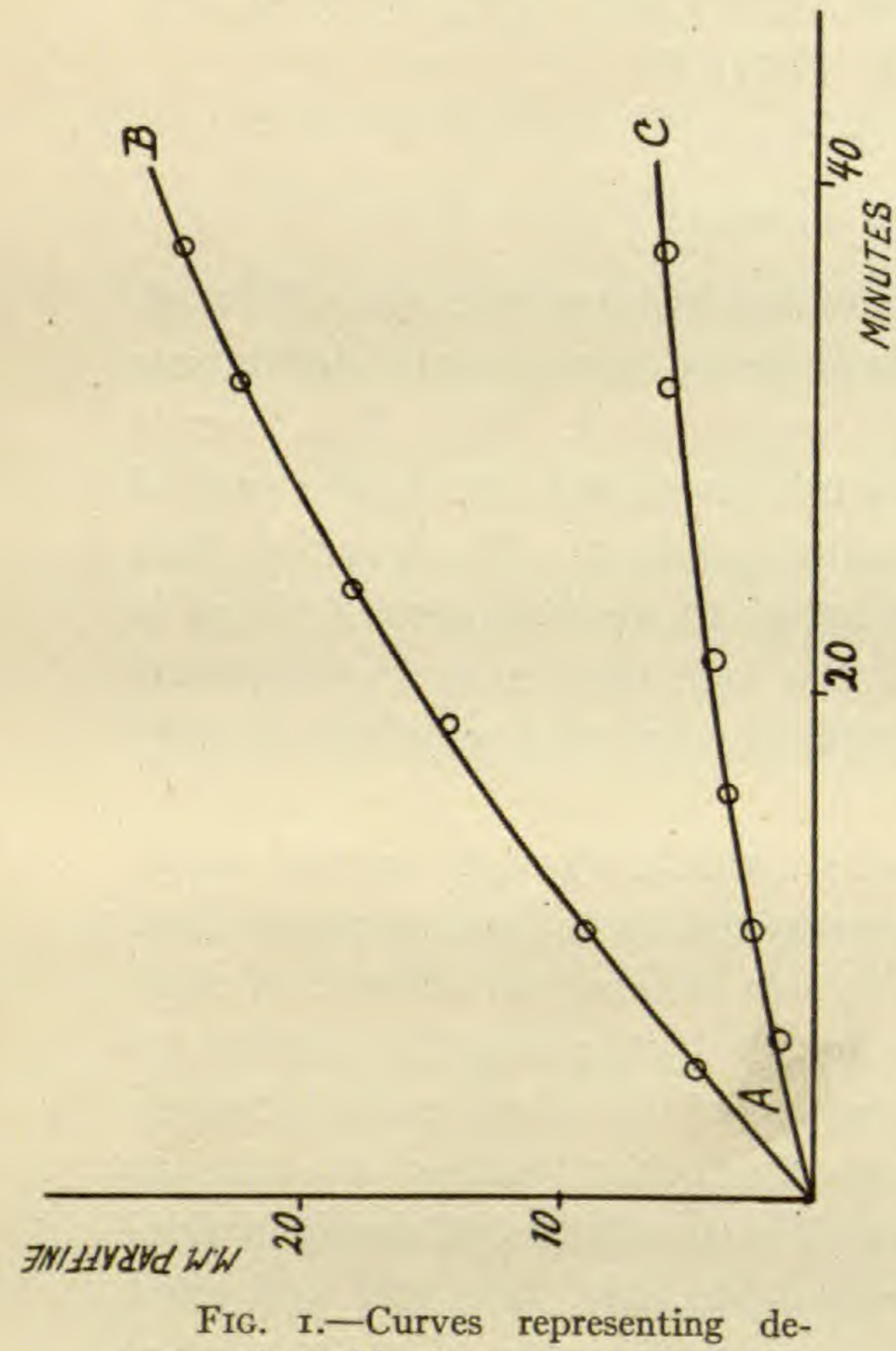

FIG. 1.—Curves representing decomposition of hydrogen peroxide: upper curve *AB* represents action of an extract of a well ripened pineapple; lower curve *AC* represents action of an extract of a partially green pineapple; ordinates represent pressure of oxygen produced (expressed in mm. of liquid paraffin in a paraffin manometer); abscissae represent time in minutes.

at atmospheric pressure.[5] The glass wall of the tank allowed easy reading of the manometer.

The curve *AB* (fig. 1) shows the rate of decomposition of the peroxide (as shown by the manometer readings) with the juice of the ripe fruit. Comparing this with *AC* of the same figure, which represents the rate of decomposition by the juice of the partially ripe fruit, it will be apparent that the latter has much less catalase activity than the former. Moreover, the juice of the green fruit showed no action on this concentration of hydrogen peroxide, or on a stronger solution, whether measured by the pressure method or by simply observing the evolution of bubbles of gas. As might be expected from these results, different fruits exhibit great variation in their catalase activity, but in every case the juice of those in a more or less green condition showed no action in decomposing hydrogen peroxide.

All of these fruits, however, showed approximately the same peroxidase activity, as the following determinations indicate. From each kind of fruit 10 cc. of juice was mixed separately with 100 cc. of 2 per cent pyrogallol solution containing 0.05 M hydrogen peroxide, placed in open beakers and maintained at 18° C. for two hours. The purpurogallin formed from the oxidation was then filtered off, dried, and weighed, after the method of BACH and CHODAT.

Comparing these results, stated in table I, with the previous measurements on the rate of hydrogen peroxide decomposition by

TABLE I

AMOUNT OF PURPUROGALLIN FORMED IN OXIDATION OF 100 CC. OF 2 PER CENT PYROGALLOL CONTAINING 0.05 M HYDROGEN PEROXIDE AND 10 CC. OF EXTRACT

Peroxidase from	Gm. purpurogallin formed in 2 hours
Ripe pineapple	0.986
Partially ripe pineapple	1.020
Green pineapple	1.096
Control; boiled pineapple	0.081

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[5] The simpler method of titrating the unused hydrogen peroxide with permanganate could not be satisfactorily employed under these conditions, since the extract of the ferment had a reducing action on the permanganate.

the same extracts, it is evident that the peroxidase activity of pineapple juice is not dependent upon the rate of decomposition of hydrogen peroxide.

From the writer's studies on the behavior of platinum black (*loc. cit.*), in which it was shown that factors which influenced the catalase action have no effect on the oxidative activity, and from the fact that in certain stages of their development pineapples contain oxidases but no catalase, we may conclude that the substances which effect the decomposition of hydrogen peroxide are not of necessity concerned with the enzymes which accelerate peroxide oxidations. It may be added that the fact that catalase is not universally present in living cells, as LOEW and others suppose, has considerable theoretical interest.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

BRIEFER ARTICLES

A NEW HYBRID COLUMBINE

The year before last my wife crossed *Aquilegia chrysantha* Gray (a garden strain) with *A. desertorum* (Jones) Ckll. from Santa Fé Canyon, New Mexico. The hybrids have bloomed this year, and are found to present some interesting characters. In order to make these clear, it is first necessary to indicate the principal diagnostic features of *A. desertorum.*

Aquilegia desertorum (Jones) Ckll.—WOOTON and STANDLEY, in their *Flora of New Mexico,* do not distinguish *A. desertorum* from *A. elegantula* Greene. Direct comparison of living plants has not yet been possible, but *A. elegantula* is properly a forest-loving species of higher altitudes, essentially a mesophytic plant. *A. desertorum,* as its name indicates, is xerophytic, living on rocky slopes in the transition zone, and is remarkable for its enormous root and long life. The early leaves have a strong red suffusion, and the plant comes into flower long before the other Rocky Mountain species, so that only a few belated blossoms can be obtained for crossing with *A. chrysantha* or *A. caerulea.* As I write (June 1), *A. desertorum* has still one or two flowers, while the first flower of *A. chrysantha* is opening. *A. caerulea* is in full flower, however, coming between the periods of *A. desertorum* and *A. chrysantha.* The following description is from a characteristic flower of *A. desertorum.*

Sepals short, about 9 mm. long, 3.5 mm. broad, lanceolate, reddish or partly pale yellowish, with purplish tips; upright, and extending about 2 mm. beyond ends of petals. Petals 25 mm. long (to end of spur), spurs only moderately divergent; about apical 5 mm. of petals light yellow, the rest bright scarlet; this scarlet appearance is wholly due to an anthocyan pigment, which looks pink by transmitted light, and readily passes into solution in weak nitric acid; with liquor potassae it first turns lilac, and then gives the usual green reaction; the lilac can be observed under the microscope fringing the edge of the alkaline fluid. Stamens exserted 7 mm. beyond ends of petals; anthers yellow. Pistils exserted 4 mm. beyond stamens.

Aquilegia desertorum × *chrysantha,* new hybrid.—The early leaves showed more or less distinct reddish or purple color, which is wholly wanting in *A. chrysantha*. The time of flowering is intermediate between that of the two parents; the plants were in full flower by the second half of May. The flowers are much broader than those of *A. desertorum*, with relatively short spurs (that is, in relation to the breadth), which is not a character of either parent. The sepals are strongly divergent, a character of *A. chrysantha*. The following description is from a characteristic flower.

Flower nodding. Sepals about 19 mm. long and 8 mm. broad, pink, with a faintly purplish shade, or the apex distinctly purplish. Petals 30 mm. long (to end of spur), 8 mm. wide near apex, broadly truncate and sub-marginate apically; apical 10 mm. cream color, spur rose-pink; spurs much broader basally than in *A. desertorum*.

Two plants, representing each parent as seed-producer, are essentially alike; but a third, recorded as from *A. chrysantha* pollen on *A. desertorum*, is distinctly different, having longer spurs (petals 34 mm., the truncate apex 7 mm. wide), and longer, narrower sepals (21 mm. long, 6 mm. wide), while the color of the spurs is more purplish, owing to less acidity. It is just possible that this represents *A. caerulea* pollen on *A. desertorum*, due to some unobserved insect, although the heads were covered and the records do not indicate any such cross as having purposely been made.

Thus it appears that the color of the flowers and the time of flowering of the F_1 hybrid are clearly intermediate; but the form of the flowers departs from both parents in the direction of the *A. vulgaris* group. The form of the flower, however, may be given a simple Mendelian interpretation, if we say that the dominant characters are the spur-length of *desertorum* and the flower-width of *chrysantha*.

We also have a row of *A. chrysantha*×*A. caerulea* hybrids, but this form has long been known in gardens. It blooms with *A. caerulea*, and has the flowers paler than *A. caerulea*, with the white replaced by light yellow, which fades to white as the flowers grow old. On the whole, it is nearly *A. caerulea*, with the yellow of *A. chrysantha* superimposed upon it.—T. D. A. COCKERELL, *Boulder, Colorado*.

AN ALTERNARIA ON SONCHUS

(WITH ONE FIGURE)

During recent studies on *Alternaria* and *Macrosporium* much material was sent to me by various botanists. Among this was an *Alternaria* collected by Dr. DAVIS near Madison, Wisconsin, which

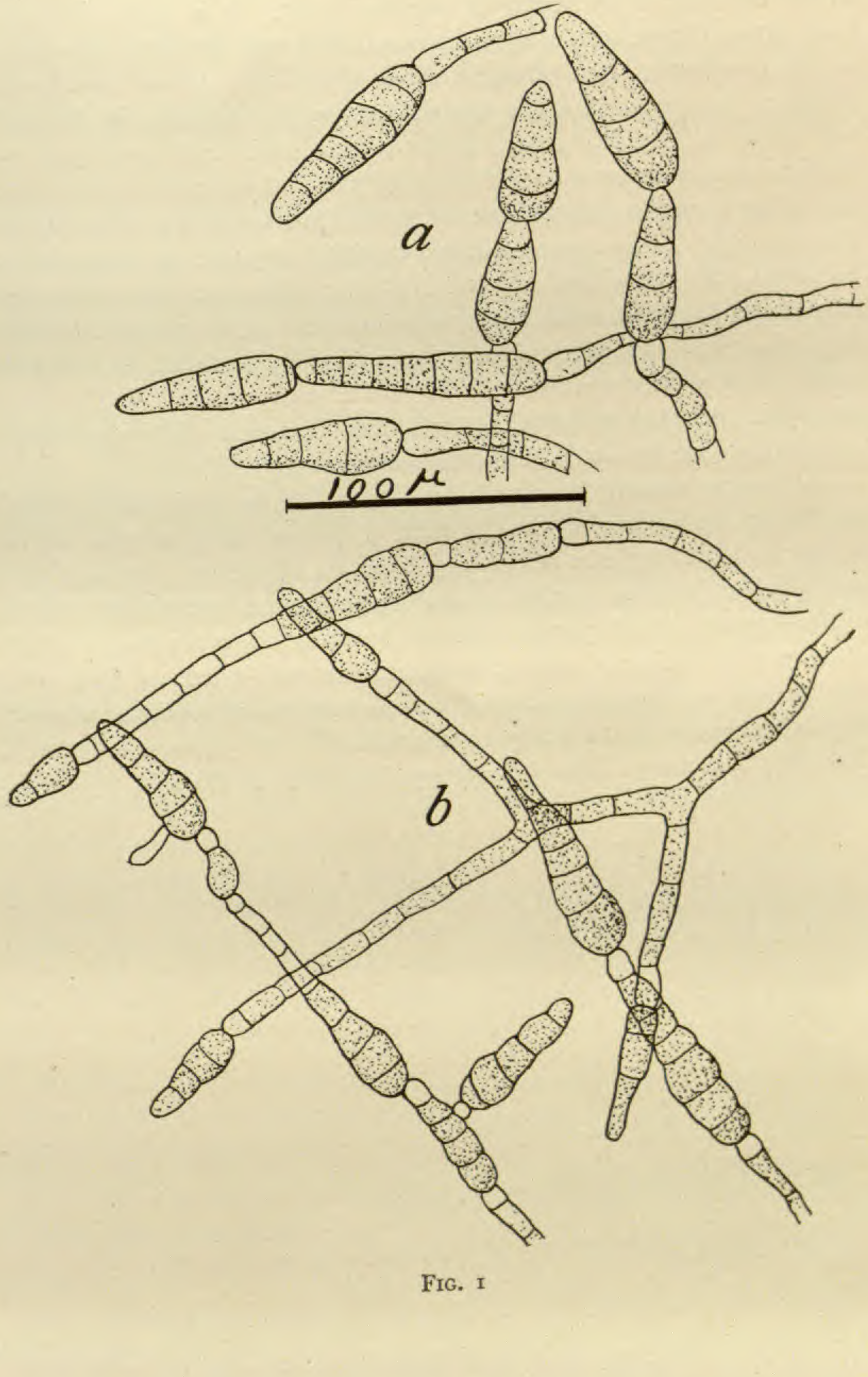

FIG. 1

caused spotting of the leaves of *Sonchus asper*. It is quite different, in so far as I have been able to discover, from any described species.

Pure cultures were obtained from spots on *Lactuca canadensis*, produced by means of crude inoculations with fragments of diseased leaf tissue of *Sonchus asper*. Spots were consistently produced on *Lactuca canadensis* by spores of the fungus from pure culture, and were not in any essential feature different from those occurring naturally on *Sonchus asper*. The spots enlarged rapidly (2–3 cm. in diameter in 10 days), the parasitism apparently being vigorous. On cultivated lettuce and dandelion small flecks were produced by the spores on plants kept under bell jars, but on these hosts the fungus failed to maintain itself. This species was the only one under study which failed to grow at 30° C., although at 20° C. growth was vigorous. The description submitted to me by Dr. J. J. DAVIS is as follows:

Alternaria Sonchi Davis, sp. nov.—Spots definite, orbicular to irregular, brown to cinereous, usually with a narrow dark margin above, darker and immarginate below, 0.5–1 cm. in diameter; conidiophores hypophyllous, effused, straight or slightly geniculate, cylindrical, obtuse, dilute brown, apical portion nearly hyaline, $18–55 \times 7–8 \mu$; conidia obclavate with obtuse apex, 5–8-septate, second, third, or first cells, one or all, occasionally divided by a vertical or oblique septum, $80–110 \times 18–20 \mu$, borne singly or in chains of 2 or 3 spores.—JOHN A. ELLIOTT, *Laboratory of Plant Pathology, University of Illinois.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Photosynthesis.—We are indebted to Willstätter and his co-workers[1] for a series of contributions on the chemistry of the chloroplast and the mechanism and behavior of the photosynthetic apparatus. It is a promising sign that so eminent a chemist as Willstätter recognizes that the chemistry of the cell is not the chemistry of the test tube and flask of the "true chemist," but rather the chemistry of a highly complex colloidal substrate with its enzymes. Physiologists can well look forward with keen anticipation to further notes from his laboratory, since we have this statement concerning his future work: "Analyse physiologisch bedeutsamer Stoffe ist nicht Selbstzweck, sondern Vorbereitung für physiologische Forschung."

The leaf pigment chlorophyll is a mixture of two green pigments, a blue-green (chlorophyll *a*) and a yellow-green (chlorophyll *b*), and two yellow pigments, carotin and xanthophyll. In the Phaeophyceae a third carotinoid (fucoxanthin) is found. The chlorophylls of all plants investigated (almost 200) are neutral substances carrying a complex magnesium compound whose molecule is built up of 4 freely substituting pyrrol nuclei and are of the following compositions respectively: $(C_{31}H_{29}N_3Mg)$ $(CONH)$ $(COOCH_3)$ $(COOC_{20}H_{39})$ and $(C_{31}H_{27}ON_3Mg)$ $(CONH)$ $(COOCH_3)$ $(COOC_{20}H_{39})$. These compositions were determined by a judicious and cautious treatment of chlorophyll extracts with proper alkalies and acids in proper concentrations. It was found that splitting with acid made it possible to preserve and find in the products of hydrolysis that compound which is separated by alkalies, and, conversely, that the alkali derivatives exhibited a characteristic group which is destroyed with extraordinary ease by acids. Saponification of the pigment with alkali yields green, water soluble salts, the chlorophyllins. Progressive removal of

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[1] Willstätter, R., and Stoll, A., Untersuchungen über Chlorophyll. 8vo. pp. viii+424. *pls. 11.* Berlin: Julius Springer. 1913; see review of recent work on the pigments of the green leaf and the processes connected with them in New Phytol. **14**:281–294. 1915.

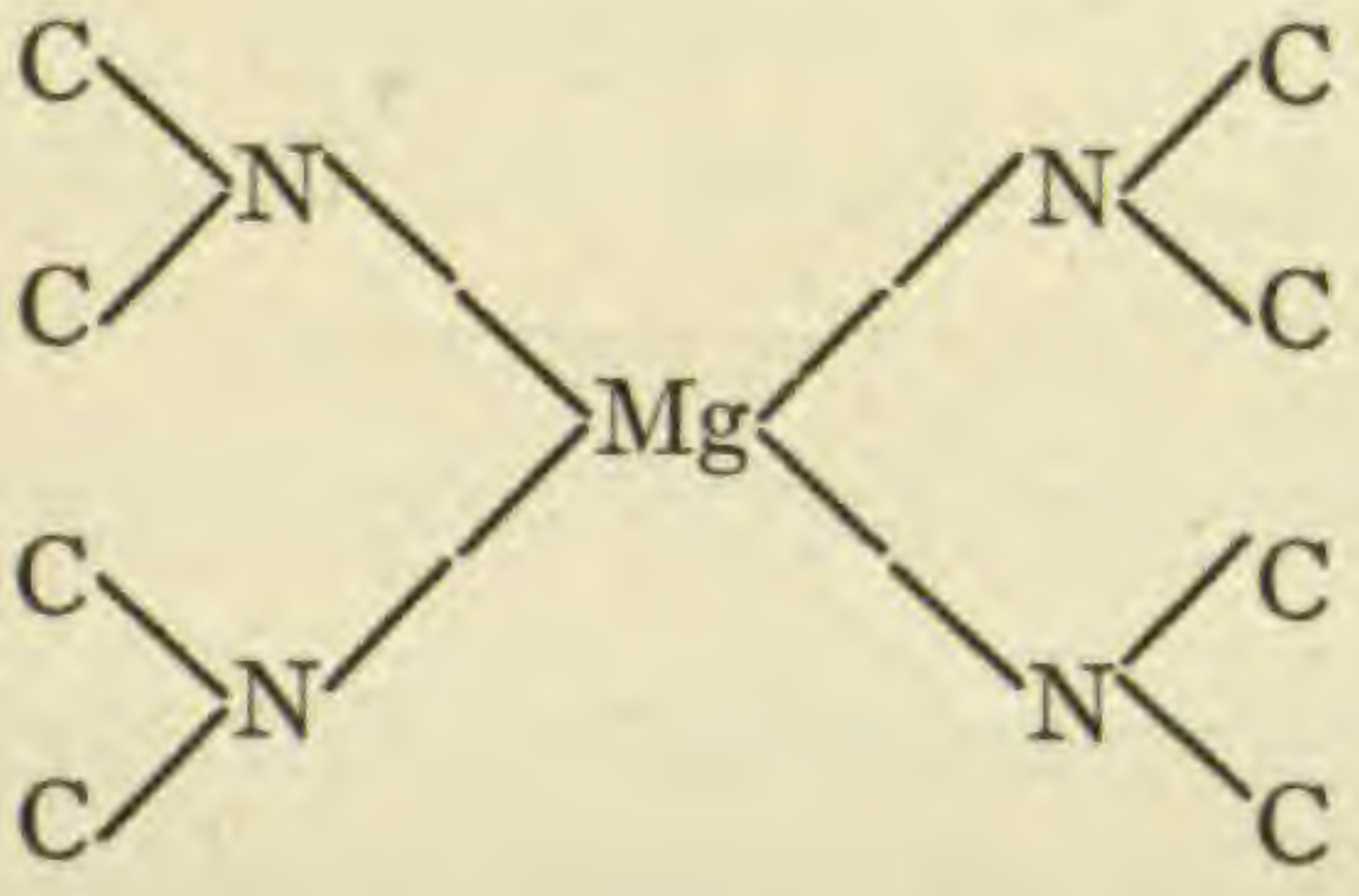
———, Über die chemischen Einrichtungen des Assimilationsapparates. Sitzb. Kgl. Preuss. Akad. Wiss. **20**:322. 1915.

———, Über die Assimilation ergrünender Blätter. Sitzb. Kgl. Preuss. Akad. Wiss. **36**:523. 1915.

———, Untersuchungen über die Assimilation der Kohlensäure. Ber. Chem. Gesells. **48**:1540–1564. 1915.

Wilstätter, R., Sitzb. Kgl. Preuss. Akad. Wiss. **32**:484. 1915.

the carboxyl groups with alkali yields a series of phyllins, the last oxygen free one being aetiophyllin, which carries the Mg group intact, has the composition $C_{31}H_{34}N_4Mg$, and hypothetically the structural nucleus



Acid treatment of the phyllins removes the Mg and yields the porphyrins, aetioporphyrin ($C_{31}H_{36}N_4$), the equivalent of aetiophyllin, being the last of the series.

Treatment of chlorophyll with acids attacks another group, no saponification takes place, and a waxlike Mg-free chlorophyll derivative, phaeophytin, is obtained. Its solution differs in color from that of chlorophyll, but the green returns upon introduction of a metal, such as Zn, Cu, or Mg. Saponification of phaeophytin with alkali yields nitrogen-containing acids and a nitrogen-free alcohol phytol ($C_{30}H_{39}OH$), which may constitute one-third of the weight of chlorophyll and varies widely in amount in various chlorophylls. Chlorophyll is associated with an enzyme, chlorophyllase, active in alcoholic media, which causes an alcoholysis of the pigment and yields the chlorophyllides, the crystalline chlorophyll of the literature, according to the equation

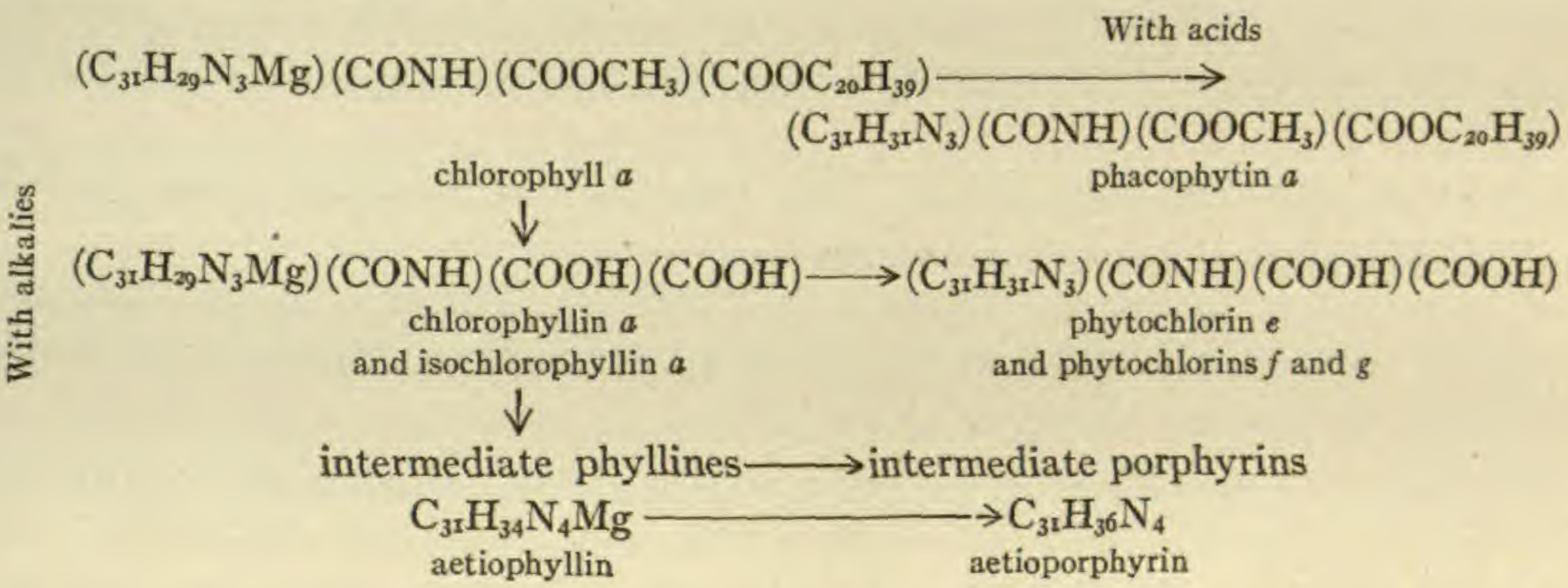
$$\underset{\text{chlorophyll } a}{(C_{32}H_{30}ON_4Mg)\ (COOCOC_3)\ (COOC_{20}H_{39})} + C_2H_5OH \rightarrow \underset{\text{phytol}}{C_{20}H_{39}OH} +$$

$$\underset{\text{ethyl chlorophyllid } a}{(C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOC_2H_5)}.$$

Plants can be divided into chlorophyllase-rich, such as *Heracleum Spondylium*, and chlorophyll-rich, such as *Urtica* sp.

The substance originally named phaeophytin by WILLSTÄTTER always yielded two distinct groups of decomposition products, one olive green in solution, the phytochlorins, the other red, the phytorhodins. Phytochlorin *e*, typical of the former series, is a tricarboxylic acid with two free carboxyl groups and one bound as a lactam; while phytorhodin *g* is a tetracarboxylic acid, only two or three of the carboxyls being in the free state. They are separated by a method based upon their different solubilities in ether and hydrochloric acid. Determination of the molecular weight of these substances, their occurrence in definite weight proportions, and their non-interconvertibility convinced WILLSTÄTTER that phytochlorin and phytorhodin arise from separate and distinct phaeophytins (*a* and *b* respectively), and that these in turn are derived from two distinct chlorophylls (chlorophyll *a* and *b*). These chlorophylls are obtainable in microcrystalline form and differ somewhat in their solubilities in various reagents.

The extraction of the chlorophylls can be carried out with dried or fresh material and is based on the use of solvents containing a moderate content of water, the latter forming salt solutions with some of the cell contents which alter the condition of the chlorophyll and render it soluble in the organic solvent. Separation and purification of chlorophyll *a* and *b*, carotin, and xanthophyll is based on their differing solubilities in solvents. Little is known of the chemistry of the carotinoids. They are hydrocarbons, both give non-fluorescent solutions, stable toward alkali but easily dissociated by acids, and both undergo auto-oxidation. Carotin and xanthophyll have the formulae $C_{40}H_{56}$ and $C_{40}H_{56}O_2$ respectively.



$$\begin{tikzcd}
\underset{\text{chlorophyll } a}{(C_{31}H_{29}N_3Mg)(CONH)(COOCH_3)(COOC_{20}H_{39})} \arrow[r, "\text{With acids}"] \arrow[d, "\text{With alkalies}"'] & \underset{\text{phacophytin } a}{(C_{31}H_{31}N_3)(CONH)(COOCH_3)(COOC_{20}H_{39})} \\
\underset{\substack{\text{chlorophyllin } a \\ \text{and isochlorophyllin } a}}{(C_{31}H_{29}N_3Mg)(CONH)(COOH)(COOH)} \arrow[r] \arrow[d] & \underset{\substack{\text{phytochlorin } e \\ \text{and phytochlorins } f \text{ and } g}}{(C_{31}H_{31}N_3)(CONH)(COOH)(COOH)} \\
\underset{\substack{C_{31}H_{34}N_4Mg \\ \text{aetiophyllin}}}{\text{intermediate phyllines}} \arrow[r] & \underset{\substack{C_{31}H_{36}N_4 \\ \text{aetioporphyrin}}}{\text{intermediate porphyrins}}
\end{tikzcd}$$

WILLSTÄTTER and STOLL reinvestigated the question of the relation of chlorophyll content to photosynthetic activity of leaves in normal, chlorophyll-poor, vernal, and autumnal leaves. They used a modified Kreussler method and determined the assimilation number, $\frac{CO_2 \text{ assimilated in 1 hour (in gm.).}}{\text{chlorophyll (in gm.)}}$ They find not only that chlorophyll is absolutely essential for photosynthesis, but also that it acts in conjunction with a second indispensable inner factor, an enzyme, which has its seat in the contact zone between plastid and plasma. If the chlorophyll suffers more because of external factors, the assimilation number rises; if the enzyme suffers more, the number falls. The enzyme occurs in greatest proportion in juvenile, greening, etiolated leaves and in yellowish green leaves, and in all of these illumination is the limiting factor, while temperature is the limiting factor in chlorophyll-rich leaves in the photosynthetic process. Conclusions as to various steps in assimilation are drawn from the behavior of isolated chlorophyll as well as non-illuminated leaves toward carbon dioxide. Chlorophyll is not constantly decomposed and regenerated, as asserted by STOKES, neither do chlorophyll *a* and *b*, nor carotin and xanthophyll change into each other to any extent, as assumed originally by WILLSTÄTTER. The ratio $Q\frac{a}{b}=3$ is about constant under all conditions, though the ratio $Q\frac{c}{x}$ falls during the course of extended experiments. A striking difference was noted in the behavior of chlorophyll towards CO_2 when in true solution in ether or alcohol, and in colloidal solution, as watery acetone. The former is exceedingly stable toward alkali and acids, while the latter is stable toward alkali, but so sensitive to acids that CO_2 splits it readily into $Mg(HCO_3)_2$ and phaeophytin ($C_{55}H_{72}O_5N_4Mg + 2CO_2 + 2H_2O \rightarrow Mg(HCO_3)_2 + C_{55}H_{74}O_5N_4$). Such a solution loses half of its metal in 4 days when treated with 5 per cent CO_2. Before the Mg is displaced, however, an intermediary dissociable compound is formed, maximum in quantity at low temperatures. The time factor is much more active in the chlorophyll *a* than in the other compound. Leaves are thus endowed with a mechanism for storing CO_2 much as the blood is. Instead of the albumin of the serum holding the CO_2, however, it is possible that carbamino compounds of amino acids or proteins are involved here. This dissociable CO_2 chlorophyll compound may take up the light energy and give an isomer of greater energy content which is suitable for spontaneous decomposition. This may be the substance that is split enzymatically.

Interesting in this connection is a paper by SPOEHR.[2] In an excellent critical review of the literature he points out certain facts that seem to weigh heavily against the theory of formaldehyde as an intermediate product in photosynthesis and in favor of formic acid as such. Discussing the work of LOEB with CO_2 and silent discharge, USHER and PRIESTLY, BERTHELOT and GUADECHOW, and STOCKLASA with CO_2 and ultraviolet light, he concludes that we have no clear record of a reduction of carbonic acid on its salts to formaldehyde which is applicable to the process going on in the plant leaf or is compatible with conditions met in the leaf. NEF's success in condensing formaldehyde to sugar might be of significance, he believes, if an enzyme could be substituted for the strong alkali and high temperature used. WILLSTÄTTER's work shows clearly that enzymes and the colloidal state of chlorophyll and the plasma are important factors in this process. It also shows that the greatest progress in physiology is to be made by work that combines chemical and physiological technique judiciously. SPOEHR finds that while formaldehyde yields sugars in light or dark only in the presence of relatively strong bases, such as do not exist in the plant, CO_2 in aqueous solution in ultraviolet light produces formic acid as the only product, and exposure of this to sunlight or ultraviolet light gives a substance having several properties and the nutritive value of sugars. He believes formic acid may well be the intermediary product of photosynthesis, as suggested by ENGELMANN, especially since organic acids in sunlight give rise to formaldehyde and vice versa. The formaldehyde reported in green leaves, as shown by WAGER and WARNER, may be due to the activity of light on chlorophyll and other plant substances. SPOEHR feels that the findings of GRAFE, BOKORNY, and BAKER, that formaldehyde is assimilated by the green leaf only in presence of sunlight, introduces an entirely new

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[2] SPOEHR, H. A., The theories of photosynthesis in the light of some new facts. Plant World **19**:1–16. 1916.

factor into the BAEYER hypothesis, to which altogether too much attention has been paid by chemists and physiologists.

The reviewer feels that the results of the chemist WILLSTÄTTER, who, to paraphrase a statement of JOHANNSEN'S, carries on his physiological studies not as chemistry, but with chemistry, deserve the notice of all physiologists and chemists, and are full of promise of a new attack upon the most fundamental material problem of mankind, namely, the increase of the food supply of the world.—G. K. K. LINK.

The bubble method in photosynthesis.—KNIEP[3] has made a critical study of the value of the bubble method for comparing the rate of photosynthesis in water plants under various conditions. From his work it is evident that the method must be applied with great caution. Part I deals with the percentage of oxygen in eliminated gas bubbles. This percentage rises as the rate of bubbling increases, but not in direct proportion. If one were to find the total oxygen production due to photosynthesis, he would need to determine (1) the volume of the eliminated gas with its percentage of oxygen, and (2) the amount of oxygen that diffused into the bathing water. For the analysis of eliminated gas KNIEP used Krogh's apparatus, by which the percentage of oxygen and carbon dioxide can be determined in very small samples of 4–6 mm.[3] The amount of oxygen diffusing into the bathing water was determined before and after a period of photosynthesis by WINKLER'S titration method. The percentage of oxygen in the eliminated gas varied from 22.8 per cent in slow photosynthesis to 45 per cent in rapid photosynthesis.

The whole matter is complex. The oxygen production in photosynthesis not only increases the volume of gas in the intercellular spaces and leads to elimination of gas from the cut surface of the stem, but it increases the oxygen percentage in these spaces and thereby steepens or sets up diffusion gradients (falling gradient of oxygen from intercellular spaces to bathing water and of nitrogen in the opposite direction). Carbon dioxide diffusion gradients due to its consumption must be considered also. Hence the amount of gas given off as bubbles and its richness in oxygen and other gases is the resultant of all these processes acting together, a given set of conditions giving one equilibrium and a change in conditions gradually leading to another set of conditions.

Part II presents the influence of water movement upon the bubble outgo. With 1 per cent $KHCO_3$ in distilled water as the bathing medium, water movement temporarily stops the bubble outgo. With tap water (supersaturated) the outgo of bubbles increases and continues in darkness even in dead plants. This behavior is all explained on the basis of the effect of the water movement upon the gas gradients. On the basis of these results KNIEP pointed out the

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[3] KNIEP, HANS, Über den Gasaustausch der Wasserpflanzen. Ein Beiträge zur Kutik der Blazenzählmethode. Jahrb. Wiss. Bot. 56:460–509. 1915. PFEFFER'S Festschrift.

probable errors in the use of the method for studying the rate of photosynthesis as affected by carbon dioxide concentration.

Part III deals with the effect of short periods of darkening of varying length upon the time needed for resumption of bubbling after reillumination. The longer the darkening, within certain limits, the longer the time required for bubble resumption. This is explained by diffusion (during darkening, as determined by the gas gradients) contracting the gas volume in the intracellular spaces and thereby drawing water into these spaces. The longer the period of darkening the greater the contraction. On the basis of these results KNIEP develops in part IV a method for determining the minimum light intensity necessary to produce oxygen elimination into the intracellular spaces, or to give an excess of photosynthetic gas exchange over the respiratory exchange. It is 2.8 candle meters. This paper deals mainly with methods that are to be used later in answering various questions in photosynthesis.—WILLIAM CROCKER.

Life histories in Laminariaceae.—In his recent investigation of *Saccorhiza bulbosa*, SAUVAGEAU[4] found that germinating zoospores behave quite at variance with results reported by other workers. Instead of a protonema, as found by WILLIAMS,[5] DREW,[6] and KILLIAN,[7] he found that the erect plant is developed directly, and also[8] that *Saccorhiza* shows heterogamy and distinct alternation of generations.

After swimming about for a time, the zoospore comes to rest, rounds out, and develops a membrane, becoming an "embryospore." Subsequent behavior determines whether the embryospore is to be a male or female gametophyte. In the latter case the embryospore doubles its diameter, multiplies its chromatophores without division of the nucleus, and in a short time a tube puts out, of about the same diameter as the embryospore, though its length may greatly exceed the diameter; into the tip of this tube the entire contents of the embryospore migrate. Finally the membrane is ruptured and there escapes a single naked uninucleate mass, slightly elongated in form, which remains attached to the mouth of the empty embryospore tube. This naked mass, which is the

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[4] SAUVAGEAU, C., Sur le développement et la biologie d'une Laminaire (*Saccorhiza bulbosa*). Compt. Rend. Acad. Sci. **160**:445–448. 1915; **161**:740–742. 1915.

[5] WILLIAMS, J. L., Germination of the zoospore in the Laminariaceae. Rept. British Ass. Adv. Sci. (Bradford) 1900.

———, The zoospores of the Laminariaceae and their germination. Rept. British Ass. Adv. Sci. (Dundee) 1912.

[6] DREW, G. H., The reproduction and early development of *Laminaria digitata* and *L. saccharina*. Ann. Botany **24**:177–190. 1910.

[7] KILLIAN, K., Beiträge zur Kenntnis der Laminarien. Zeitschr. Bot. **3**:433–494. 1911.

[8] SAUVAGEAU, C., Sur la sexualité hétérogamique d'une Laminaire (*Saccorhiza bulbosa*). Compt. Rend. Acad. Sci. **161**:796–799. 1915.

egg, soon develops a wall about itself, separating it from the abandoned tube. Sometimes the embryospore nucleus divides, giving two cells; then the anterior cell behaves like an ordinary embryospore; or if a monosiphonous filament of several uninucleate cells is formed, the anterior cell again functions. Only in weak and poorly developed cultures did SAUVAGEAU observe erect branches from the embryospore; in these cases each erect branch behaves in the manner described.

If the embryospore is to develop a male gametophyte, its diameter is increased little or none, elongation is very rapid, but the entire structure remains very minute. The antheridia develop as rounded colorless buds, each enclosing a single sperm, which after its escape is seen to be motile, colorless, and laterally biciliate.

Fertilization was not observed, but SAUVAGEAU assumes that it occurs and that from the fertilized egg the plantlet or sporophyte develops immediately. His account from this stage on differs in few essentials from the accounts of other workers, the stipe and blade being differentiated at a very early stage.

It is to be regretted that at the critical stages details are not more clearly given. However, it is evident from this account, as also from that of DREW, that there are certain phases in the life history of the Laminariaceae which are in need of critical investigation, especially from a cytological point of view. DREW concluded that as a result of conjugation of isogamous gametes a filament (the sporophyte) developed which in turn gave rise to the plantlet (the gametophyte). SAUVAGEAU concludes that the gametophyte comes very early in the cycle; in fact, it is practically a walled zoospore, and the plantlet which is produced as the result of fertilization of the egg is the sporophyte. It would seem that a careful investigation might clear up the haziness which still remains about these phases in the life history.—MABEL L. ROE.

Tannins.—MOELLER[9] has noted peptization phenomena in tannin solutions, and believes that they form the basis of the process of tanning. A tannin extract contains two sorts of substances: (1) a tannin that is soluble in water, and (2) an accompanying substance, a gel, which is insoluble in water. With pyrogallol tannins this accompanying substance is called ellagic acid, and with pyrocatechin tannins it is called phlobaphene. The ellagic acid and phlobaphene, while insoluble in water, are soluble in the tannin solution; that is, the tannin peptizes the ellagic acid gel and the phlobaphene gel, the tannin being called the peptizator and the gel the peptized substance. In the process of tanning the hide adsorbs the peptizator and the gel coagulates out, surrounds the fibers of the hide, and leather is formed. He says a true solution cannot tan a hide, but only a peptized solution, and that all tanning solutions

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[9] MOELLER, W., Die Peptisationserscheinungen in Gerbstofflösungen. Kolloid. Zeitsch. 16:69–76. 1915.

contain a gel held in solution as a hydrosol by the action of a peptizator. Purified tannin solutions do not tan, and the more "impure" the solution the better the tanning effect. Watery tanning solutions on standing in the air undergo changes that increase their tanning value, owing to the formation of an insoluble product that becomes peptized.

MOELLER finds a similar situation exists in the artificially prepared tanning solutions. In chinon tanning solutions the chinon takes the place of a peptizator, while hydrochinon is the peptized gel. In the mineral tanning solutions an insoluble metallic hydroxide is peptized into the solution condition by the peptizing action of the acid salt solution. According to MOELLER'S view, the properties of a tanning extract depend upon the equilibrium between the peptizator and the peptized substance, and the character of the leather depends upon the nature and quantity of the peptized gel that is taken up by the hide fibers.—F. E. DENNY.

Taxonomic notes.—Miss GILKEY[10] has published a revision of the Tuberales of California, preceded by an account of the distribution of truffles in California, their economic importance, and their morphology and phylogeny. Accompanied by very full discussion, 11 genera are presented, including 32 species, the largest genus being *Tuber*, with 12 species. A new genus (*Hydnotryopsis*) and 15 new species are described, distributed among the following genera: *Hydnocystis*, *Genea* (3), *Hydnotrya*, *Tuber* (6), *Piersonia*, *Geopora* (2), and *Hydnotryopsis*.

WEST[11] has described a new genus of mycorrhizal fungi associated with the roots of the various genera of Marattiaceae. He names it *Stigeosporium*, and regards it as of special interest inasmuch as it produces "under natural conditions distinct reproductive bodies within the tissues of the host root."

WILLIAMS[12] has published a list of Peruvian mosses based upon two collections. It includes 71 species, among them 6 new species in the following genera: *Leptodontium*, *Globulina*, *Tortula*, *Grimmia*, *Bryum*, and *Hygrohypnum*.—J. M. C.

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[10] GILKEY, HELEN MARGARET, A revision of the Tuberales of California. Univ. Calif. Publ. Bot. **6**:275–356. *pls. 26–30.* 1916.

[11] WEST, CYRIL, *Stigeosporium Marattiacearum*, gen. et sp. nov. Ann. Botany **30**:357. 1916.

[12] WILLIAMS, R. S., Peruvian mosses. Bull. Torr. Bot. Club **43**:323–334. *pls. 17–20.* 1916.

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SOME RECENT INVESTIGATIONS IN SUGAR-BEET BREEDING

FREDERICK J. PRITCHARD

(WITH FIFTY-ONE FIGURES)

Introduction

This investigation was made for the purpose of ascertaining certain facts and principles which have an important bearing upon methods of breeding. The introduction of more economical methods, based upon correlated characters or upon a better knowledge of scientific principles, would aid materially in the improvement of beets.

The efficiency of breeding methods depends largely upon the soundness of the scientific principles on which they are based. Unfortunately, many principles of breeding are still obscure, and even doubtful principles are employed as the basis of improving or originating a variety. Biologists who believe that improvement results from a gradual accumulation of small variations practice continuous selection, while those who adhere to a belief in improvement by the use of a few exceptional, prepotent individuals work with larger numbers and depend upon the isolation of mutations. In studying the effects of our own methods we have endeavored to determine whether improvement is accomplished through continuous selection, and also whether prepotent plants of exceptional quality occur within the limited range of our cultures.

A sugar-beet variety as ordinarily developed and maintained differs from a variety of other crops in that it consists of a number of families which are preserved from generation to generation by continuous selection. Whenever a family shows deterioration it is discarded, but, as new families are annually added, the number composing the variety is not materially altered.

One of the most costly operations practiced in sugar-beet breeding is the determination of the so-called best roots, either for starting families or as representatives of families already established. This is accomplished by chemically analyzing the individual roots of each family and grading them upon the basis of size, shape, and percentage of sugar. The few best roots are saved to preserve the family and continue the process of improvement, while the remainder are thrown into a mixed lot and used for growing stock seed. A fairly good conception of the number of analyses made in commercial work of this kind is afforded by the records of a single European beet-seed company who analyze over 300,000 individual beets a year. The cost of making these analyses forms a large part of their operating expenses. Yet, aside from the theory that "like begets like," there seems to be little evidence that these highly selected roots are better for breeding purposes than the discards. It has apparently been assumed upon theoretical grounds that a high percentage of sugar tends to be transmitted, and therefore is the most important quality of a mother root without regard to the possibility of its being a fluctuating character and nowise indicative of the average quality of the plant's progeny.

Real differences between individuals and varieties are often obscured by variations caused by irregularities of the soil, which makes it difficult to distinguish hereditary differences from temporary differences and thus complicates the question of methods. The comparative efficiency of different methods has been treated somewhat in detail in another paper,[1] but the effect of environment on the behavior of consecutive check and progeny rows will be graphically illustrated here, as it presents a number of interesting phenomena which have an important bearing on the results of this investigation.

[1] A contribution to the use of checks and repeated plantings in variety tests.

While only a few of the beet breeders' problems have been outlined, they are fundamentally important and need investigation for the purpose of developing more economical methods.

Character of material employed

The material employed consisted of (1) an American variety of sugar-beets, known as Morrison's Kleinwanzleben; (2) an unnamed variety originated at Madison, Wisconsin, in 1912 by making selections from 11 foreign varieties and designated for convenience in reference as Madison Original Selections; (3) 5 South Dakota varieties bred for several years at Brookings, South Dakota; and (4) an old, well-established European variety, Kleinwanzleben's Original.

Morrison's Kleinwanzleben is composed of a large number of separate strains or families which have been preserved and improved by continuous selection. Ten years were devoted to this work at Fairfield, Washington, and 3 years at Madison, Wisconsin. The records obtained from these families and their individual components have furnished all the progeny row data not otherwise accounted for in the tables and figures.

Madison Original Selections are composed of beet families which originated from roots containing 18–26 per cent sugar. As no records of their performance were available until 1914, they have contributed few data to this paper.

The 5 South Dakota varieties used in our experiments at Brookings were originated by selecting rich roots from foreign stock and making subsequent tests and selections primarily upon the basis of a high percentage of sugar. About an equal number of roots were taken at random from each of these varieties to compile the data from this material, which are used in table I and graph A of fig. 51.

Kleinwanzleben's Original was the variety planted in the check rows. It is one of the most uniform, most highly bred, and most widely used varieties of sugar-beets on the market. The seed was taken from an unopened bag, sealed by the Rabethge & Giesecke Company at their plant, in order to increase the probability of its all being grown in one locality under similar environmental conditions, and thoroughly mixed before being used, to enhance its uniformity.

Investigation

CONTROL OF CROSS-FERTILIZATION.—Undesirable cross-fertilization was avoided by planting the seed-beets of approximately equal qualities in isolated groups, a practice commonly followed in beet breeding. At Fairfield the quality was determined from the percentage of sugar, but at Madison a somewhat different method of classifying the roots was employed. As small beets are richer on the average than large beets, the regression coefficient was determined between weight and percentage of sugar and a correction made for size. Roots of equivalent value, as determined from both size and percentage of sugar, were then planted together at distances of 40–80 rods from other groups. However, at Brookings all the selected seed-beets of each variety were planted in a group and allowed to cross-fertilize with one another but not with beets of another group.

CHEMICAL CONTROL.—All the chemical work was done by experienced sugar chemists, the personnel consisting of JOSEPH F. REED at Fairfield, GUY YOUNGBERG at Brookings, and W. B. CLARK and assistants at Madison.

The sugar determinations of beet families were made at Fairfield by collecting roots at regular intervals in the row, grinding them to a fine pulp, and making composite analyses of the samples. A somewhat different method of procedure was followed at Madison. The quantity of sugar was first determined for each individual root and the sum of the quantities was then divided by the total weight of roots.

FIELD CONDITIONS UNDER WHICH THE MATERIAL WAS GROWN.—The experimental fields were fairly uniform with respect to soil-type, fertility, drainage, and previous cropping, and all field operations, as plowing, planting, thinning, cultivating, etc., were always performed in as short a time as circumstances would permit, in order to prevent the introduction of unequal factors.

SELECTION OF FOUNDATION STOCK.—The choosing of a variety as foundation stock often has as much to do with the improvement of a crop as subsequent selections. Sugar-beet varieties are seldom distinguishable from one another morphologically, however, and as they have nearly the same descent, some doubt may be expressed regarding the existence of real variety differences. In order to determine the truth of this hypothesis, 78 families of Morrison's Kleinwanzleben,[2] which made the best records for percentage and yield of sugar at Madison in 1912, and 52 families of Madison Original Selections were used in a comparative merit test in 1914.

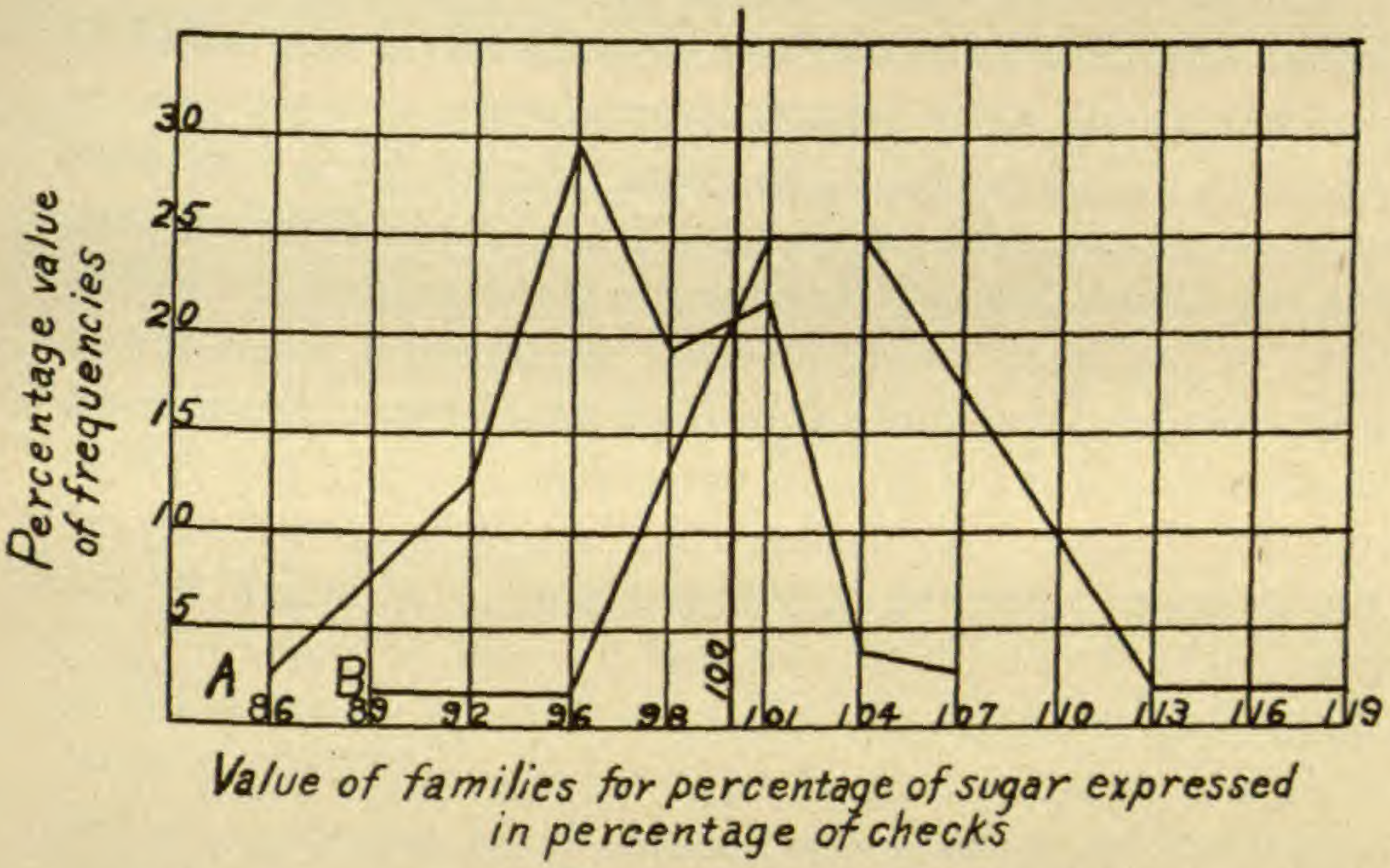

FIG. 1—Comparison of two kinds of sugar-beet material used as foundation stock.

A progeny row of each family (seed of a single beet) was planted between two check rows of Kleinwanzleben's Original. When the beets were harvested, each progeny row was compared with the average of its two contiguous checks by giving the latter a value of 100 and computing the value of the progeny row upon this basis. In fig. 1 the vertical line represents the values of the check rows for percentage of sugar; the frequency polygons *A* and *B*, Morrison's Kleinwanzleben and Madison Original Selections, respectively. The frequencies were reduced to percentages by

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[2] In 1912 these families were grown from seed of Fairfield roots containing 20–22 per cent sugar.

dividing each class by the total number of variates in the population; hence the graphs are comparable.

Fig. 1 shows the superiority of Madison Original Selections over Morrison's Kleinwanzleben at Madison, where the percentage of sugar is normally low and a higher percentage is the chief desideratum. Hence the foreign varieties from which the Madison Original Selections were made are better as foundation stock at Madison than the variety Morrison's Kleinwanzleben. One or two plantings of each variety would hardly suffice to show this difference, but the large number of replications used were sufficient to establish it with a fair degree of certainty.

Morrison's Kleinwanzleben has been selected for a high yield of sugar, and in tests with foreign varieties in several different localities in the United States it has usually stood preeminent in this respect. In a 5 years' test at Fairfield, Washington, where it was bred for 10 years, it was equal to Kleinwanzleben's Original and better than 11 other varieties in percentage of sugar and ranked first in yield of sugar. At Madison, however, it is not well adapted to the breeders' needs as foundation stock. Although it was superior to Kleinwanzleben's Original in yield of sugar in 1913 (cf. fig. 30), it was inferior to it in 1912. It was relatively low in percentage of sugar in comparison to the checks (KWO) in 1912, and continued to maintain this position in its second year's test after all the supposedly poor families had been eliminated.

SELECTION OF MOTHER ROOTS.—The selection of mother roots for the purpose of starting a new family of beets or improving one already established is usually made upon the basis of size, shape, percentage of sugar, and relative freedom from mineral substances which interfere with the extraction of sugar. The coefficient of purity is not usually determined for breeding purposes, however, as only roots containing a high percentage of sugar are saved and they are almost invariably characterized by high purity. As size and shape are distinguished by physical selection, percentage of sugar is the only character determined chemically.

VARIABILITY OF MATERIAL FROM WHICH MOTHER ROOTS WERE SELECTED.—The variability of beet roots in percentage and quantity of sugar is ample for selection, as may be seen in table I.

The average variability of the roots as shown by the standard deviations was 1.25–1.59 per cent sugar and 19.28–37.99 grams of sugar. Measured by the coefficient of variability, this amounts to 6–12 per cent of the mean for percentage of sugar and 18–50 per cent for quantity of sugar, which are fairly large differences for averages.

TABLE I

VARIABILITY OF SUGAR-BEET ROOTS IN PERCENTAGE AND QUANTITY OF SUGAR

Station	Year	Number of roots analyzed	Mean	Standard deviation	Coefficient of variability
Percentage of sugar in roots					
Fairfield......	1907	230	20.15±0.056	1.26±0.039	6.25
“	1909	400	20.37±0.048	1.47±0.035	7.22
“	1910	400	17.34±0.044	1.31±0.031	7.55
“	1910	400	18.76±0.042	1.25±0.029	6.66
Brookings.....	1911	3784	17.67±0.017	1.59±0.012	8.99
Madison......	1912	500	12.86±0.047	1.55±0.033	12.06
“	1913	500	14.24±0.041	1.35±0.029	9.46
Quantity of sugar per root in grams					
Madison......	1912	500	98.46±1.146	37.99±0.810	38.58
“	1913	500	54.02±0.807	26.76±0.570	49.53
Fairfield......	1910	126	104.61±1.191	19.85±0.850	18.97
“	1910	147	104.90±1.077	19.28±0.770	18.38
“	1909	231	113.68±1.134	25.43±0.790	22.37
Brookings.....	1911	3784	79.87±0.266	23.94±0.186	29.97

The range of variation for percentage of sugar in individual beets is not shown in table I, but varies ordinarily from about 8 to 24. Small, immature beets frequently contain less than 8 per cent sugar, and occasionally a root is found which contains more than 24 per cent. We found one beet at Madison which showed 26 per cent sugar in 2 separate analyses. Another small root, so small in fact that it had to be bored lengthwise through the center to obtain a sample of pulp, contained 30 per cent sugar.

RELATIONSHIPS BETWEEN A FEW COMMONLY DETERMINED CHARACTERS OF THE ROOT AND ITS YIELD OF SEED.—A knowledge of the relationship of such characters as root weight and percentage of sugar to seed production is essential to intelligent selection. Very frequently beet roots are separated into selects and discards on small differences. A satisfactory method of predicting seed yields would not only alter these results but would furnish a means of obtaining sufficient seed to make several plantings of each family.

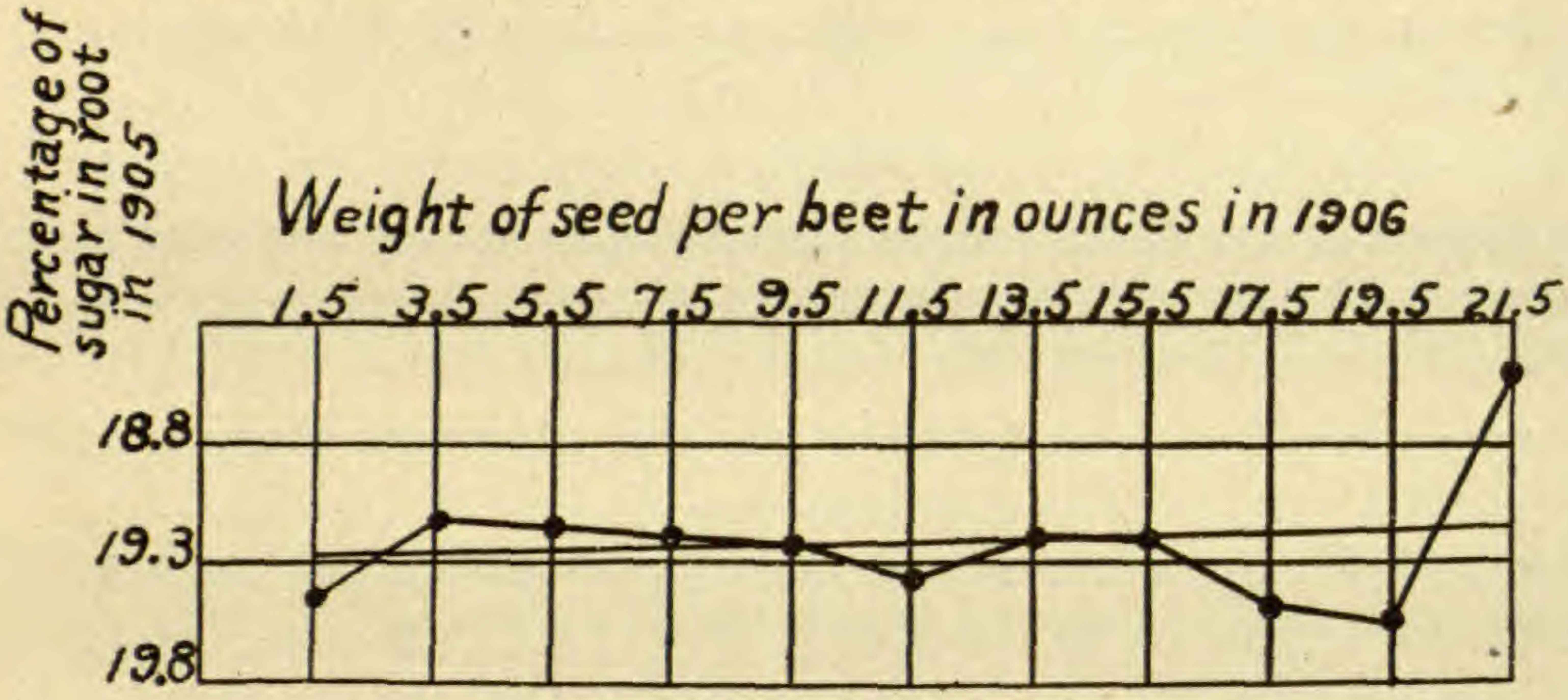

FIG. 2.—To accompany table II

Tables II–XI (summarized on p. 463) and figs. 2–11 show the relationships which obtain between these characters. Some of the tables show slight irregularities, but they are not sufficient to invalidate the results. The effects of the irregularities may be seen in

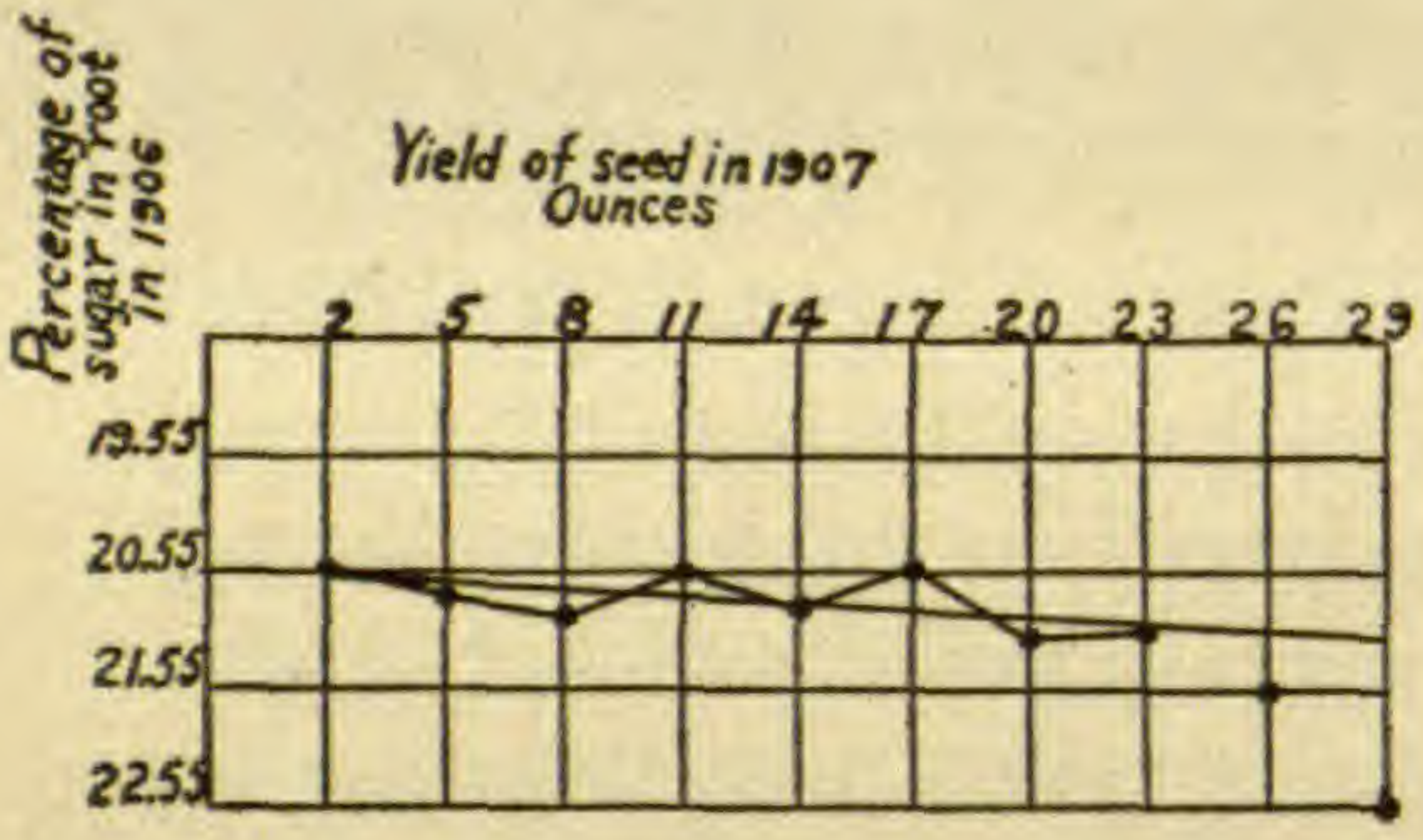

FIG. 3.—To accompany table III

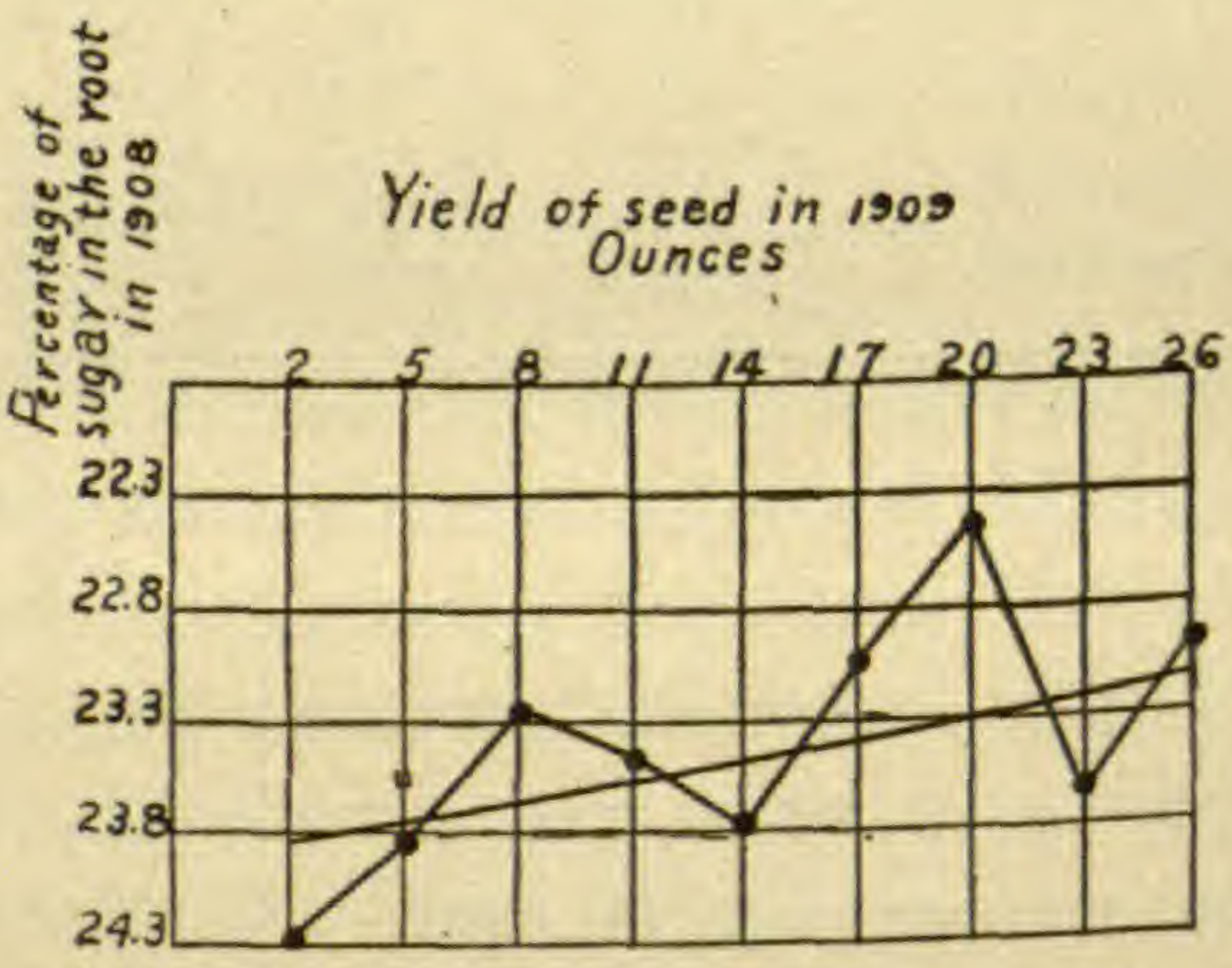

FIG. 4.—To accompany table IV

the deviation of the dots representing the empirical means of the separate classes from the straight line regressions. The divergence is not marked except where the numbers are small. Moreover, the general trends of the broken lines connecting the empirical means agree fairly well in direction with the straight line regressions. The distributions are fairly regular, therefore, and the coefficients are probably reliable.

When due consideration is given to probable errors,[3] the coefficients show no correlation between weight, percentage of sugar, or quantity of sugar of the seed beet and its yield of seed. This refers to beets of ordinary sizes such as are grown for factory use. Very small beets, 0.5–2 inches in diameter (stecklings), form a little less seed per plant than large roots, although they grow to nearly normal size while producing seed. There is little doubt regarding the absence of correlation between these characters, as the tables cover a number of years and the results all lead to the

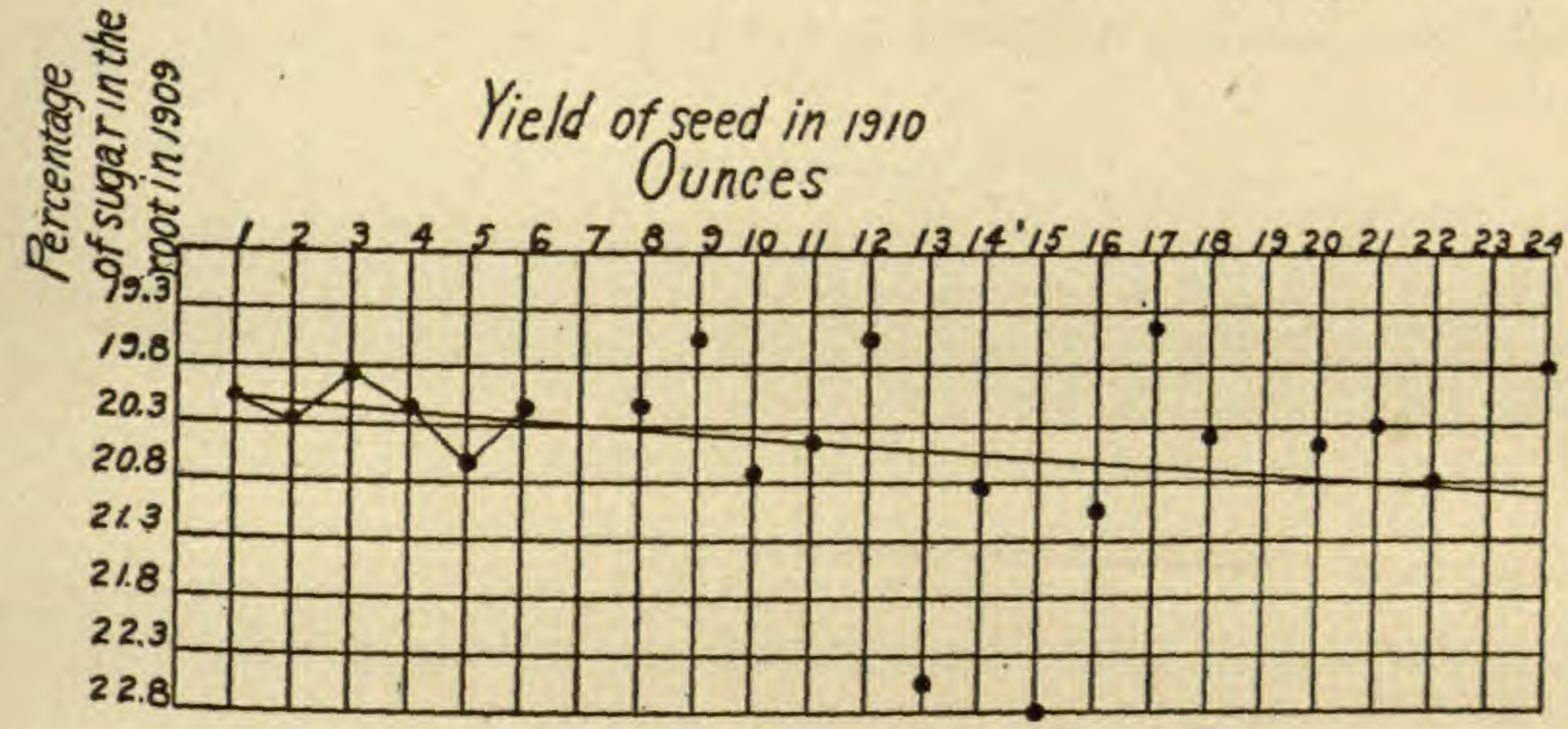

FIG. 5.—To accompany table V

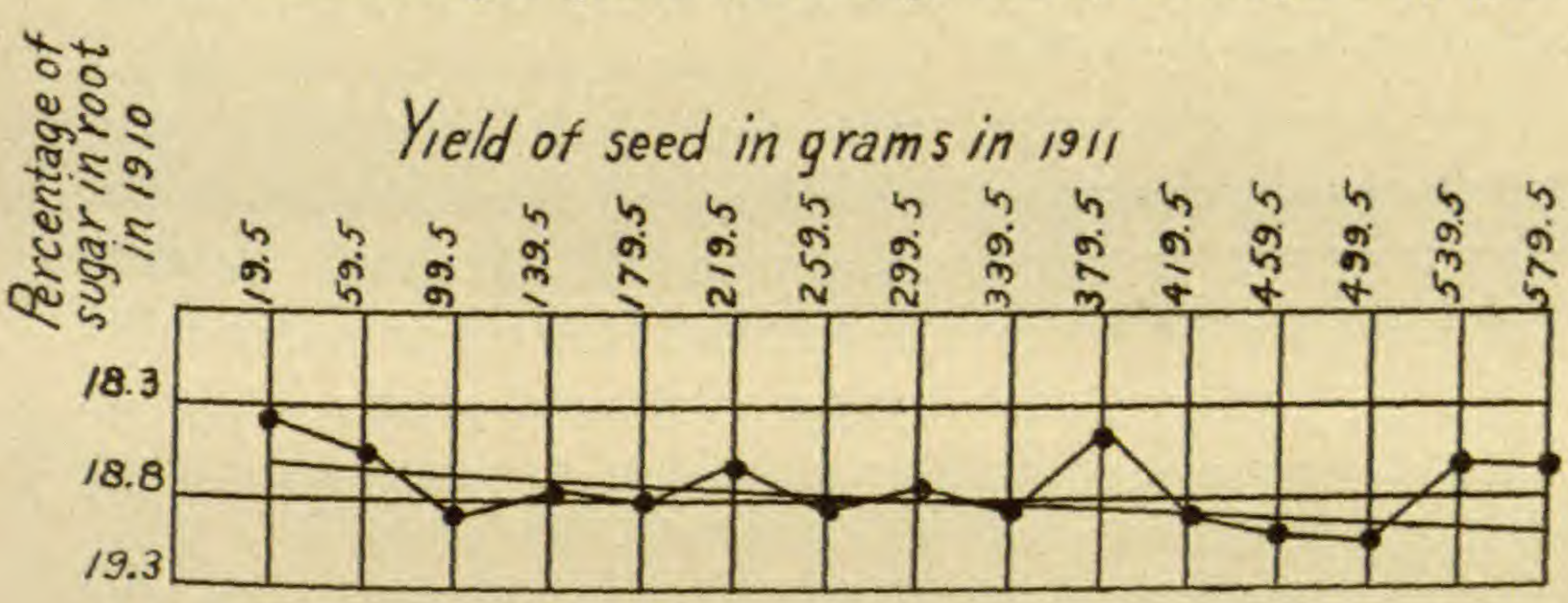

FIG. 6.—To accompany table VI

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[3] The biometrical constant should exceed 3–4 times the probable error to have significance.

same conclusion. However, BARTOS[4] has found that seed production decreases as percentage of sugar increases. His average differences are only 3.3 and 8.6 per cent, however, and in the absence of probable errors we have no means of determining whether they bear any significance.

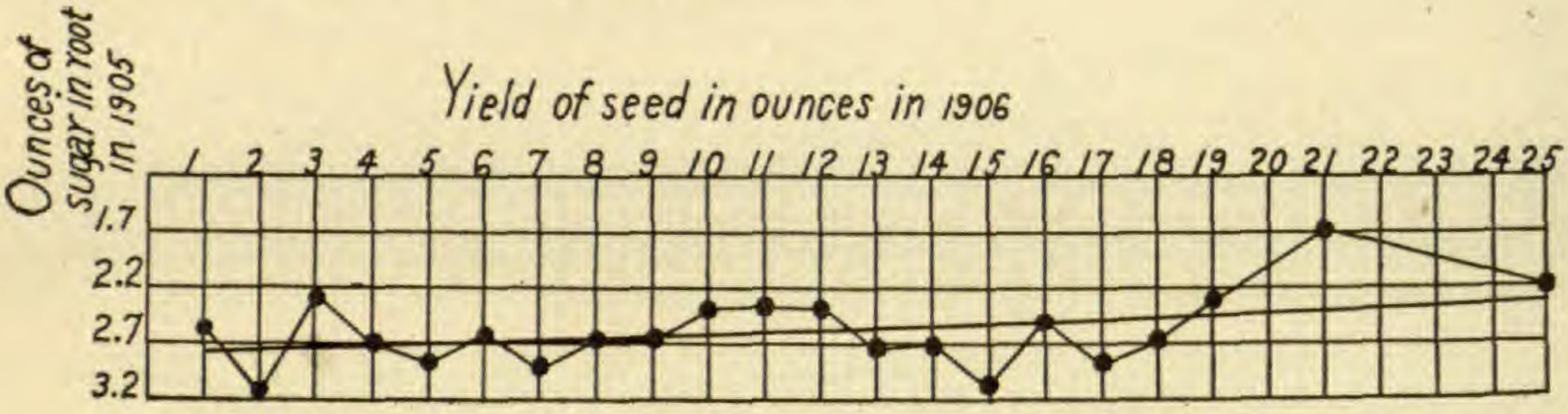

FIG. 7.—To accompany table VII

If percentage of sugar were negatively correlated with seed yield, as BARTOS believes, it would constitute an undesirable relationship. Even positive correlation between percentage of sugar and seed yield, or between weight of root and seed yield, would form a less desirable relationship than the absence of correlation, as high percentage is correlated with low yield and high tonnage with low purity. Maximum sugar production is dependent upon both percentage and tonnage, and consequently cannot run to either extreme. Positive correlation between extractable sugar and seed yield, however, would constitute a very desirable relationship, as selection for maximum sugar production would also tend to increase the seed yield.

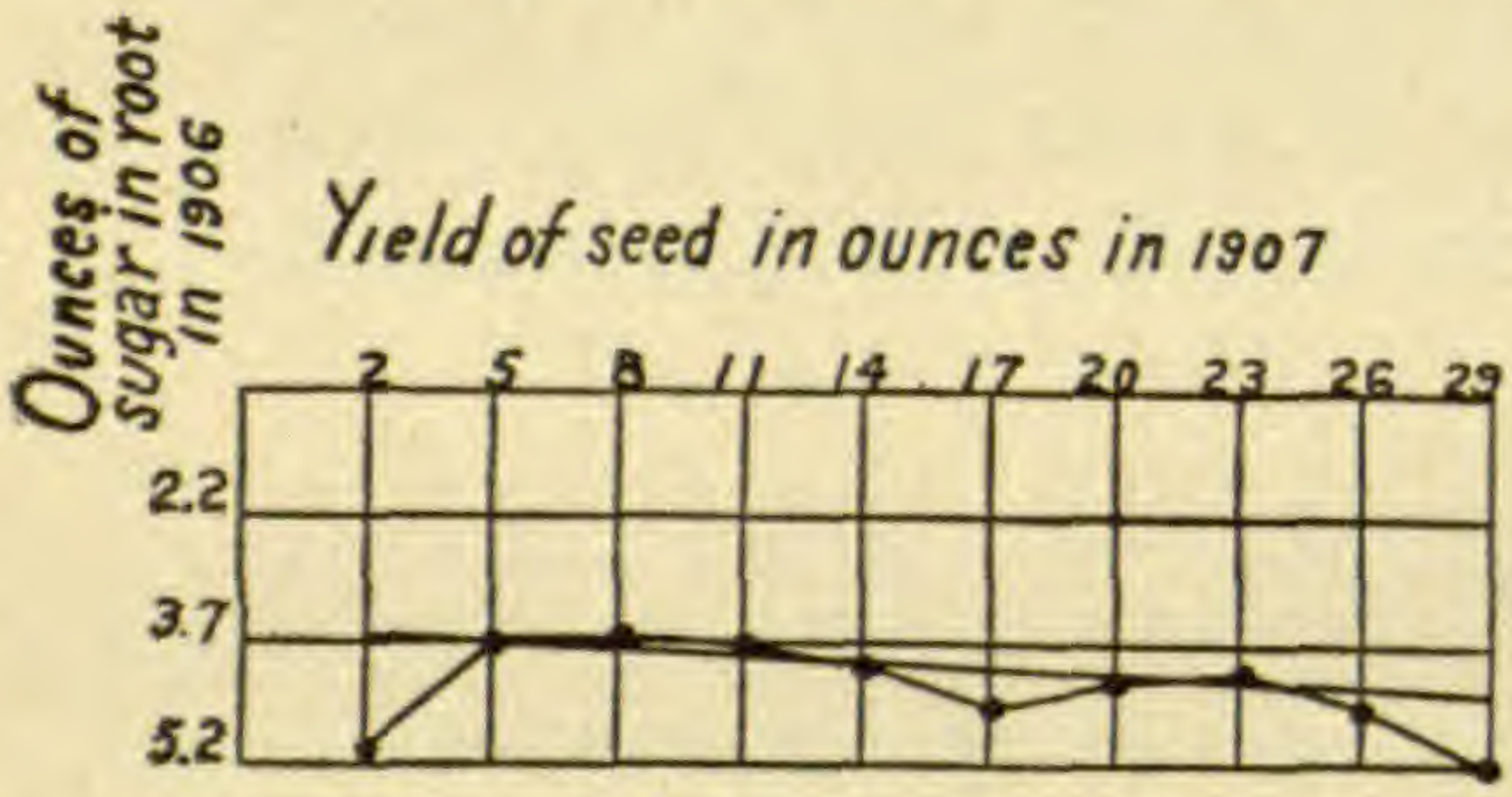

FIG. 8.—To accompany table VIII

RELATIONSHIP BETWEEN A ROOT'S YIELD OF SEED AND THE PERCENTAGE OF SUGAR IN ITS PROGENY.—No distinction between individual sugar-beet plants can safely be made in the seed generation until we learn what relationship exists between a root's yield of seed and the average sugar-producing capacity of its progeny.

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[4] BARTOS, V., Je zucherhaltiger die Rübensorte ist desto kleinere Samenfruchtbarkeit hat sie. Cukrovarnicke Listy, December, 1908; rev. Bl. Zucherrübenbau 16: 93. 1909.

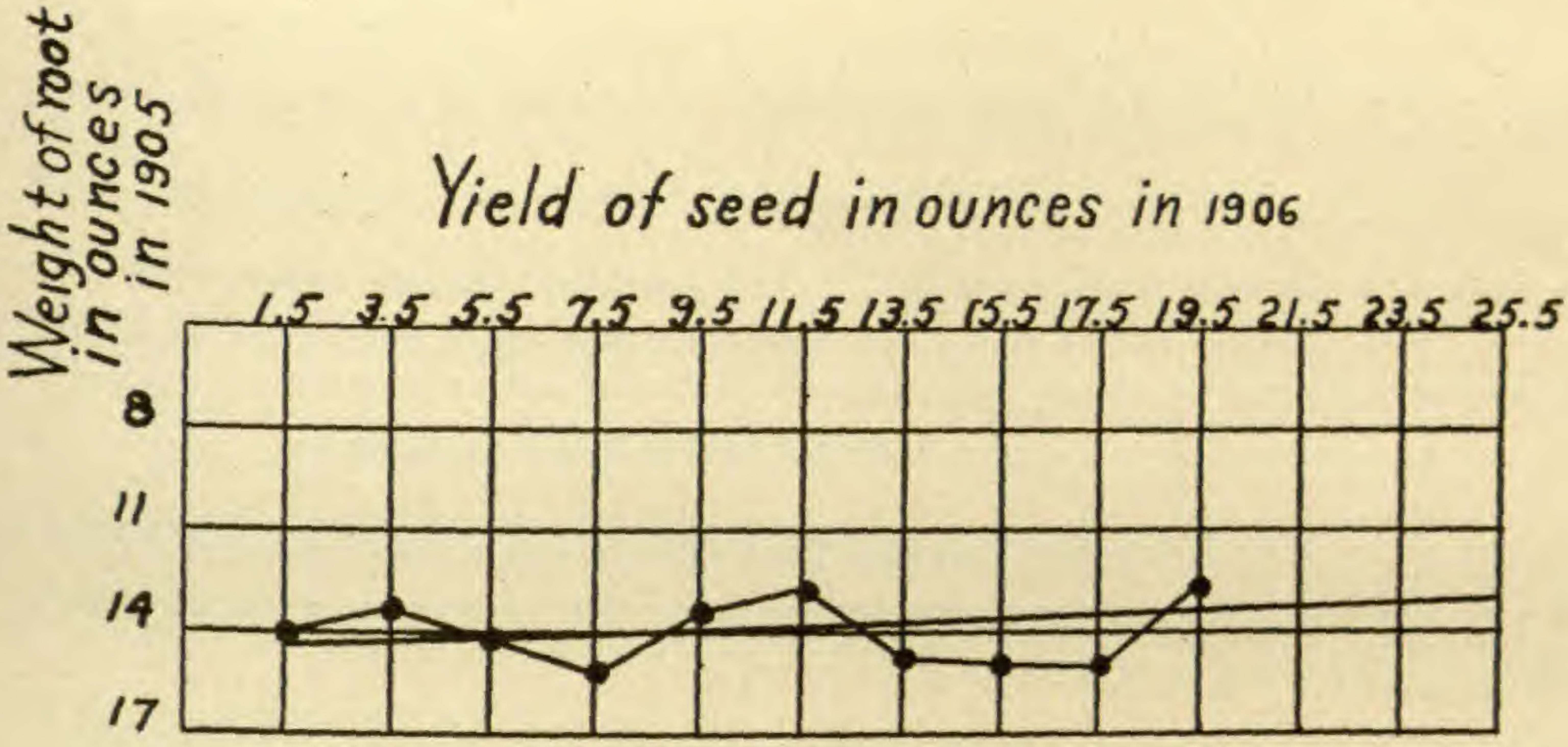

FIG. 9.—To accompany table IX

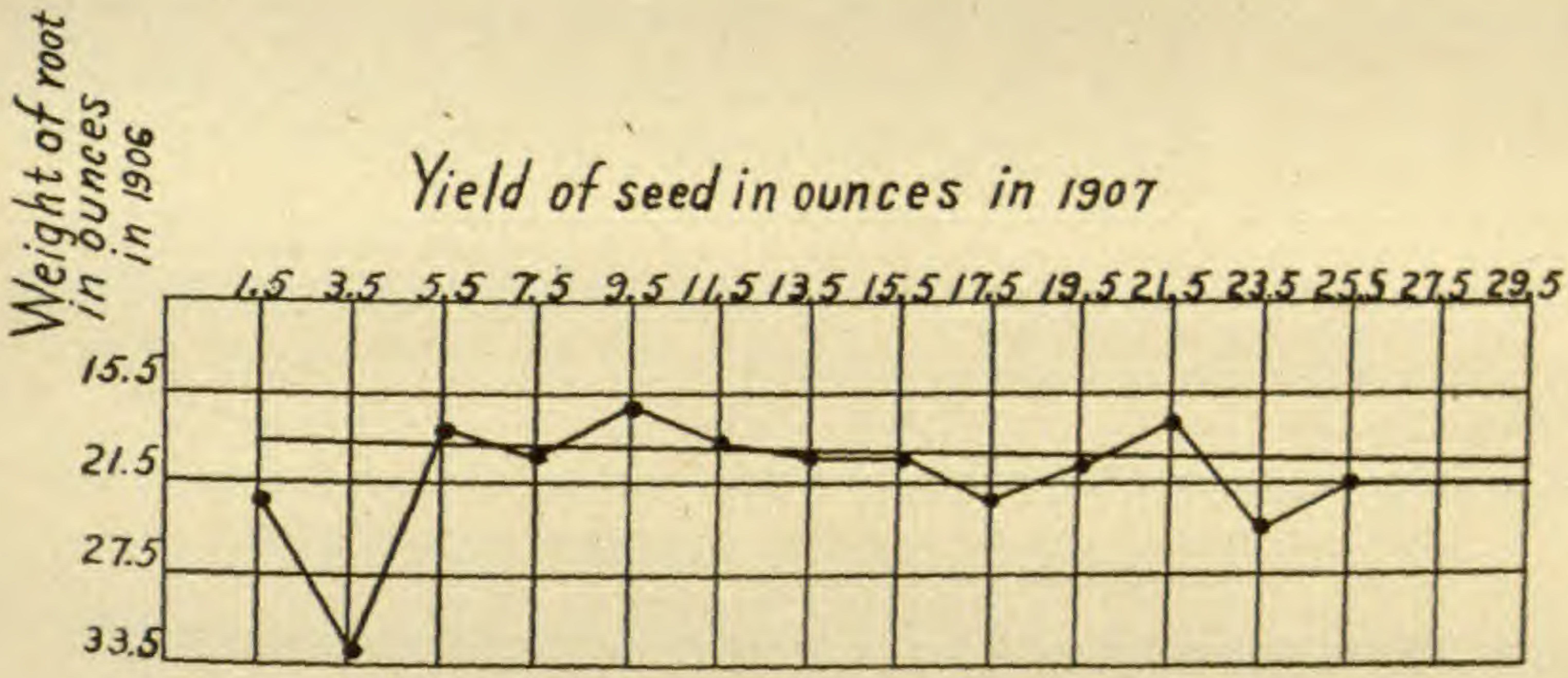

FIG. 10.—To accompany table X

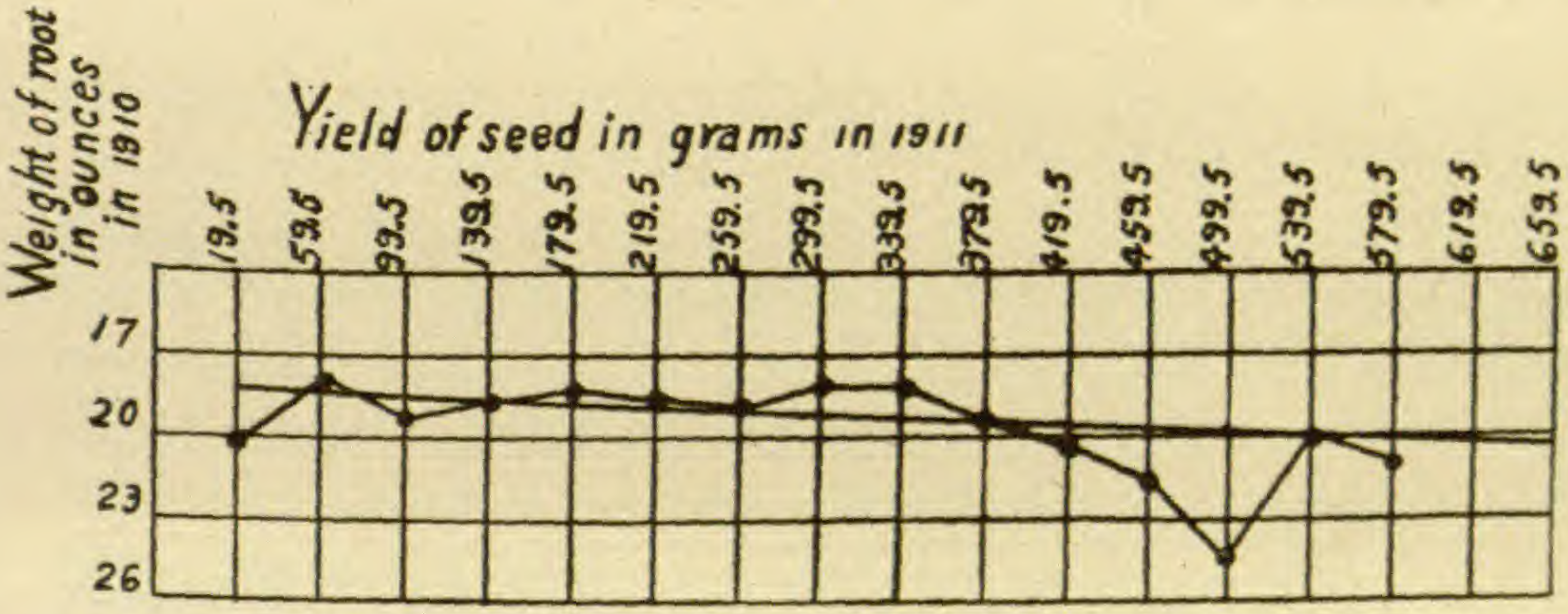

FIG. 11.—To accompany table XI

Tables XII and XIII disclose this relationship. In the experiment from which the records were obtained, the seed of each plant was

TABLE XII

RELATIONSHIP BETWEEN A BEET'S YIELD OF SEED AND THE AVERAGE PERCENTAGE OF SUGAR IN ITS PROGENY; YIELD OF SEED IN 1912

Centers of percentage of sugar classes of 0.5 per cent range in progeny of 1913	Centers of weight classes of 25 gm. range											
	162.5	187.5	212.5	237.5	262.5	287.5	312.5	337.5	362.5	387.5	412.5	Totals
13.0	1*	1	0	0	0	0	0	0	0	0	0	2
13.5	4	1	0	1	0	0	0	0	0	0	0	6
14.0	3	1	1	3	3	0	0	1	0	0	0	12
14.5	9	5	1	1	0	2	0	0	0	0	0	18
15.0	6	2	4	2	1	0	0	0	0	0	0	15
15.5	10	9	5	4	1	0	0	0	0	0	0	29
16.0	3	8	2	3	0	0	1	1	0	0	1	19
16.5	8	3	3	2	1	0	0	0	0	0	0	17
17.0	3	1	0	0	0	0	0	0	0	0	0	4
17.5	1	1	0	0	1	0	0	0	0	0	0	3
Totals	48	32	16	16	7	2	1	2	0	0	1	125

Mean yield of seed, 198.5 gm.; mean percentage of sugar, 15.32; standard deviation in yield of seed, 43.405 gm.; standard deviation in percentage of sugar, 0.996; coefficient of correlation, −0.013.

* Number of beets in the classes in 1912.

TABLE XIII

RELATIONSHIP BETWEEN A BEET'S YIELD OF SEED AND THE AVERAGE VALUE OF ITS PROGENY FOR PERCENTAGE OF SUGAR EXPRESSED IN PERCENTAGE OF THE CHECKS; YIELD OF SEED IN 1912

Percentage of sugar in progeny divided by percentage of sugar in checks in 1913	Centers of weight classes of 25 gm. range											
	162.5	187.5	212.5	237.5	262.5	287.5	312.5	337.5	362.5	387.5	412.5	Totals
83.5	0	1	0	0	0	0	0	0	0	0	0	1
85.5	3*	0	3	0	0	0	0	0	0	0	0	6
87.5	4	7	0	3	1	0	0	0	0	0	0	15
89.5	6	6	3	0	1	0	0	2	0	0	1	19
91.5	8	6	2	4	1	0	1	0	0	0	0	22
93.5	13	4	4	2	2	1	0	0	0	0	0	26
95.5	5	3	2	2	1	0	0	0	0	0	0	13
97.5	3	2	1	4	0	0	0	0	0	0	0	10
99.5	3	1	1	0	1	1	0	0	0	0	0	7
101.5	3	2	0	1	0	0	0	0	0	0	0	6
Totals	48	32	16	16	7	2	1	2	0	0	1	125

Mean yield of seed, 198.5 gm.; mean value of progeny, 92.6 per cent of percentage of sugar contained in their checks; standard deviation in yield of seed, 43.405 gm.; standard deviation in value of progeny 14.07 per cent of percentage of sugar contained in their checks; coefficient of correlation, −0.010.

* Number of beets which produced seed in 1912 and progeny in 1913.

sown in a progeny row between 2 check rows of Kleinwanzleben's Original. The relationship between seed yield and percentage of sugar was determined from the data both with and without the use of checks. In table XII actual percentages of sugar are recorded in the horizontal columns, but in table XIII the values occupying these columns were obtained by dividing the percentage of sugar of each progeny row by the average percentage of sugar of its 2 contiguous checks. As the 2 tables were compiled from the same data they are alike, except that table XII shows the relationship between seed yield and percentage of sugar when no checks were used, and table XIII when every alternate row was employed as a check.

The tables show no correlation between a beet's yield of seed and the average percentage of sugar in its progeny. The application of this fact to sugar-beet breeding is obvious, as extensive selections may be made for freer seed production without danger of sugar deterioration. Moreover, it affords an opportunity to reverse the order of selection by making the chief eliminations in the seed generation and thus greatly reduce the amount of chemical work and increase the effectiveness of the working funds.

TRANSMISSION OF SELECTED QUALITIES OF MOTHER ROOTS.—Mother roots are selected chiefly on the basis of size, shape, and percentage of sugar, because these qualities are desired in the progeny and there is a popular belief that they are inherited, but we really know very little regarding the transmission of such characters. In the early period of beet breeding they appeared to improve through selection, but this was at a time when the material was very variable and full of distinct physiological species. Improvement probably resulted from the isolation of these species, although the selections were made with a view to improving the characters. Today it is quite different. The poorer physiological species have gradually been eliminated and all varieties are now much alike. Moreover, such root characters as size and percentage of sugar—and incidentally this includes total sugar content—are markedly influenced by the environment. Consequently large fluctuations occur which are indistinguishable from hereditary differences. The fluctuations probably characterize the supposedly best beets, in which case the cost of analyzing mother roots is an absolute waste of money. Tables XIV–XXV (summarized on p. 463) and figs. 12–23 show the extent to which we have found weight, percentage of sugar, and total sugar content of the root to be transmitted to the progeny.

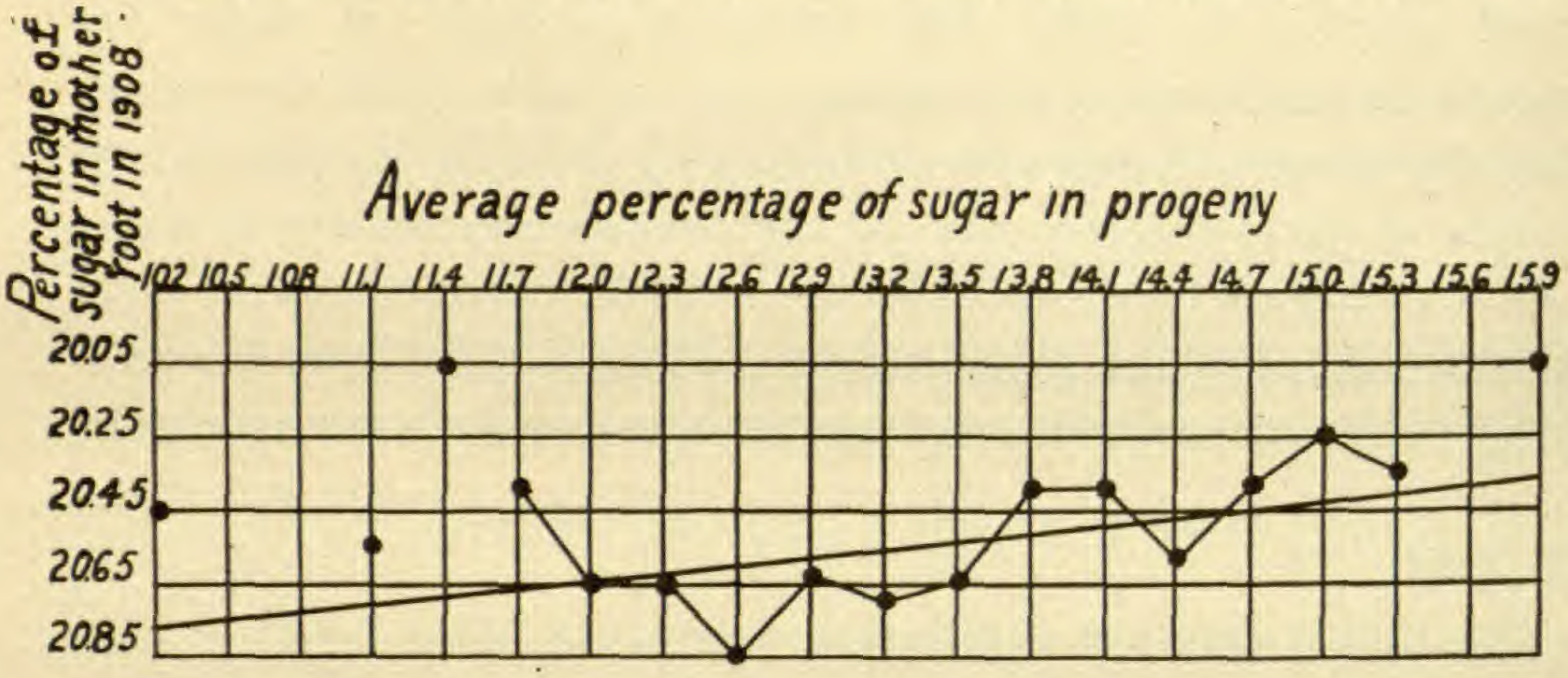

FIG. 12.—To accompany table XIV

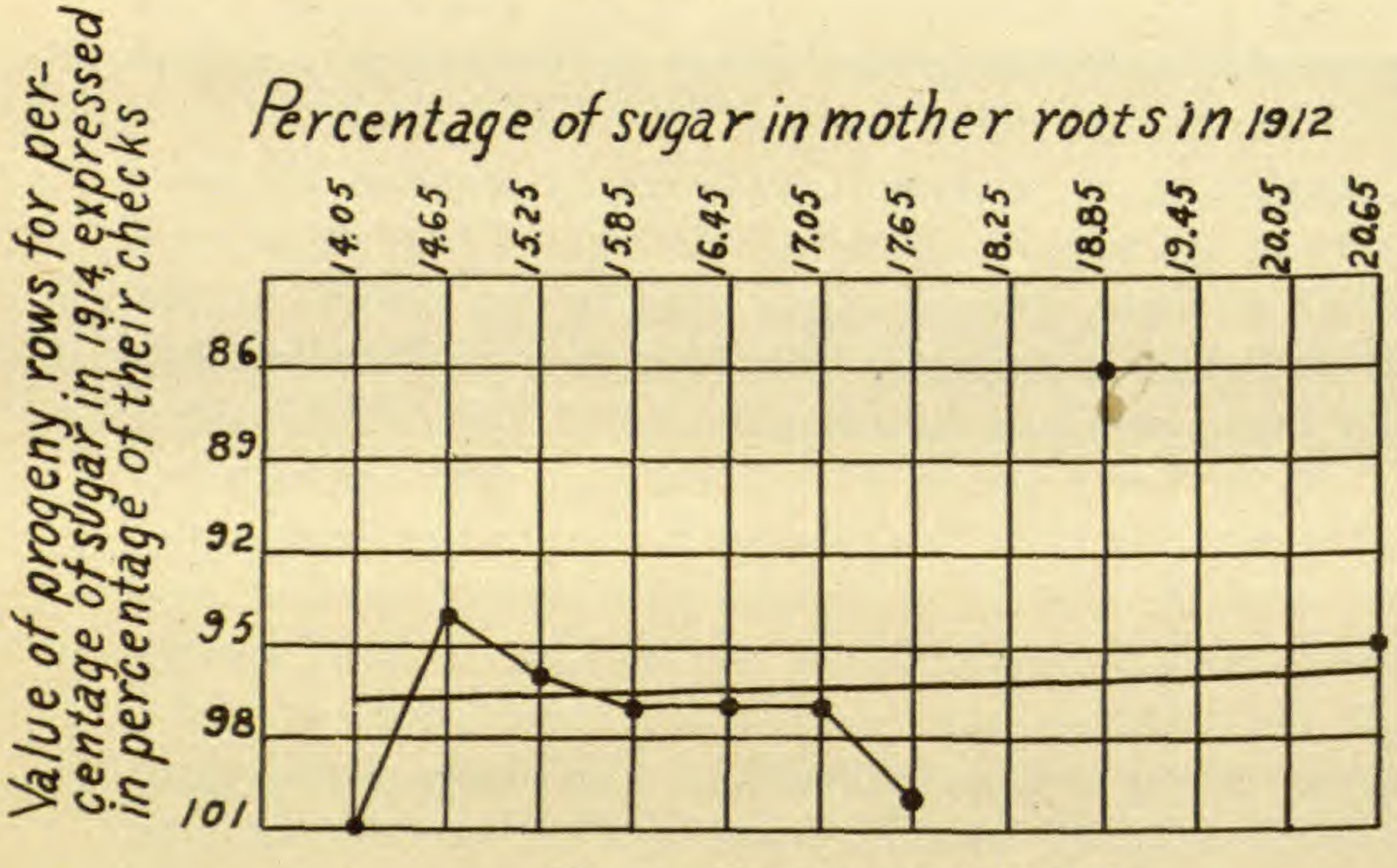

FIG. 13.—To accompany table XV

The results show no correlation between percentage of sugar, weight, or sugar content of the root and the average value of the same quality in its progeny. There is no constant relationship between the weight of the mother root and the average percentage of sugar or the average sugar content of its progeny roots. Moreover, exceptionally good roots transmit no different qualities than do average roots. In fact, the poorest roots made as good progeny records as the best roots. While cross-fertilization may have had an equalizing tendency upon the qualities, real differences could hardly be eliminated in one generation, as both good and

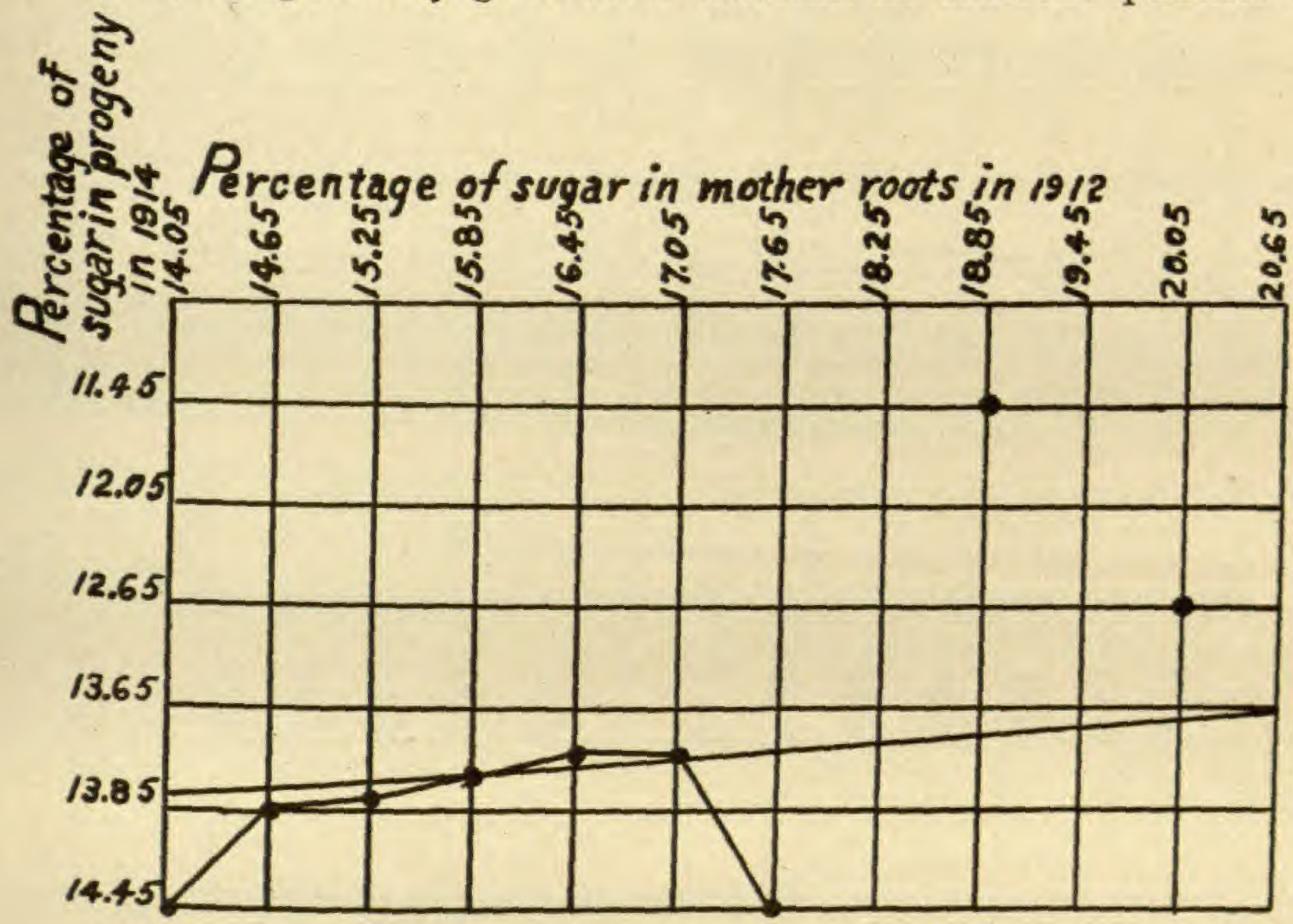

FIG. 14.—To accompany table XVI

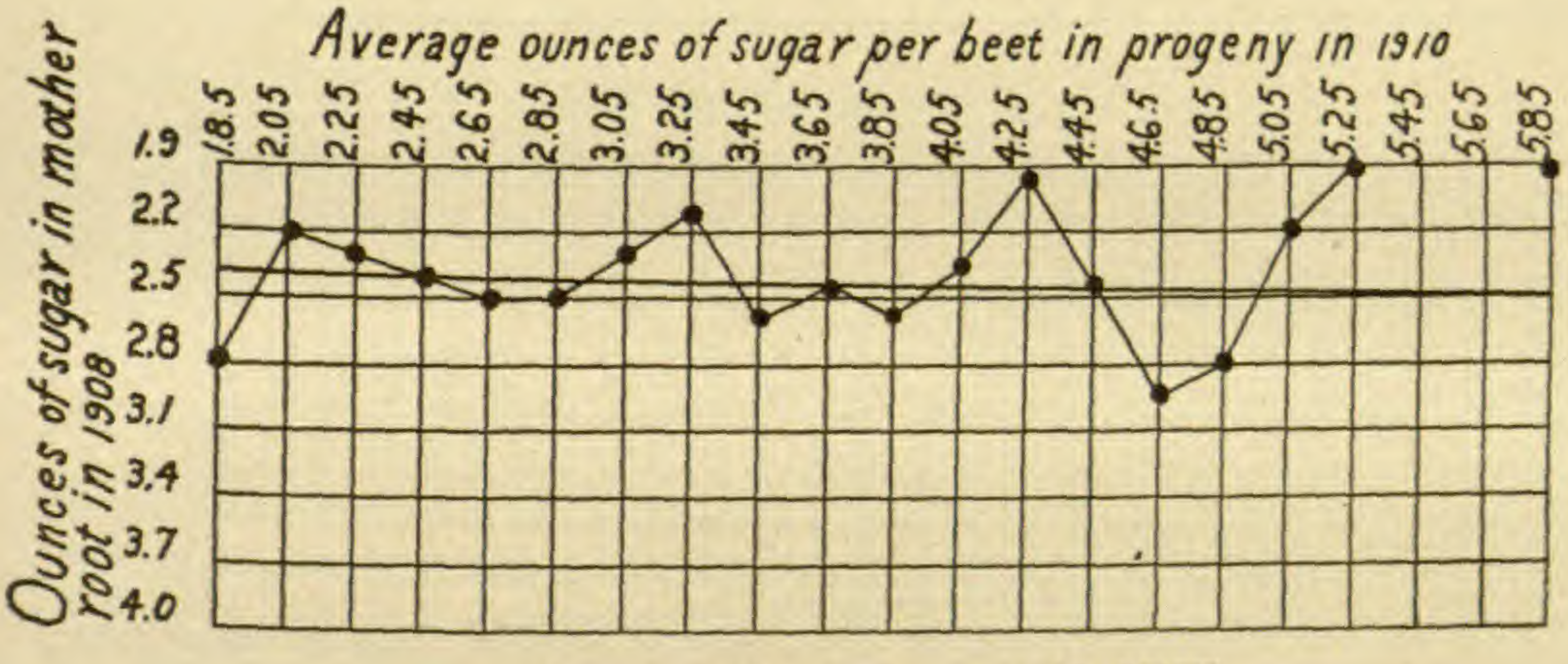

FIG. 15.—To accompany table XVII

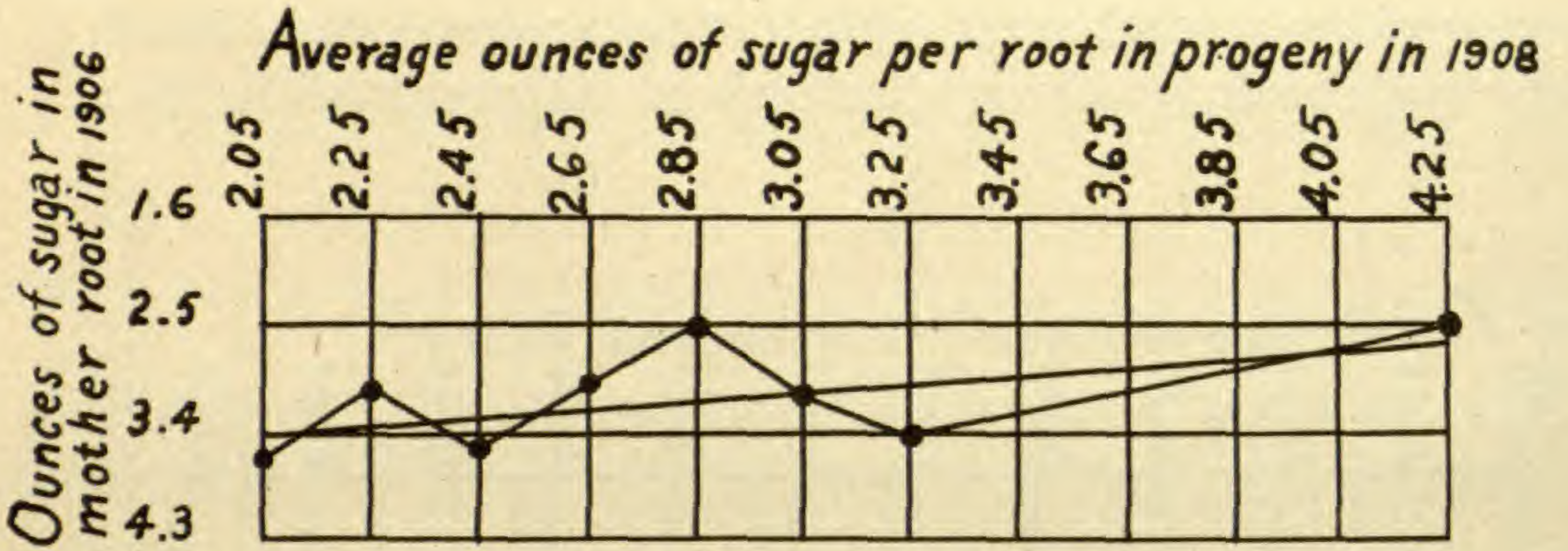

FIG. 16.—To accompany table XVIII

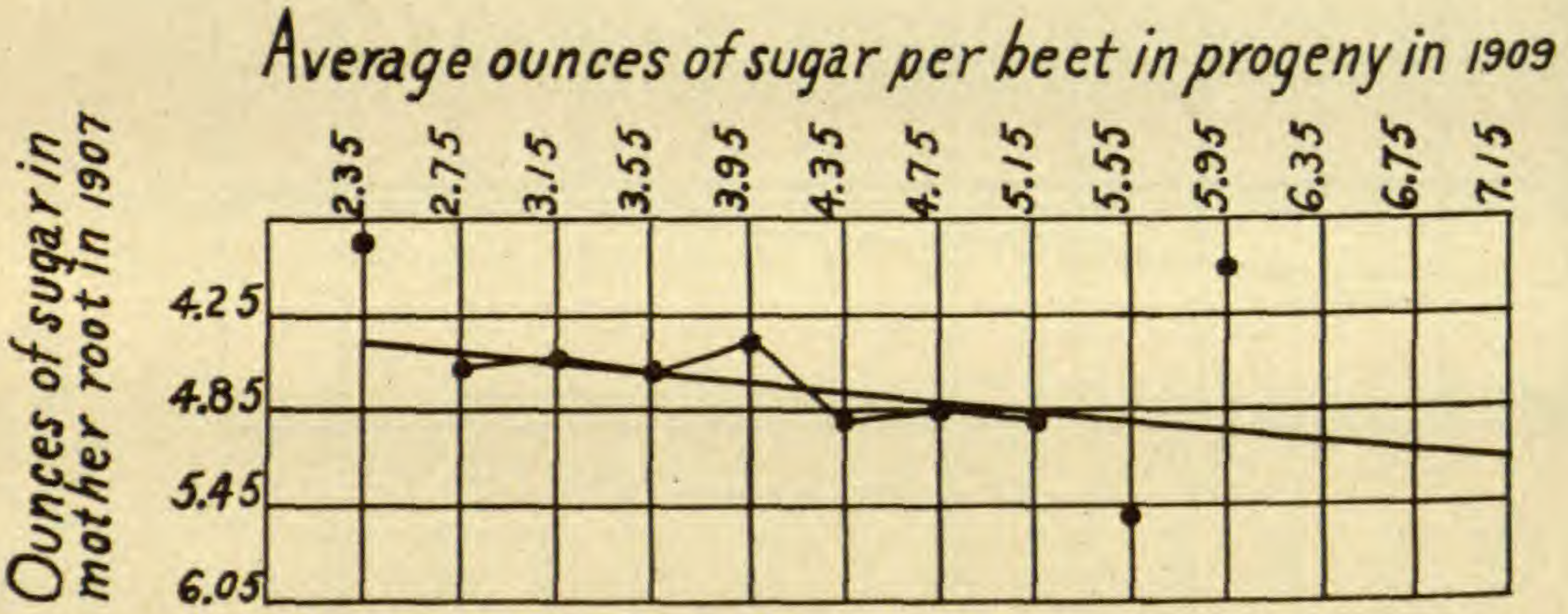

FIG. 17.—To accompany table XIX

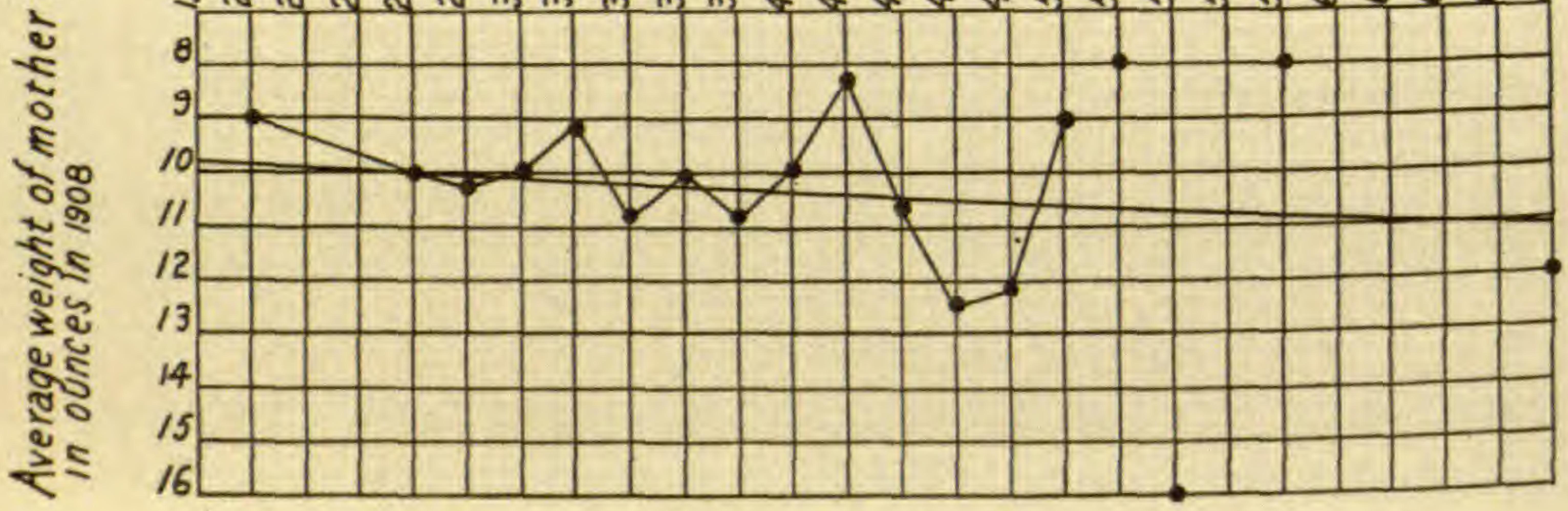

FIG. 18.—To accompany table XX

poor plants would receive pollen of equal average quality, and hence the better mothers should produce the better average progeny. Moreover, the fact that insects visit several flowers before leaving a plant probably causes a considerable percentage of selfing, even though the plant may prefer foreign pollen. If hereditary differences occur in mother roots and are transmitted to their progeny, they are certainly obscured by fluctuations caused by irregularities of the soil, which influence the qualities of both mother root and progeny, and thus baffle the breeder's attempt to discover real differences. By planting each family 10–20 times and using a considerable number of checks it may be possible to distinguish hereditary differences between their progeny, but this would hardly apply to mother roots. In fact, these results seem to condemn the customary practice of selecting mother roots by current physical and chemical means. Taking roots at random would certainly be much cheaper and apparently fully as effective.

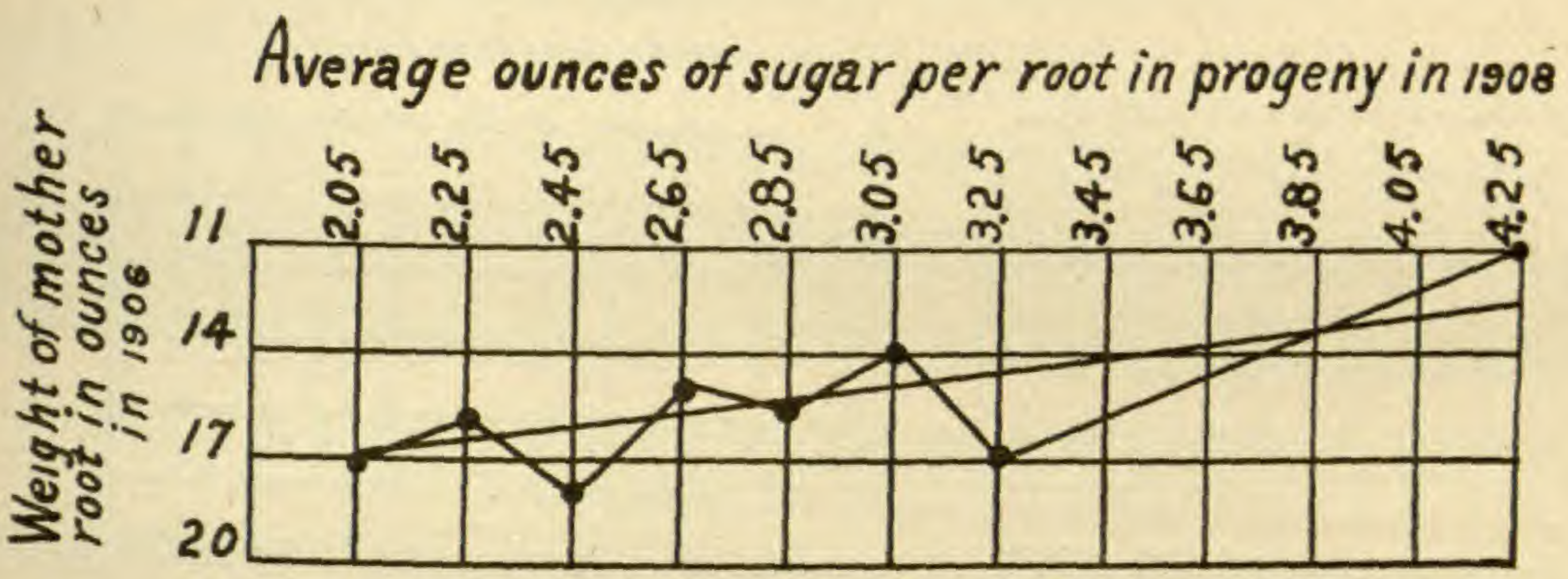

FIG. 19.—To accompany table XXI

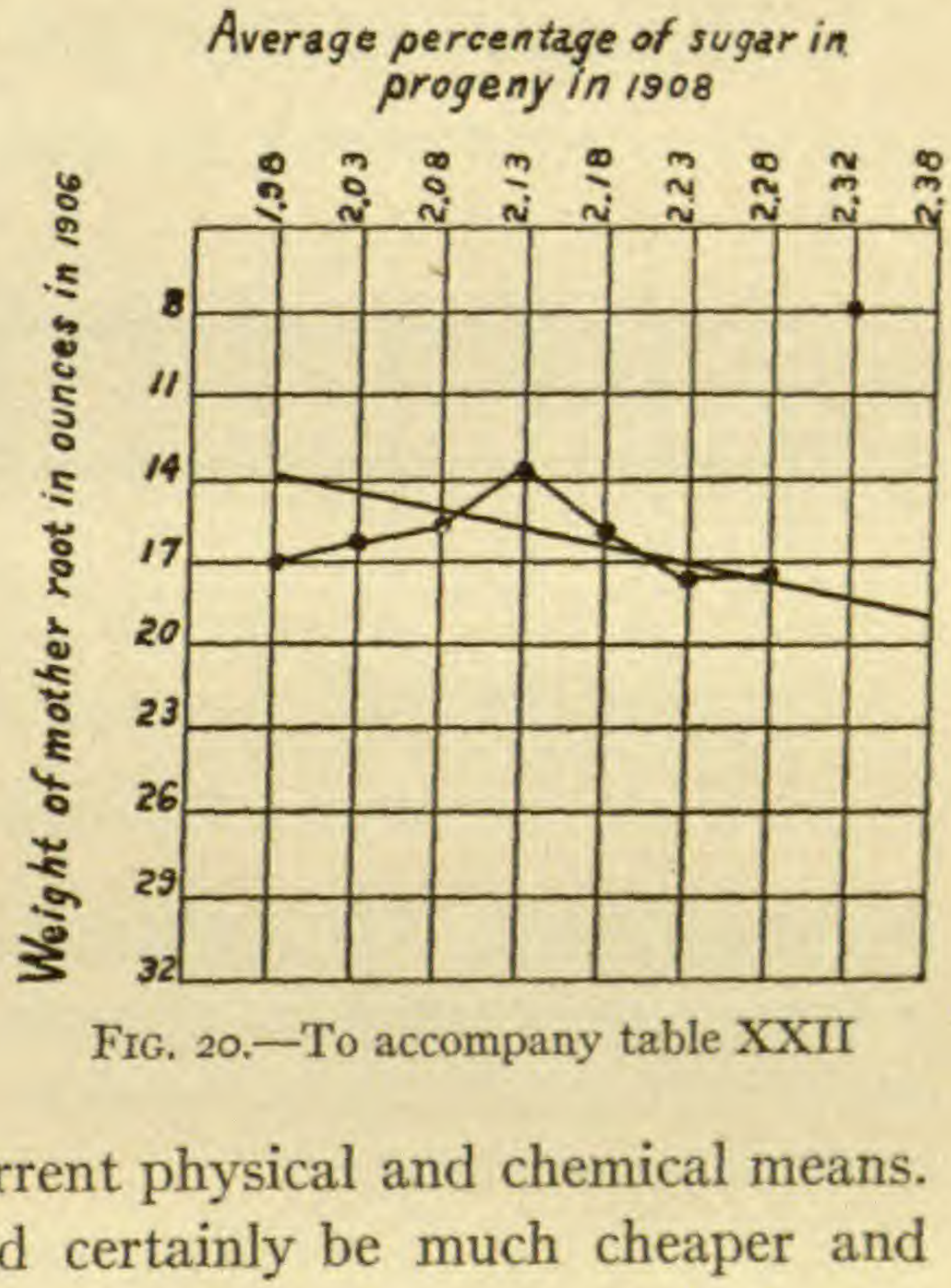

FIG. 20.—To accompany table XXII

SELECTION OF FAMILIES.—Sugar-beet families are selected upon the basis of percentage and yield of sugar. Extreme selection in either direction is avoided, as a maximum yield of sugar is usually associated with a moderately high percentage of sugar, while an extremely high percentage is generally indicative of a low tonnage. As the presence of inorganic salts lowers the percentage and prevents the extraction of about an equal quantity of sugar, selection for extractable sugar is the most ideal method to employ, but it has the disadvantage that additional chemical work is required to determine the coefficients of purity. Relative merits, therefore, are more economically determined from the percentage of sugar and total yield of sugar.

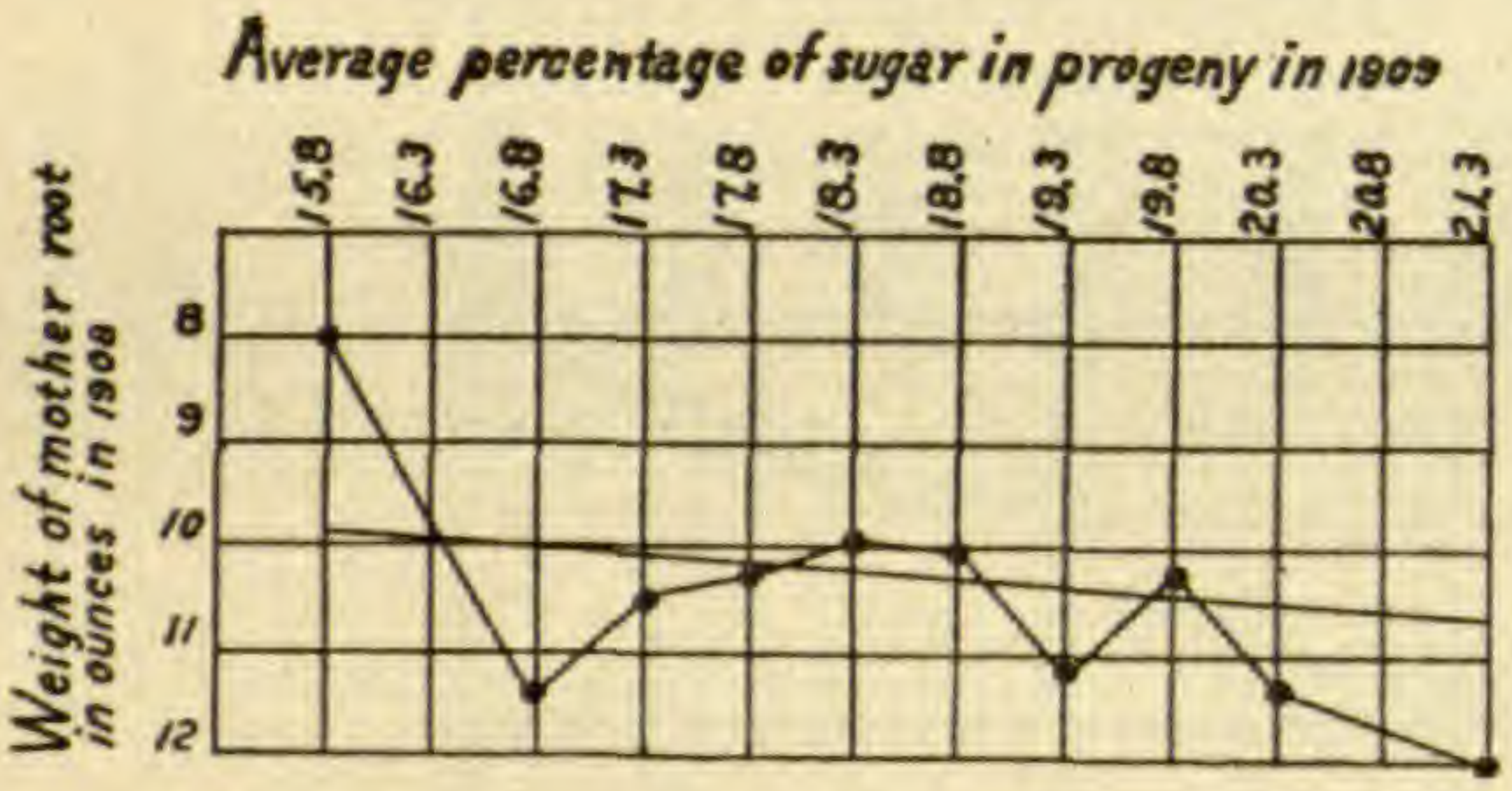

FIG. 21.—To accompany table XXIII

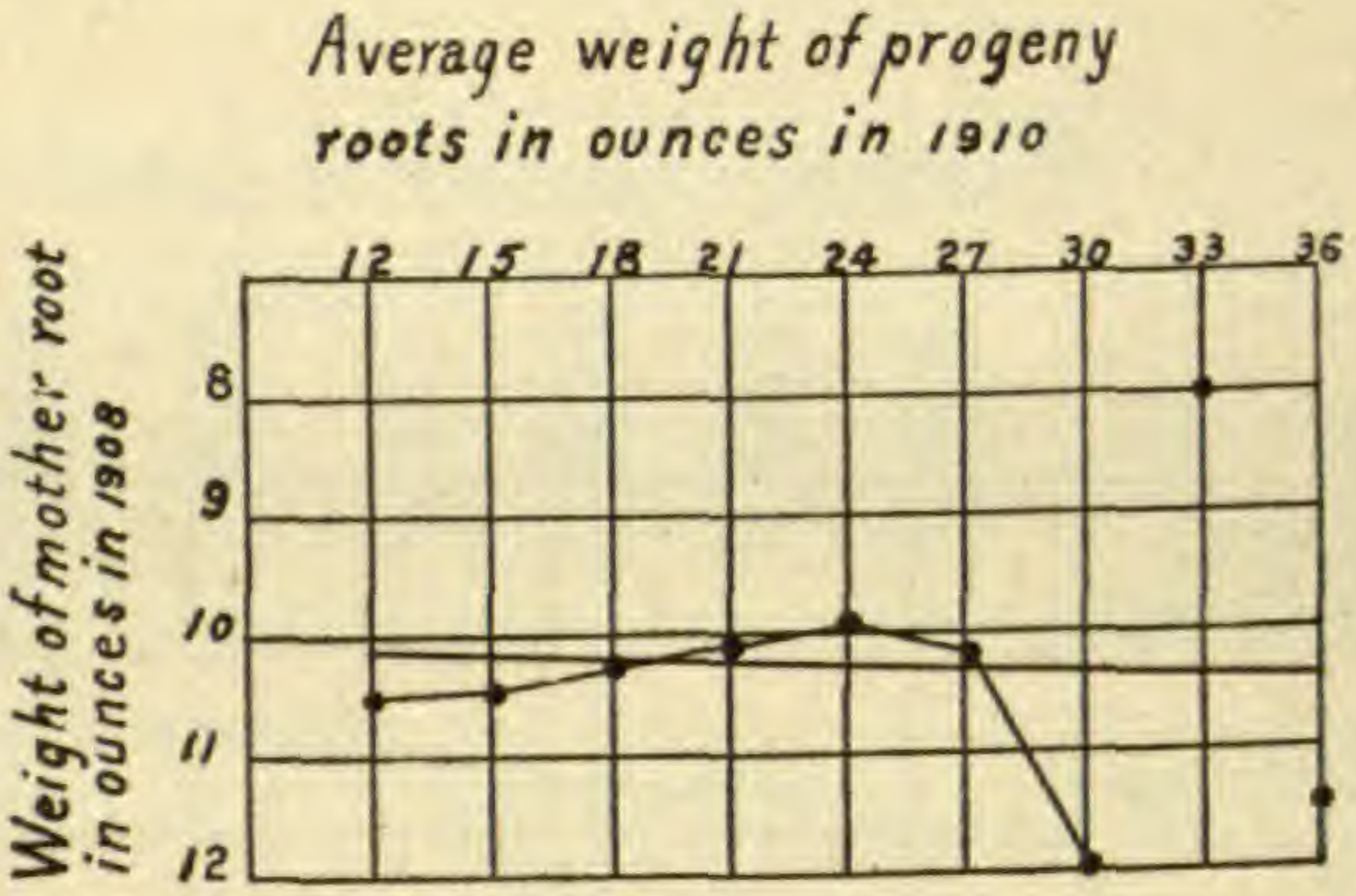

FIG. 22.—To accompany table XXIV

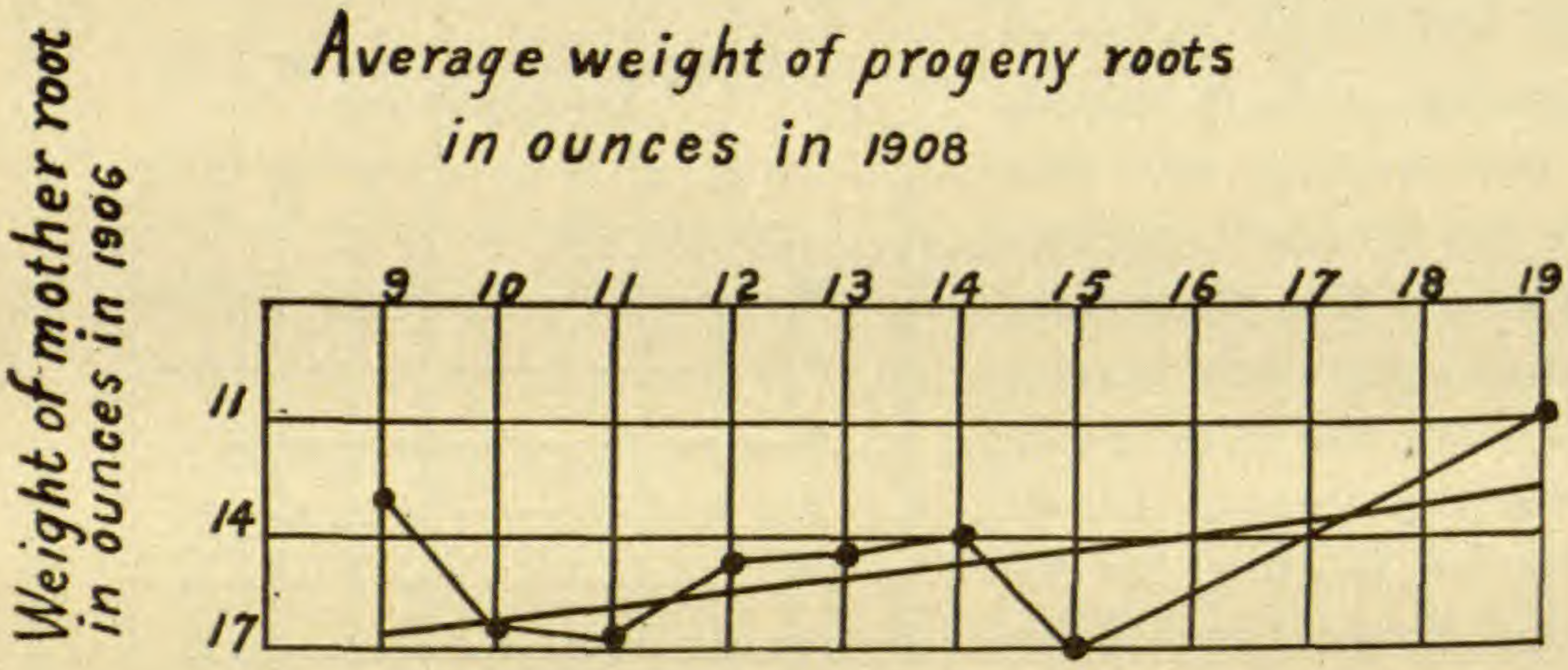

FIG. 23.—To accompany table XXV

VARIABILITY OF PROGENY ROWS.—Progeny rows show considerable variation even when the stand of beets is comparatively uniform. The coefficients of variability presented in table XXVI were determined from 173 beet families of Morrison's Kleinwanzleben grown at Madison, Wisconsin, in progeny rows of 40–50 beets each in 1912, and from 98 families of the same stock grown under similar conditions at Madison in 1913.

TABLE XXVI

VARIABILITY OF SUGAR-BEET FAMILIES

Percentage of sugar

Year	Mean	Standard deviation	Coefficient of variability
1912.....	13.60±0.068	0.998±0.048	7.34
1913.....	15.41±0.058	1.009±0.041	6.54

Yield of sugar per row

Year	Mean (gm.)	Standard deviation (gm.)	Coefficient of variability
1912.....	4276.26±32.67	637.08±23.00	14.90
1913.....	2074.70±19.64	288.18±13.88	13.89

The average variability of progeny rows expressed in percentage of the mean was 6.54–7.34 for percentage of sugar, and 13.89–14.90 for yield of sugar. Although these constants are smaller than the coefficients obtained for individual beets of the same families, 9.46–12.06 and 38.58–49.53 for percentage and yield respectively, they are fairly large for averages.

The range for percentage and yield is not shown in the table, but rows containing 80–100 per cent stand varied from 10.1 to 15.7 in percentage of sugar in 1912, and from 13.2 to 17.4 in 1913; while the extreme yields for the same periods were 2383–5654 and 1423–2746 gm. respectively. Such differences certainly offer ample opportunity for selection.

EFFECT OF SOIL IRREGULARITIES UPON THE VARIABILITY OF BEET FAMILIES.—This was determined from the records of our sugar-beet breeding experiments in which progeny rows and check rows were planted alternately.

As family differences exhibited in field tests may be due to several causes, some method had to be adopted for studying soil effects that would eliminate such factors as family hereditary differences, and the possible use of too small a number of plants to form a representative sample of the family. This was accomplished by plotting the mean values of consecutive progeny rows

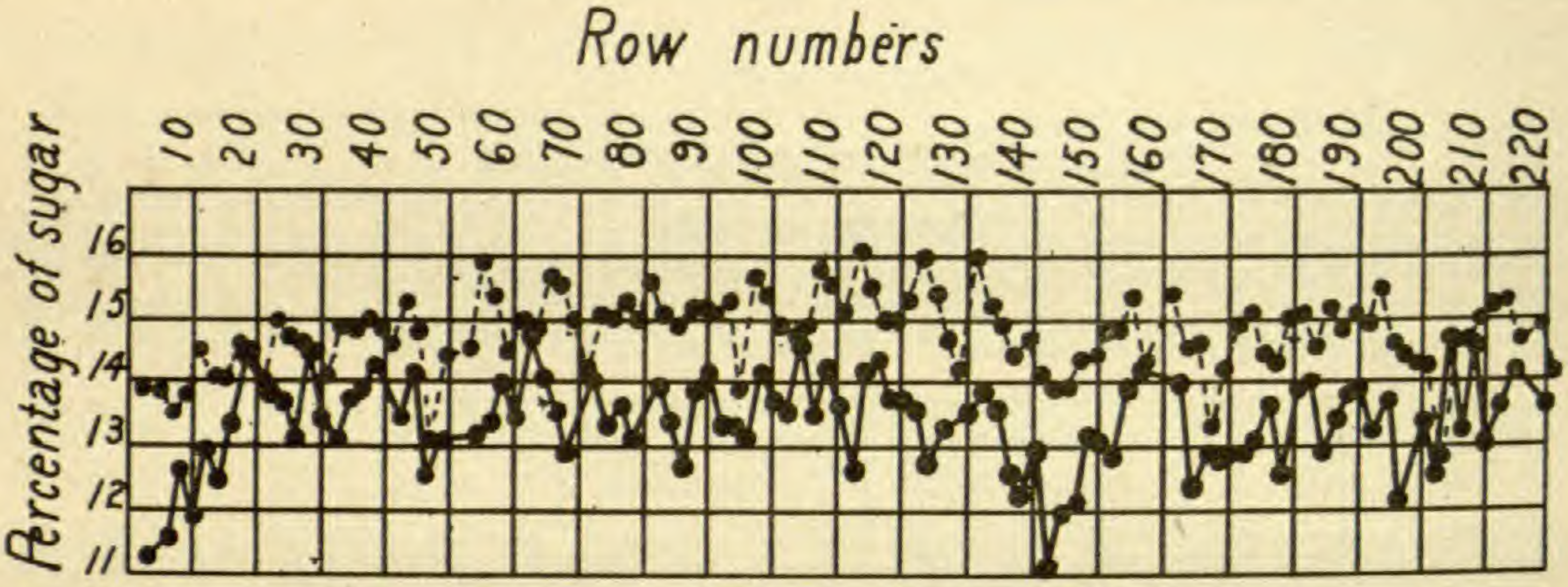

FIG. 24.—Effect of soil irregularities on percentage of sugar of check and progeny rows, strip 1, Madison, 1912; solid line indicates progeny rows; broken line, check rows.

for percentage and yield of sugar in the same order as the rows occurred in the field. Family differences in percentage or yield of sugar which show regular progression in direction are assumed

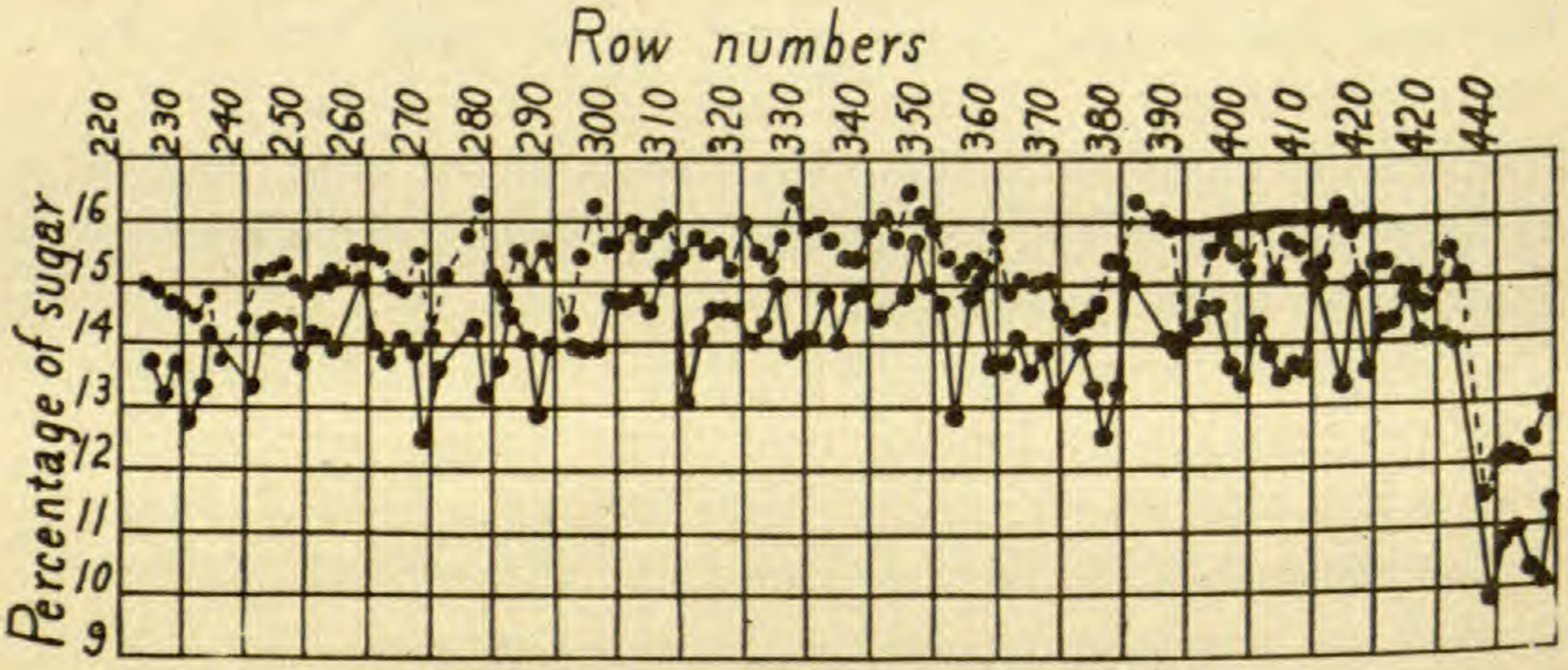

FIG. 25.—Effect of soil irregularities on percentage of sugar of check and progeny rows, strip 2, Madison, 1912; solid line indicates progeny rows; broken line, check rows.

to be due to the inequalities of the soil, while differences which show no such regularity may be due either to local soil disturbances or to other causes. A check on the results of the foregoing method is afforded by plotting the mean values of progeny rows of contiguous strips whose row ends abut, and noting whether the same relative parts of the different strips show the same irregularities. Another check better adapted to show local soil irregularities and more generally applicable consists in plotting the mean values of the check rows in regular order. If these values show the same progressions as the means of the progeny rows, the effects are doubtless due to irregularities of the soil.

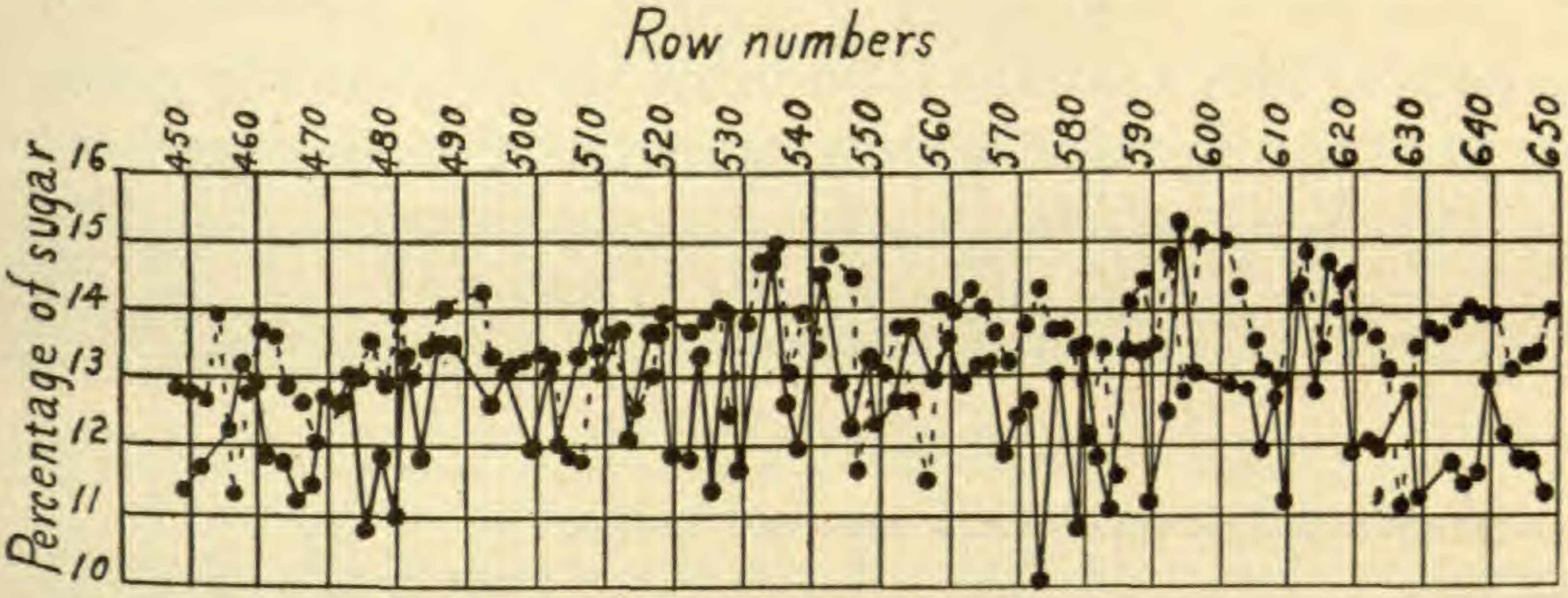

FIG. 26.—Effect of soil irregularities on percentage of sugar of check and progeny rows, strip 3, Madison, 1912; solid line indicates progeny rows; broken line, check rows.

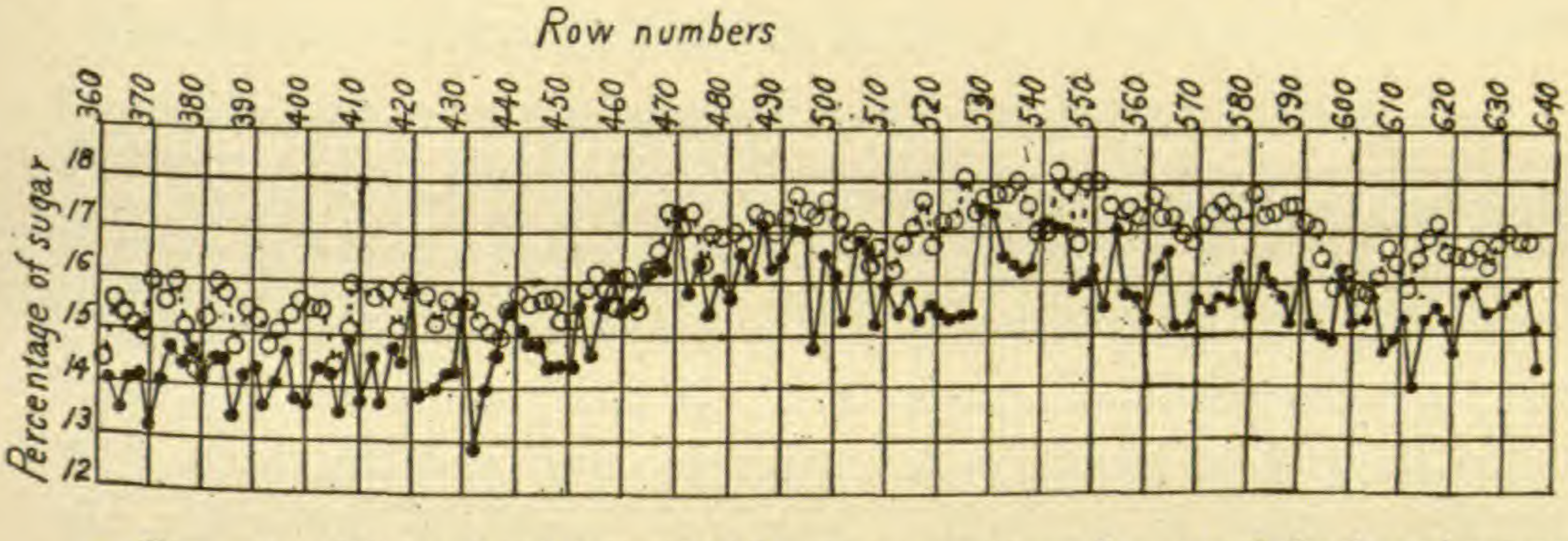

FIG. 27.—Effect of soil irregularities on percentage of sugar of check and progeny rows, Madison, 1913; solid line indicates progeny rows; broken line, check rows.

In the accompanying graphs, figs. 24–29 show the mean percentage of sugar, and fig. 30 the mean yield of sugar of consecutive progeny rows and check rows. Figs. 24–26 represent 3 contiguous strips of the same field which meet at their row ends. Strips 1 and 2 were planted on the same date, but strip 3 was plowed up and replanted on account of a poor stand. Consequently, the beets in strip 3 were not fully mature when harvested, as may be seen from their low percentage of sugar. The row numbers run consecutively, but only every tenth row is numbered in the figures.

The regularity in the trend of mean percentages of sugar in figs. 24–29 and of mean yields of sugar in fig. 30 shows that soil irregularities have a marked influence on the behavior of progeny rows and check rows. The close agreement of progeny rows and check rows is remarkable and shows the advantage of employing frequent checks over the use of the mean value of the progeny rows as a standard of comparison. If the graphs are examined closely, it will be noted that a progeny row and its check rows frequently vary in opposite directions, although following the general trend of neighboring rows. Occasionally these peculiarities may be due to the hereditary nature of the progeny row of beets, but more frequently they are the result of local soil disturbances, as may be seen from similar deviations of check rows from their general trend. On the average the consecutive deviations of check rows from their trends appear smaller than the corresponding deviations of the progeny rows. Hence real differences between progeny rows may exist, but they are small in comparison with the fluctuations.

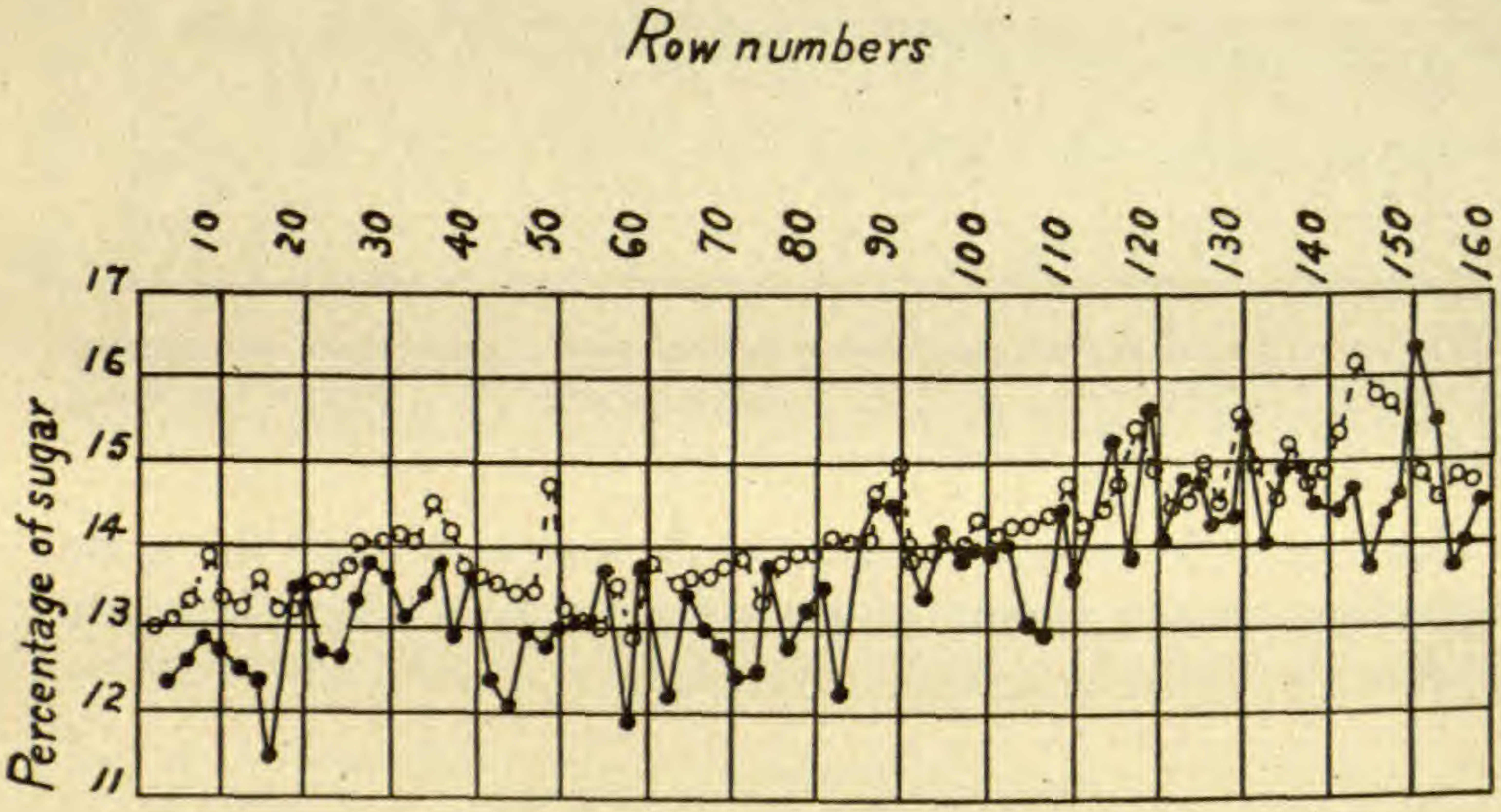

FIG. 28.—Effect of soil irregularities on percentage of sugar of check and progeny rows, Madison, 1914; solid line indicates progeny rows (Morrison's Kleinwanzleben); broken line, check rows.

The only practical method of overcoming the effects of sudden soil disturbances is the employment of replications. Repeated plantings of each family between check rows of a standard variety will give a more reliable means of comparison than a single planting.

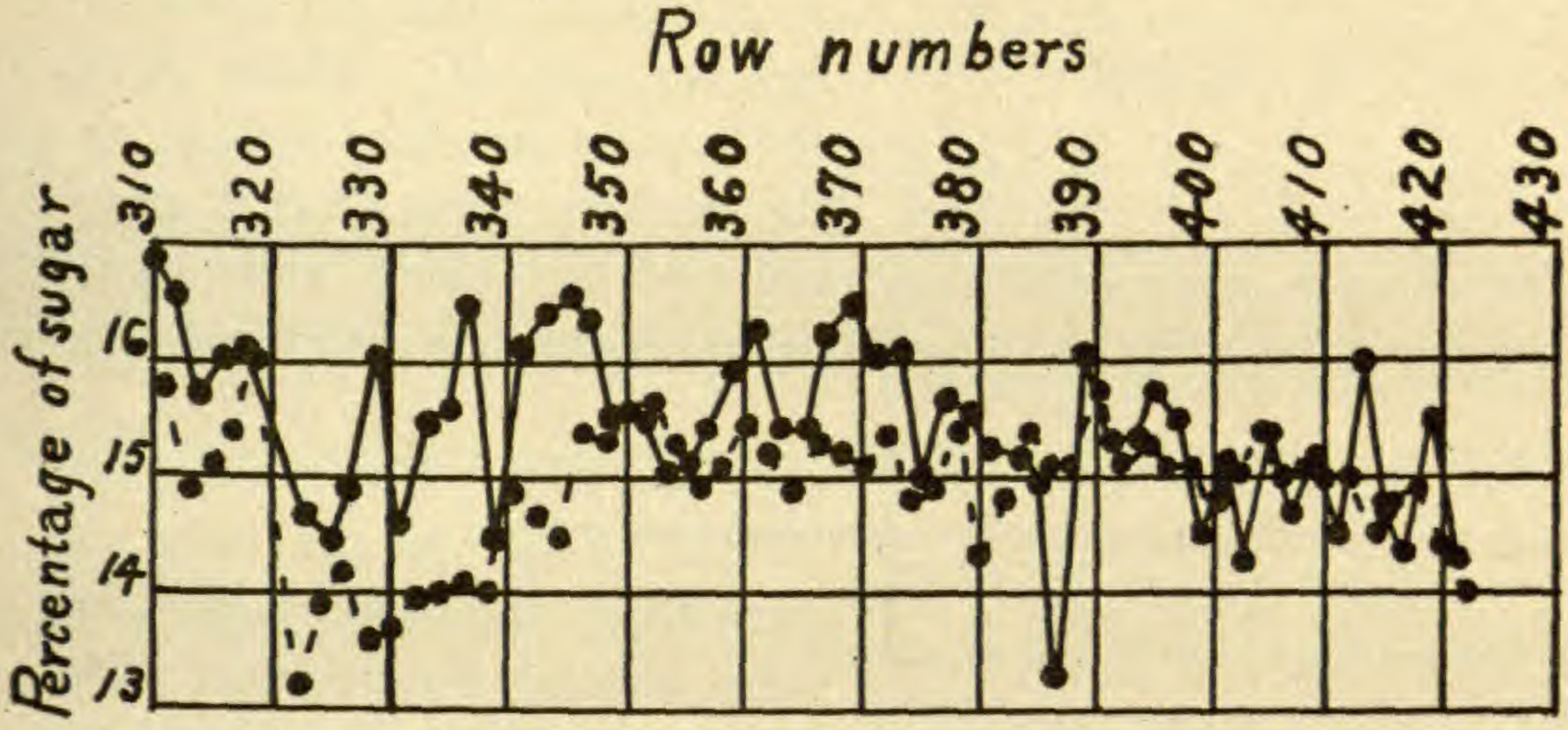

FIG. 29.—Effect of soil irregularities on percentage of sugar of check and progeny rows, Madison, 1914; solid line indicates progeny rows (Madison Original Selections); broken line, check rows.

A decided change in the direction of the graphs is shown in rows 132–155, 346–382, and 595–612 of the 3 contiguous strips in figs. 24–26. This difference is not due to the presence of a dead furrow or back furrow, as no such areas were included in the experiment. Moreover, the disturbance covers an area 30–40 ft. wide in strips 1 and 2. The remarkable drops in percentage of

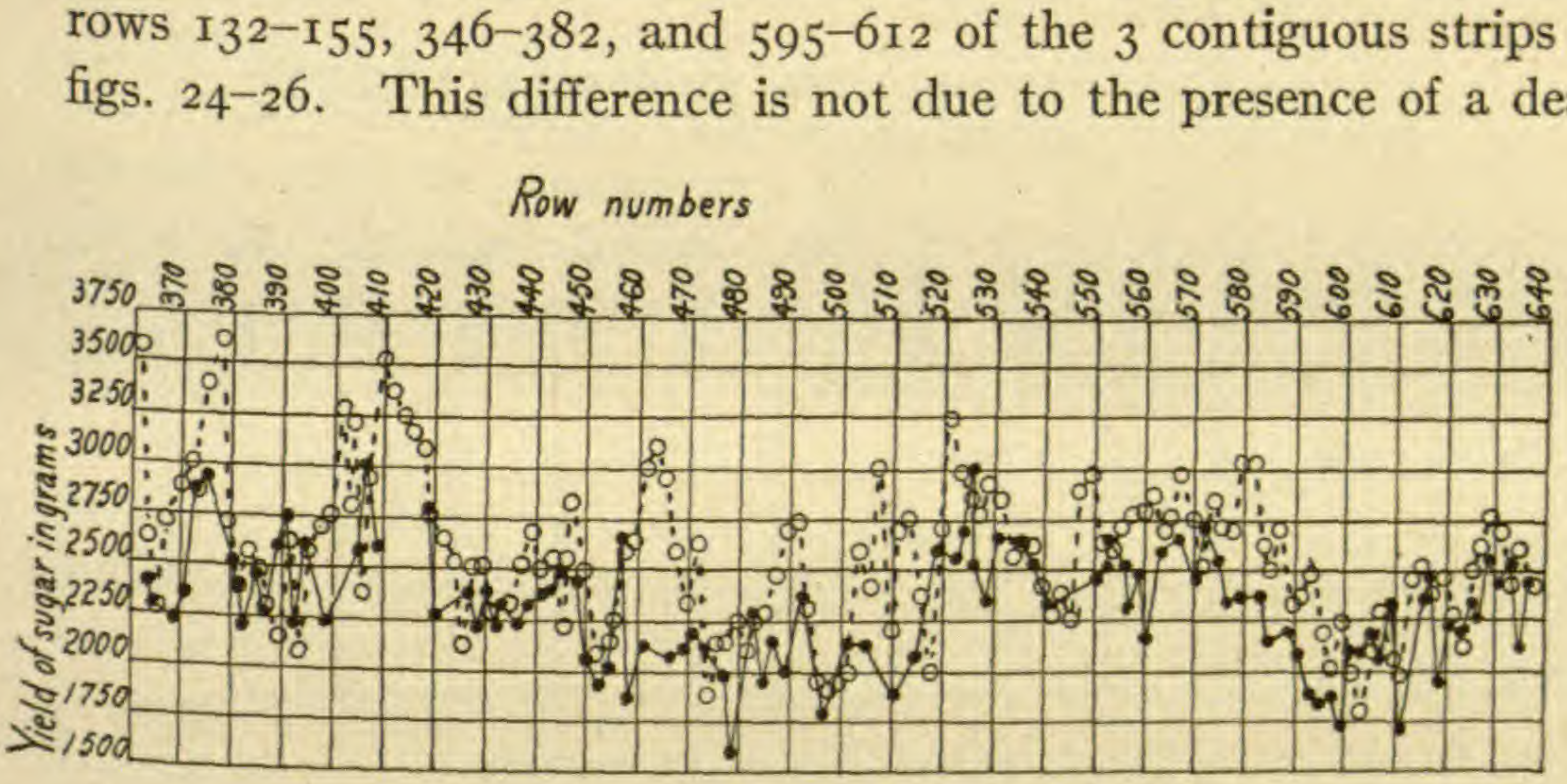

FIG. 30.—Effect of soil irregularities on yield of sugar of check and progeny rows, Madison, 1913; solid line indicates progeny rows; broken line, check rows.

sugar in rows 435–449 has not been accounted for. These rows belong to strip 2, as indicated in the figure, and not to strip 3, as might be inferred from their behavior.

A variation of 2 per cent sugar occurs between different parts of the same field, as shown in figs. 27 and 28. These local differences are not limited to the progeny rows, but appear also in the check rows, and hence cannot be attributed to accidental groupings of good or poor families. The yields exhibited in fig. 30 are fully as variable as the percentages and show striking progressions with frequent changes in direction.

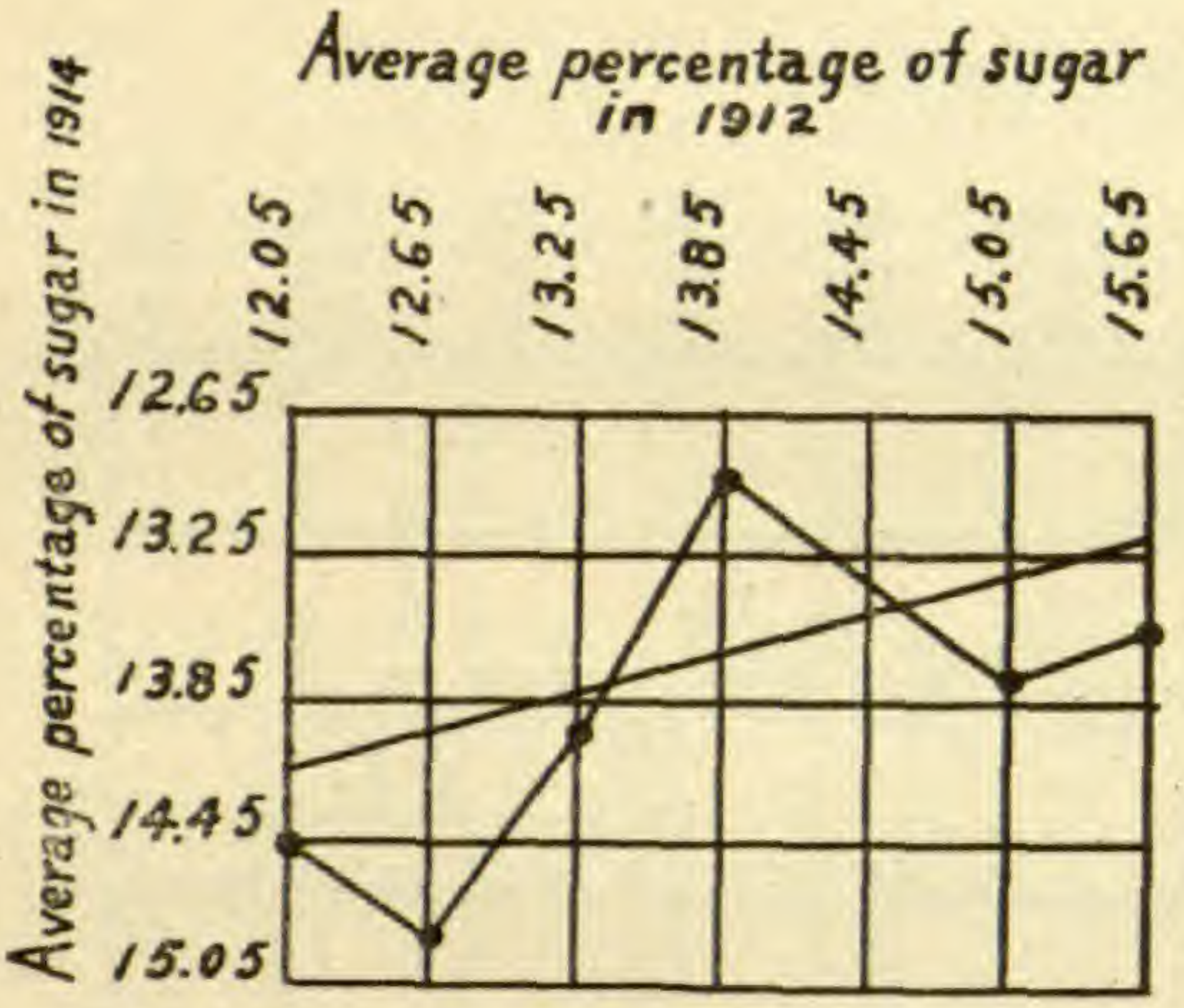

FIG. 31.—To accompany table XXVII

TRANSMISSION OF QUALITIES EXHIBITED BY SELECTED FAMILIES.—Average root weight and average percentage of sugar are the chief factors which determine the relative merits of sugar-beet families grown under uniform spacing, as they represent both yield and quality; but since these characters are very easily modified by the environment, it is doubtful whether real family differences are ordinarily distinguishable. In fact, fluctuations appearing under field conditions probably exceed the real differ-

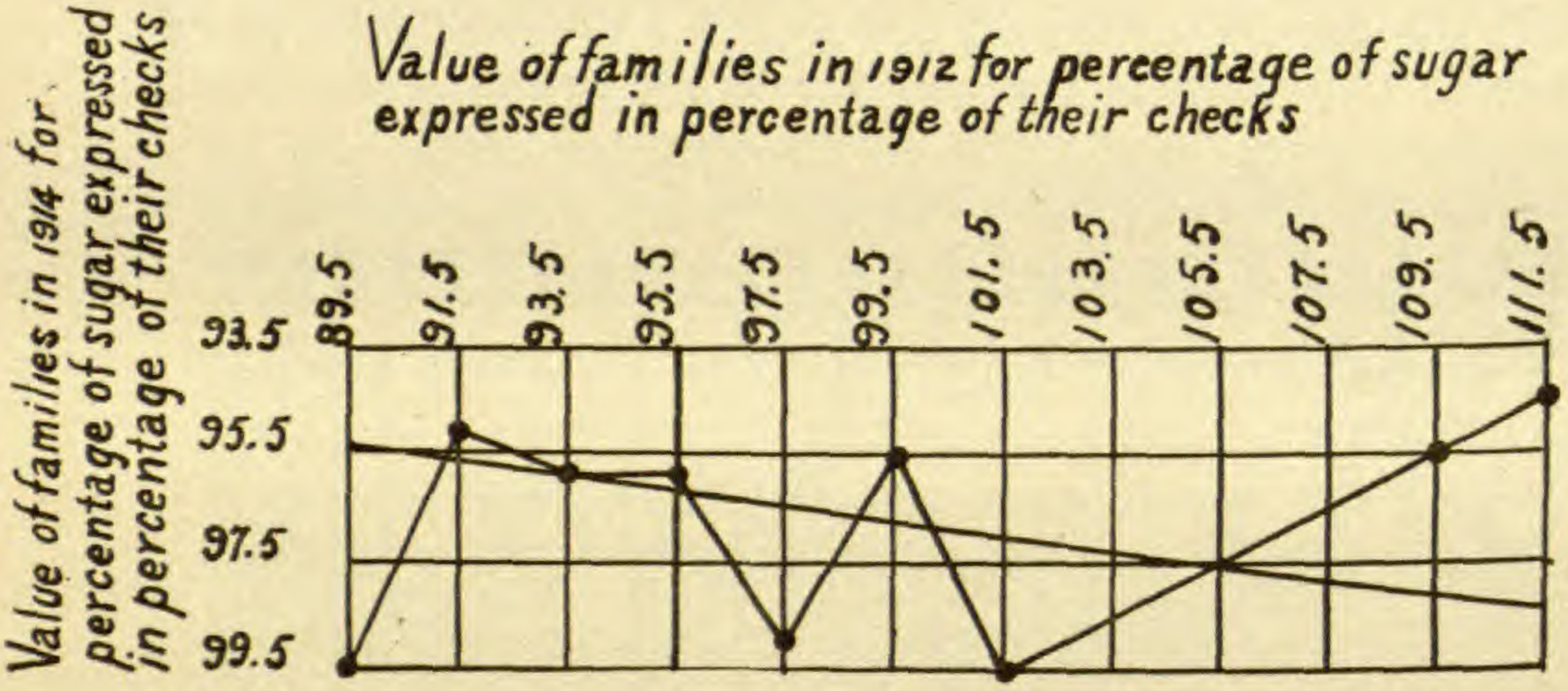

FIG. 32.—To accompany table XXVIII

ences between families. Tables XXVII–XXXII (summarized on p. 464) and figs. 31–36 show whether such family characters as percentage of sugar, average root weight, and average sugar content of the root are transmitted from generation to generation. Tables XXVII and XXVIII were compiled from the same data.

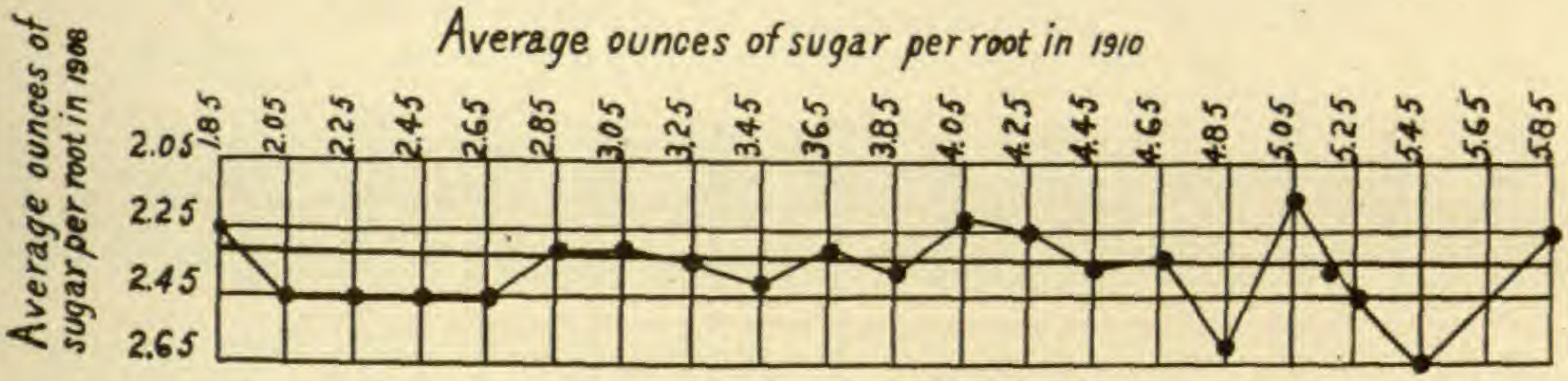

FIG. 33.—To accompany table XXIX

Table XXVII shows the relationship between the average percentages of sugar in two consecutive generations of beet families when no checks were used; table XXVIII shows the same relationship when every alternate row was employed as a check, and the value of each family was determined by dividing its percentage of sugar by the average percentage of sugar of its two contiguous checks. The families used in compiling data for tables XXIX–XXXII were planted without checks.

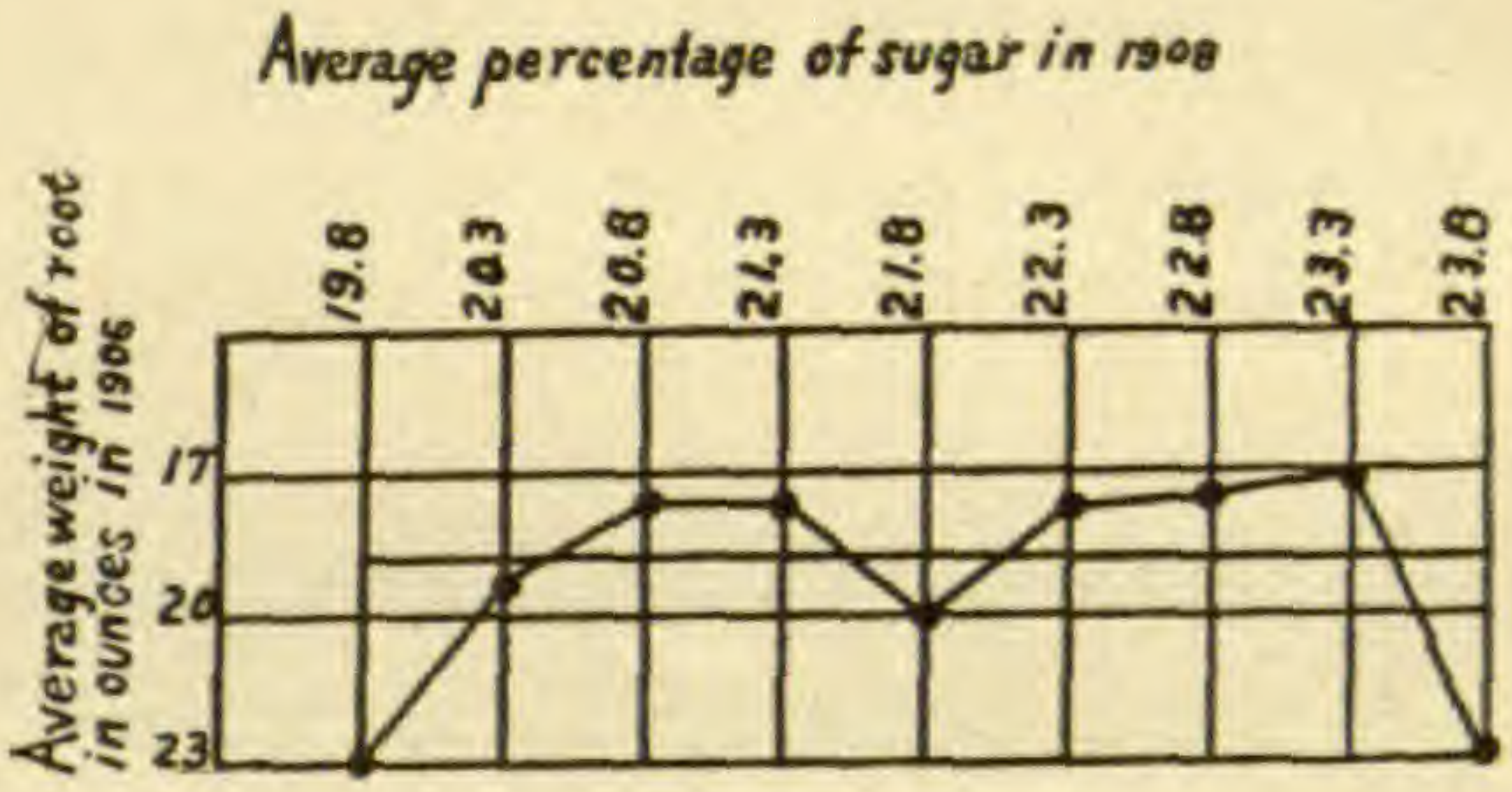

FIG. 34.—To accompany table XXX

No correlation is shown in tables XXVII and XXVIII between the percentages of sugar in two consecutive generations of a beet family. Even when every alternate row was planted as a check to offset the effects of the inequalities of the soil, the coefficient of correlation was only 0.089, while its probable error was 0.076.

Tables XXIX–XXXII furnish no evidence of correlation between average root weight or average quantity of sugar in successive generations, nor between average root weight in one generation and percentage of sugar in the following generation.

As a root's weight is negatively correlated with its percentage of sugar and positively correlated with its total sugar content, an absence of correlation between one pair of characters in tables XXVII–XXXII would signify a lack of correlation between each of the other pairs. The coefficients of correlation not only confirm this assumption but also have a corroborative effect upon one another.

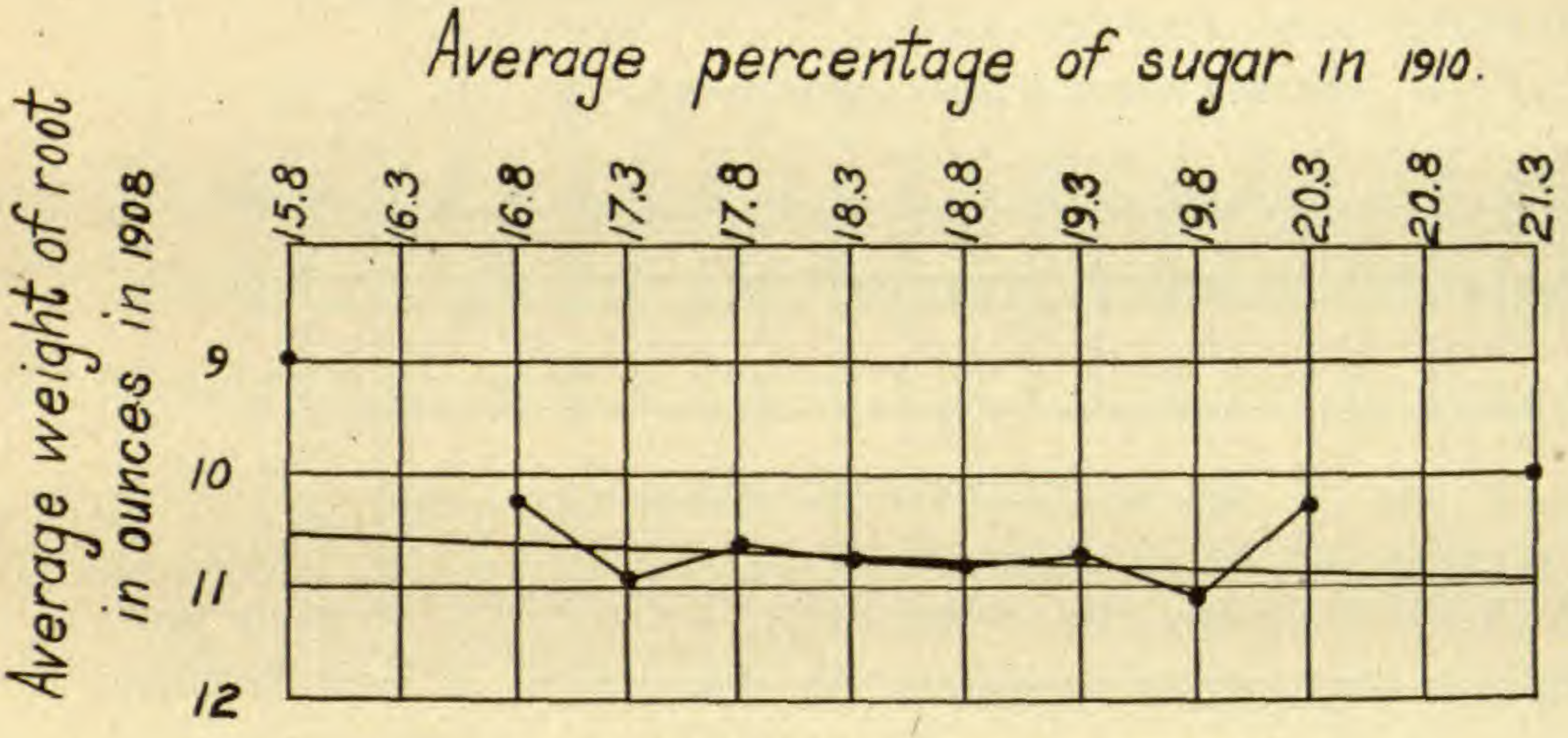

FIG. 35.—To accompany table XXXI

Figs. 31–36 furnish a graphic illustration of the character of the material used and the reliability of the results. The trends of the empirical means of the separate classes represented by dots and connected by broken lines show a fairly close agreement with the straight line regressions. Moreover, there are no marked deviations of empirical means from the straight lines except where the classes are small. Hence the distributions are fairly regular and the biometrical constants are probably reliable.

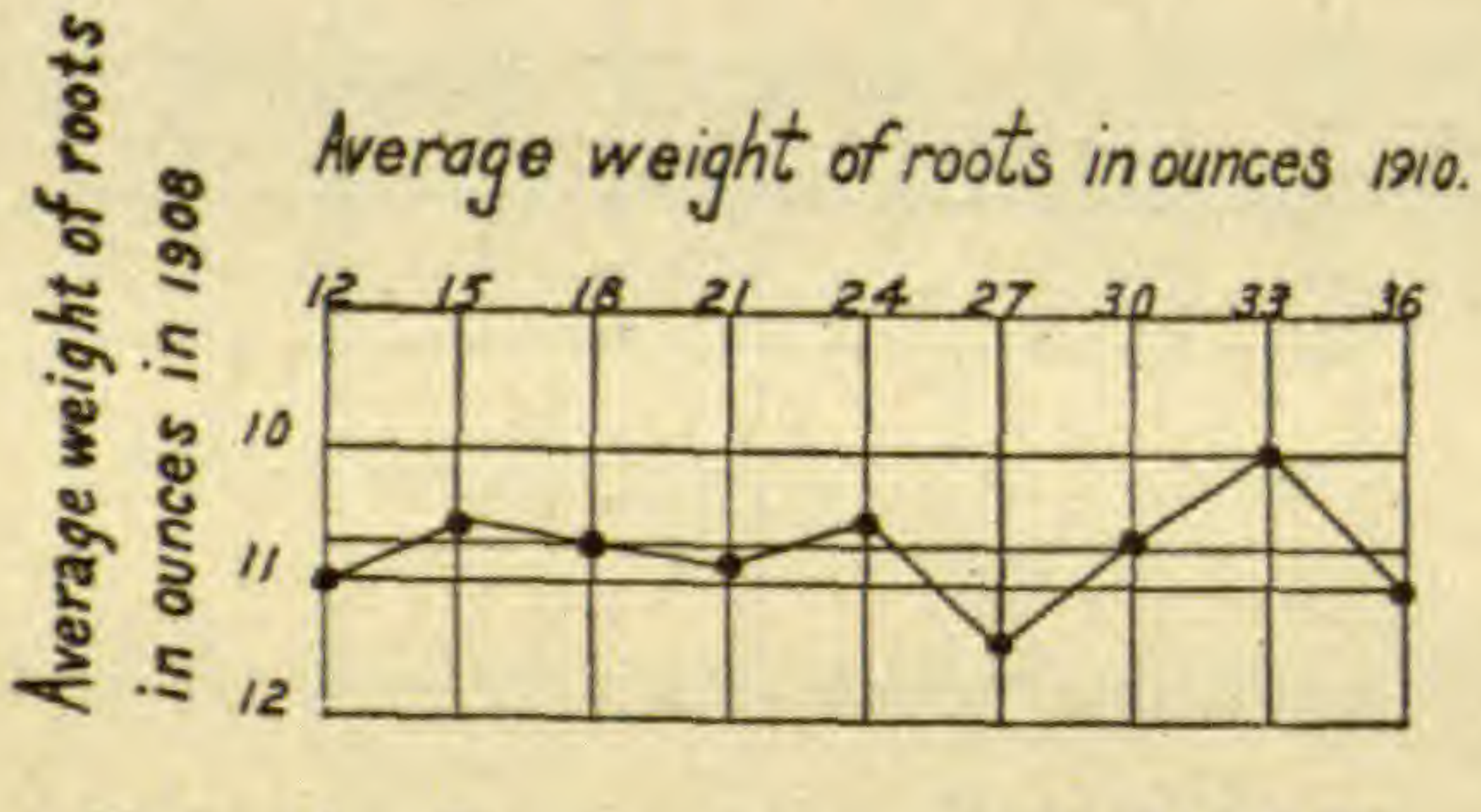

FIG. 36.—To accompany table XXXII

We are not justified in concluding from the foregoing results that no real differences in yield and quality occur, however, as real differences may be obscured by fluctuations. The most we can say at present is that family differences of this character as determined by current field methods are not permanent.

DETERIORATION FROM LACK OF SELECTION.—There is a current belief that sugar-beets deteriorate in percentage of sugar when selection is discontinued, but this has not been proved satisfactorily. A gradual decrease for a limited period is not necessarily indicative of permanent reduction, as such environmental factors as sunshine, temperature, rainfall, time of planting, fertility of the soil, drainage, and cultural methods all vary in different years and these variations affect the percentage of sugar. In fact, the percentage may fall for 2 or 3 consecutive seasons and then rise again under more favorable conditions.

If sugar-beets deteriorate from lack of selection, commercial seed should produce poorer roots than seed of selected mothers, as it is grown from an intermediate steckling generation in which no selections are made. Such a comparison was made in 1913 by mixing seed of highly selected families of Morrison's Kleinwanzleben and planting it in alternate rows beside the commercial variety of the same stock. The results are shown in table XXXIII.

TABLE XXXIII

COMPARISON OF MORRISON'S COMMERCIAL SEED WITH SEED OF MORRISON'S SELECTED MOTHER BEETS

MORRISON'S SELECTED MOTHER-BEET SEED				MORRISON'S COMMERCIAL SEED			
Row number	Number of beets in row	Weight of row of beets in pounds	Percentage of sugar in beets	Row number	Number of beets in row	Weight of row of beets in pounds	Percentage of sugar in beets
2	68	46.5	15.0	3	67	43.5	15.9
4	68	37.0	15.0	5	65	32.5	15.2
6	60	39.5	15.2	7	62	37.0	15.4
8	72	40.0	15.1	9	70	39.5	15.5
10	62	38.0	15.2	11	72	43.0	15.3
12	82	47.0	14.9	13	69	36.5	15.7
14	85	49.5	15.0	15	57	33.0	15.7
16	74	45.0	14.9	0	0	0	0
Averages	71.4	42.8	15.0		66	38.0	15.5

Average weight of root in grams: Morrison's commercial, 259.91; Morrison's selected, 271.70.

In every instance the commercial seed produced the richer roots. They were somewhat smaller than the roots from seed of selected mothers, averaging 11.79 gm. less per plant, but this is equivalent to only about 0.03 per cent sugar, while the average difference obtained was 0.5. Hence the commercial seed not only failed to show deterioration, but actually appeared to improve. Similar results were observed between the variety and its more highly selected families in 1912 which led to the planning of this experiment.

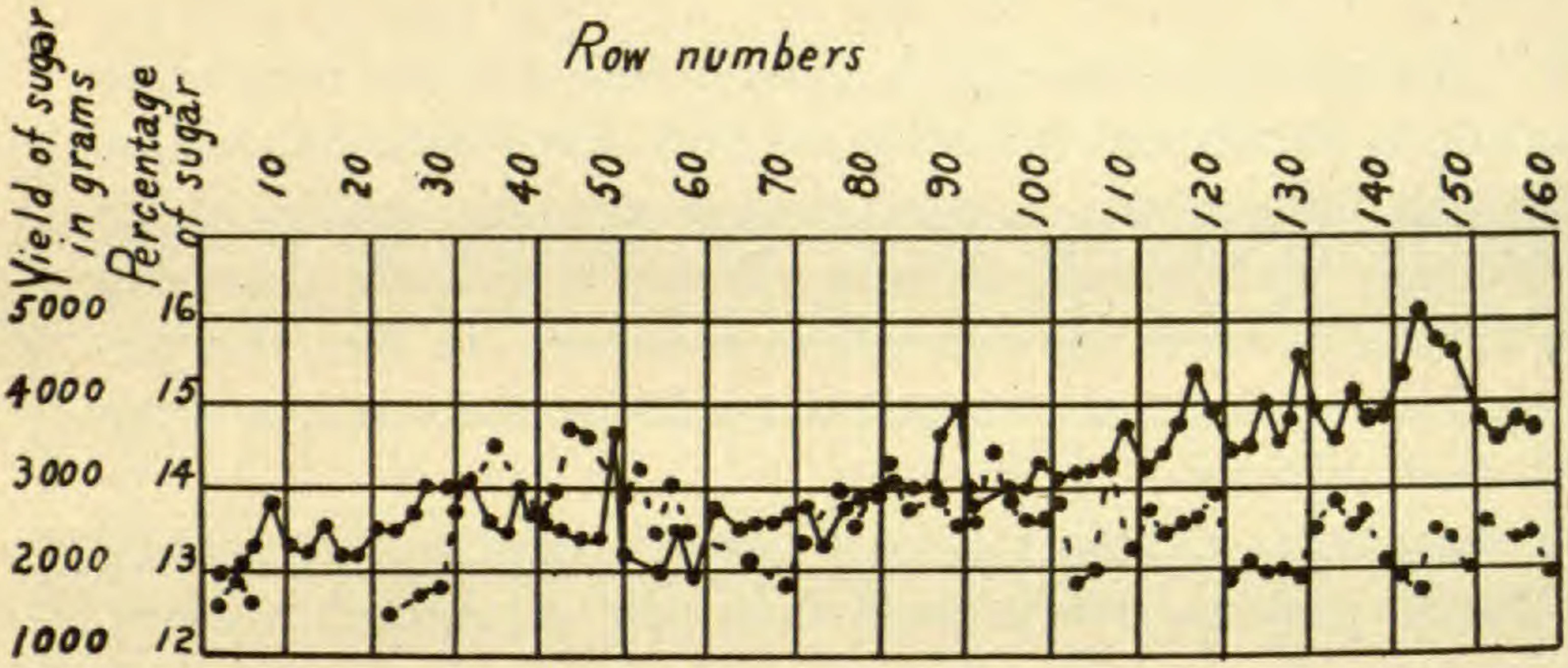

FIG. 37.—Effect of soil irregularities on relationship between percentage of sugar and yield of sugar in consecutive check rows, Madison, 1914; solid line indicates percentage of sugar; broken line, yield of sugar.

RELATIONSHIPS BETWEEN PERCENTAGE OF SUGAR, YIELD OF SUGAR, AND AVERAGE ROOT WEIGHT OF SUGAR-BEET ROWS.—As beets are grown under approximately uniform spacing, the same correlations which obtain between weight, percentage of sugar, and yield of sugar in individual roots would be expected to occur in sugar-beet rows. An ideal stand is never obtained under field

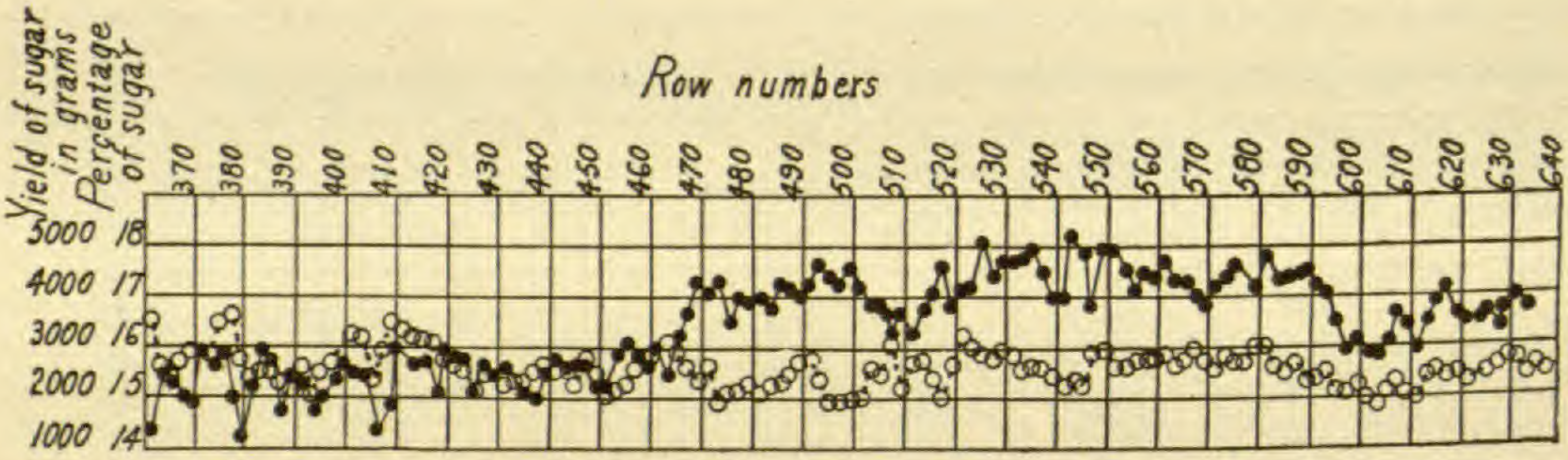

FIG. 38.—Effect of soil irregularities on relationship between percentage of sugar and yield of sugar in consecutive check rows, Madison, 1913; solid line indicates percentage of sugar; broken line, yield of sugar (corrected yield).

conditions, however, and as this may cause a deviation from the theoretical relationships, the actual relationships have been determined by plotting in figs. 37–50 the mean values of consecutive rows containing 80–100 per cent stand.

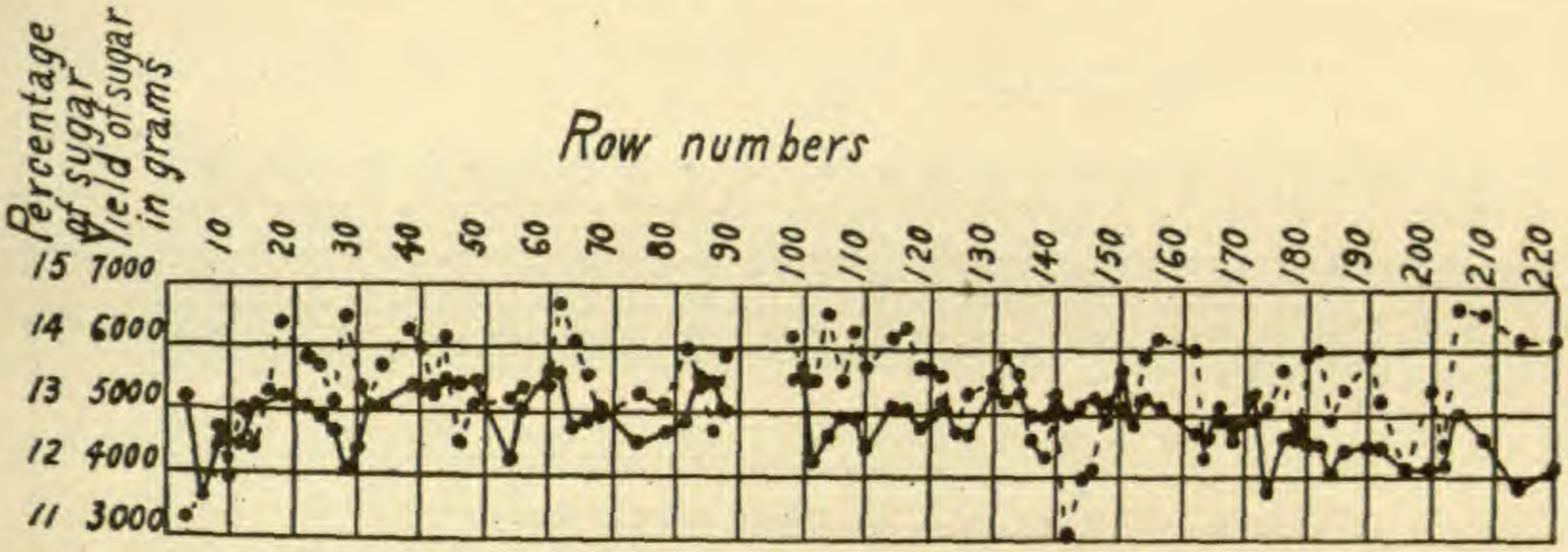

FIG. 39.—Effect of soil irregularities on relationship between percentage of sugar and yield of sugar in progeny rows, Madison, 1912; solid line indicates yield of sugar (corrected yield); broken line, percentage of sugar.

Figs. 37–41 show no correlation between percentage of sugar and yield of sugar in beet rows. In some places the graphs seem to follow a similar trend, as in rows 390–460 and 600–630 of fig. 38, and 370–440 of fig. 40; but in other places, as in rows 100–160 of fig. 37 and 450–520 of fig. 41, they apparently diverge. On the average, therefore, percentage and yield vary independently.

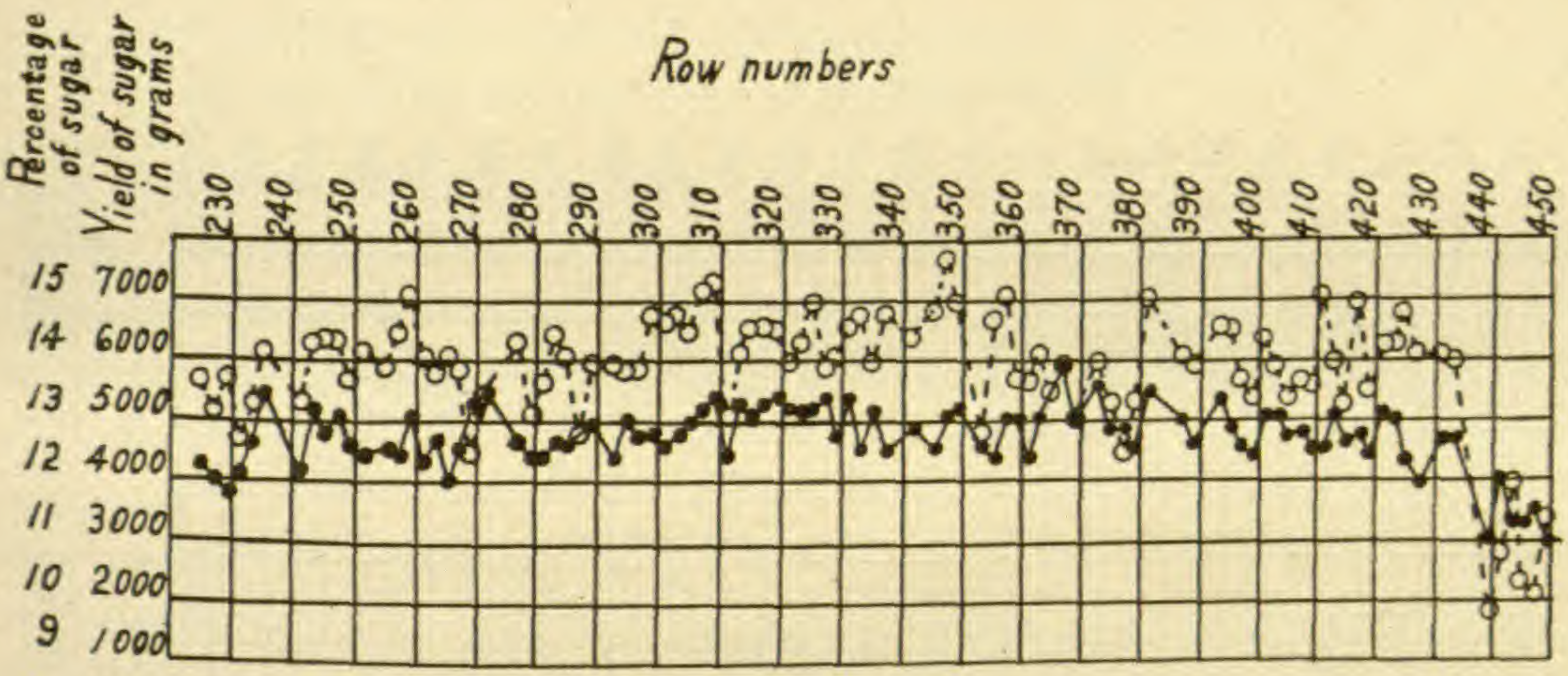

FIG. 40.—Effect of soil irregularities on relationship between percentage of sugar and yield of sugar in progeny rows, Madison, 1912; solid line indicates yield of sugar (corrected yield); broken line, percentage of sugar.

A negative correlation occurs between percentage of sugar and average weight of root per row. The most marked occurrence of this kind is shown in the trends of figs. 44 and 45. Divergence is fully as evident, however, in more localized areas and even in individual rows. Compare in this regard the divergence of graphs in rows 47, 72, 127, 157, and 162 of fig. 42, and 240–250, 255–265, and 370–380 of fig. 43.

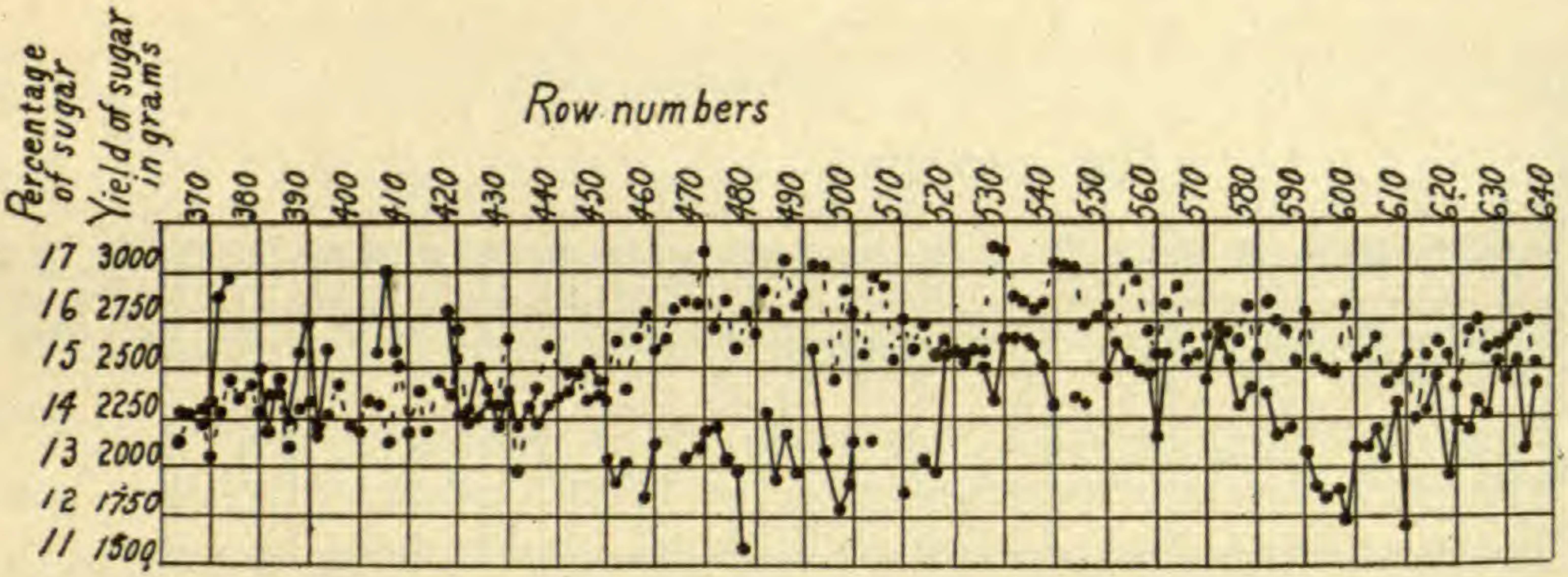

FIG. 41.—Effect of soil irregularities on relationship between percentage of sugar and yield of sugar in consecutive progeny rows, Madison, 1913; solid line indicates yield of sugar (corrected yield); broken line, percentage of sugar.

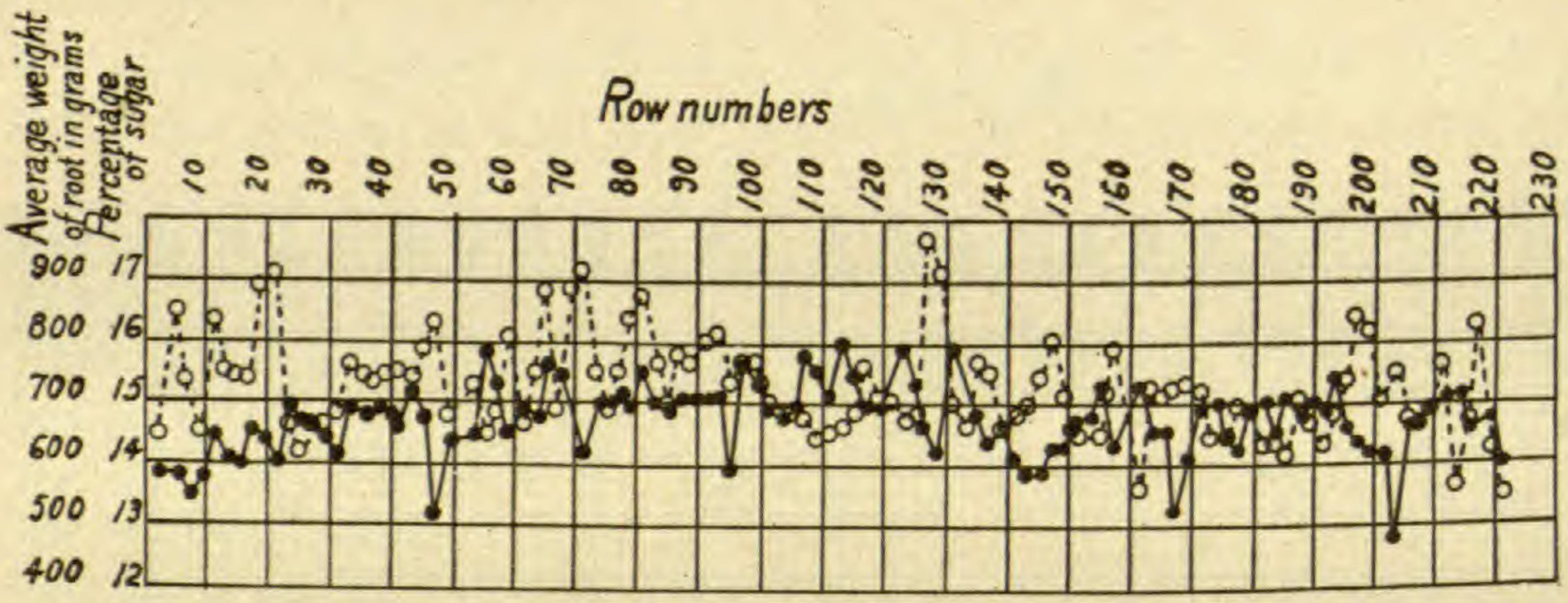

FIG. 42.—Effect of soil irregularities on relationship between percentage of sugar and average weight of root in consecutive check rows, Madison, 1912; solid line indicates percentage of sugar; broken line, average weight of root per row.

A high positive correlation is shown between average weight of root and yield of sugar per row in figs. 46–49. Whenever either graph progresses in a given direction, it is closely followed by the other member of the pair. This is most conspicuous in figs. 47 and 48.

As corrected yields, that is, yields corrected for a full stand by means of the regression coefficient between number of roots and yield of sugar per row, were employed in figs. 38–41, and actual yields in figs. 37 and 46–49, their relationship has been determined and illustrated in fig. 50. Only rows containing a stand of 80–100 per cent are connected. The *o*'s of the unconnected rows represent actual yields, and the *x*'s represent corrected yields of rows containing less than 80 per cent stand.

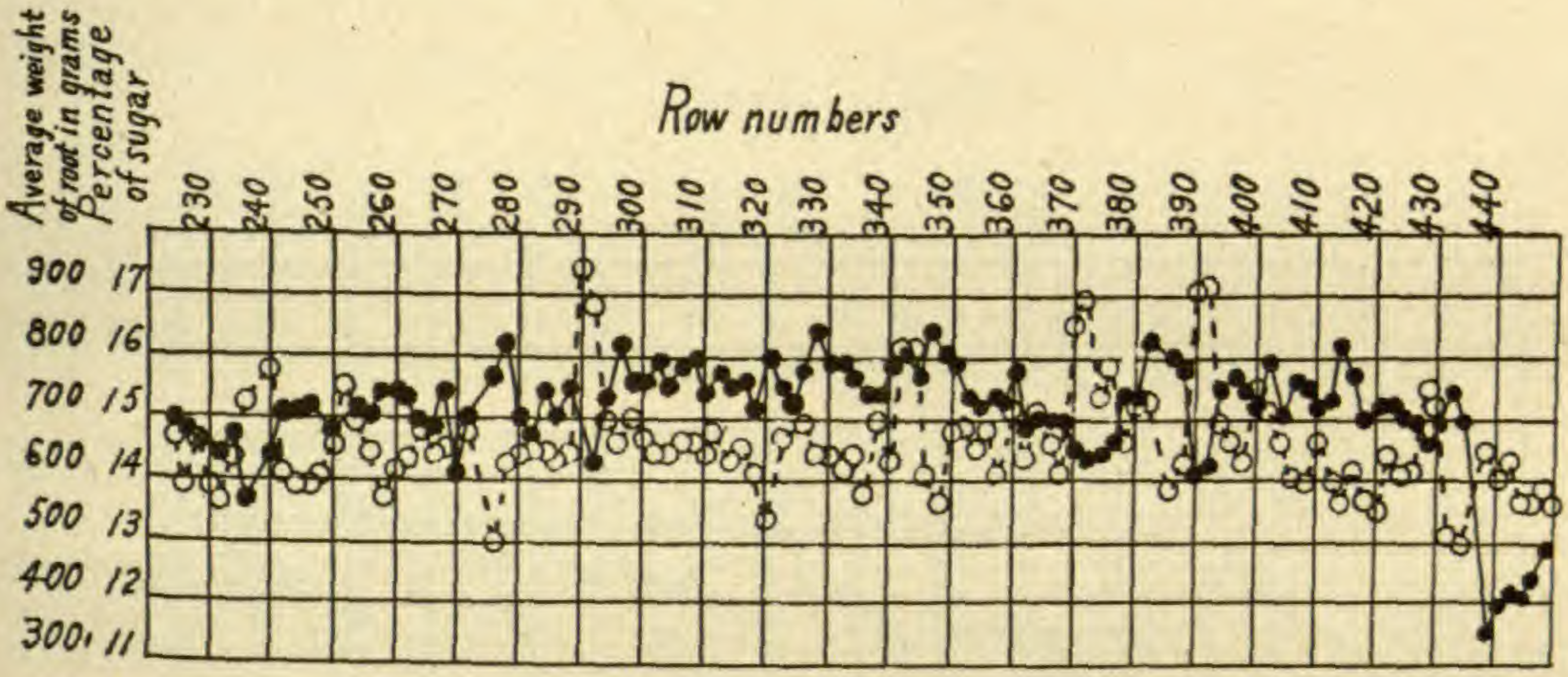

FIG. 43.—Effect of soil irregularities on relationship between percentage of sugar and average weight of root in consecutive check rows, Madison, 1912; solid line indicates percentage of sugar; broken line, average weight of root per row.

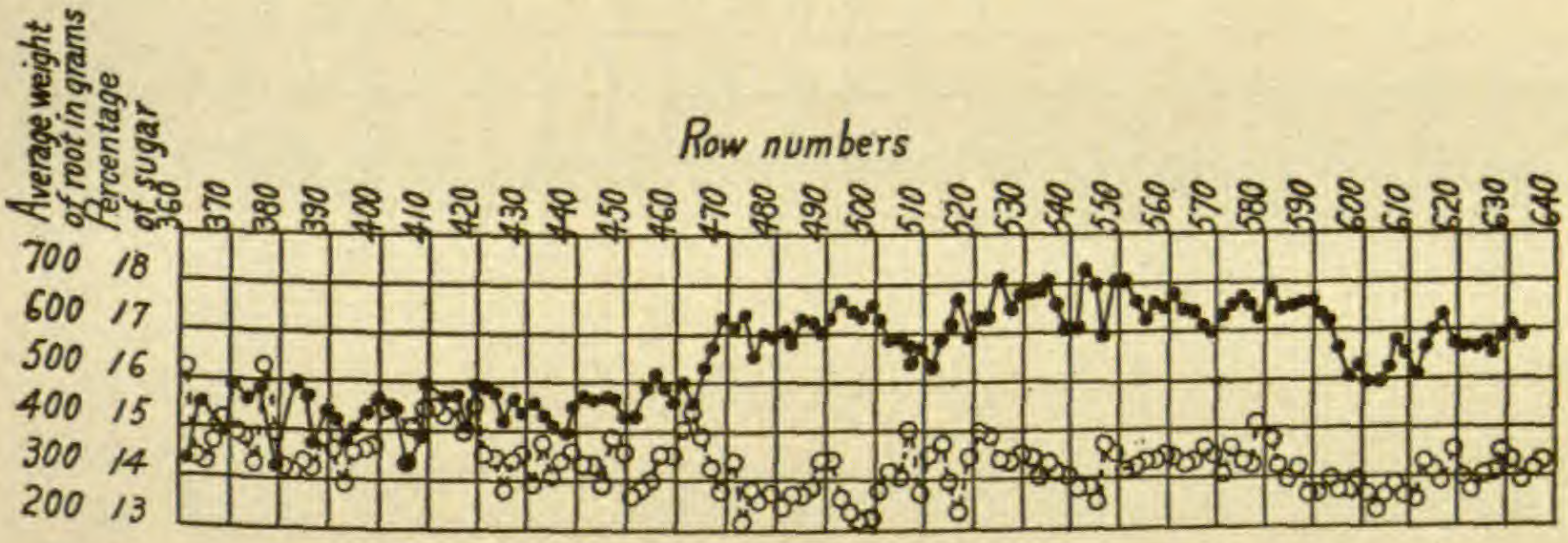

FIG. 44.—Effect of soil irregularities on relationship between percentage of sugar and average weight of root in consecutive check rows, Madison, 1913; solid line indicates percentage of sugar; broken line, average weight of root per row.

The graphs show practically the same progressions and trends. The use of actual yields instead of corrected yields, therefore, in some of the figures did not materially alter the results of comparison.

The relationships exhibited in the foregoing figures between yield and quality of beet rows have a very important bearing upon sugar-beet improvement. Selection for yield of sugar regardless of percentage would increase the size of the root but decrease the proportion of extractable sugar, as large roots have, not only a lower percentage of sugar than small roots, but also a lower coefficient of purity. On the other hand, selection for percentage alone would decrease the tonnage, as percentage of sugar is

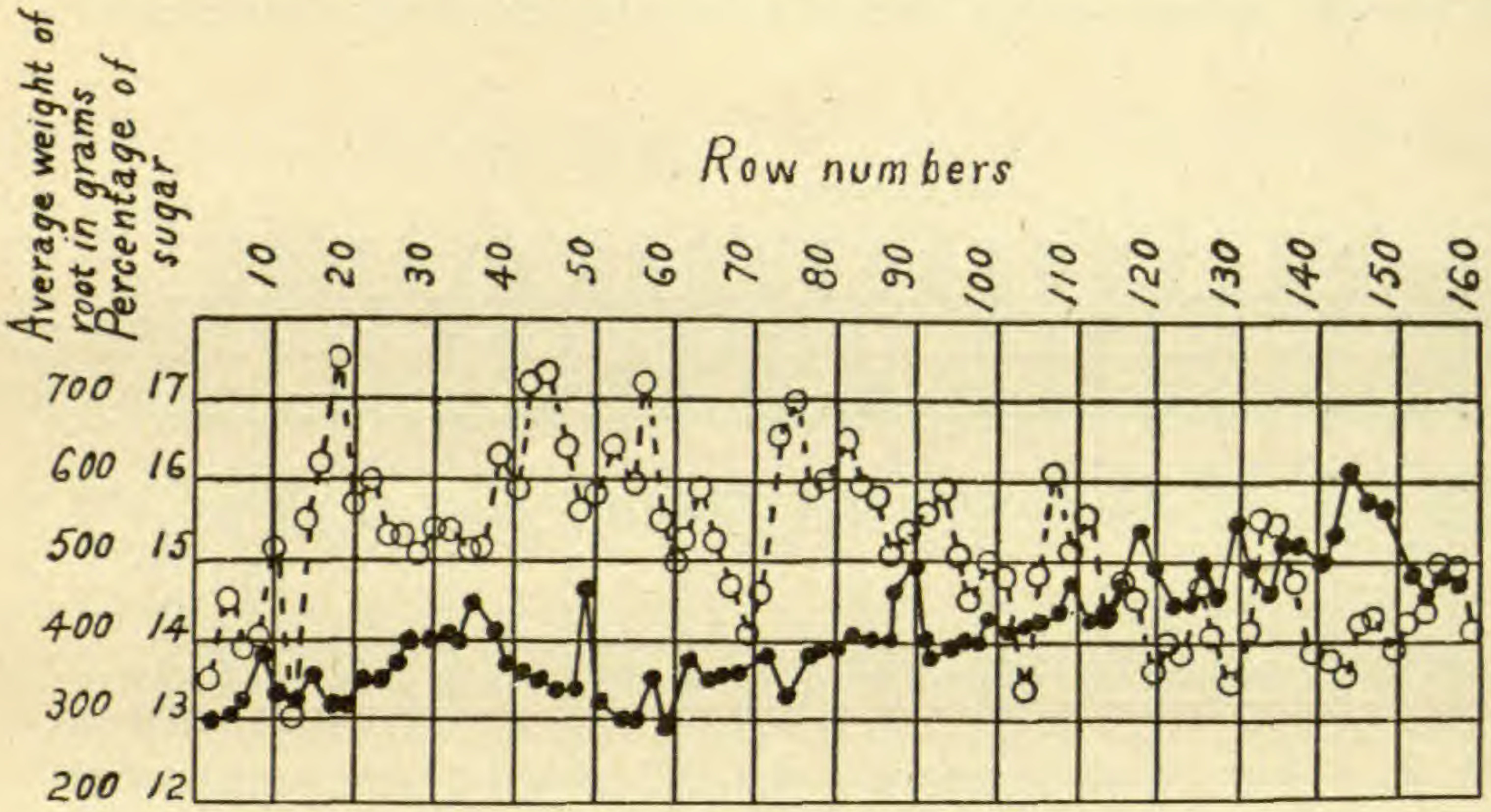

FIG. 45.—Effect of soil irregularities on relationship between percentage of sugar and average weight of root in consecutive check rows, Madison, 1914; solid line indicates percentage of sugar; broken line, average weight of root per row.

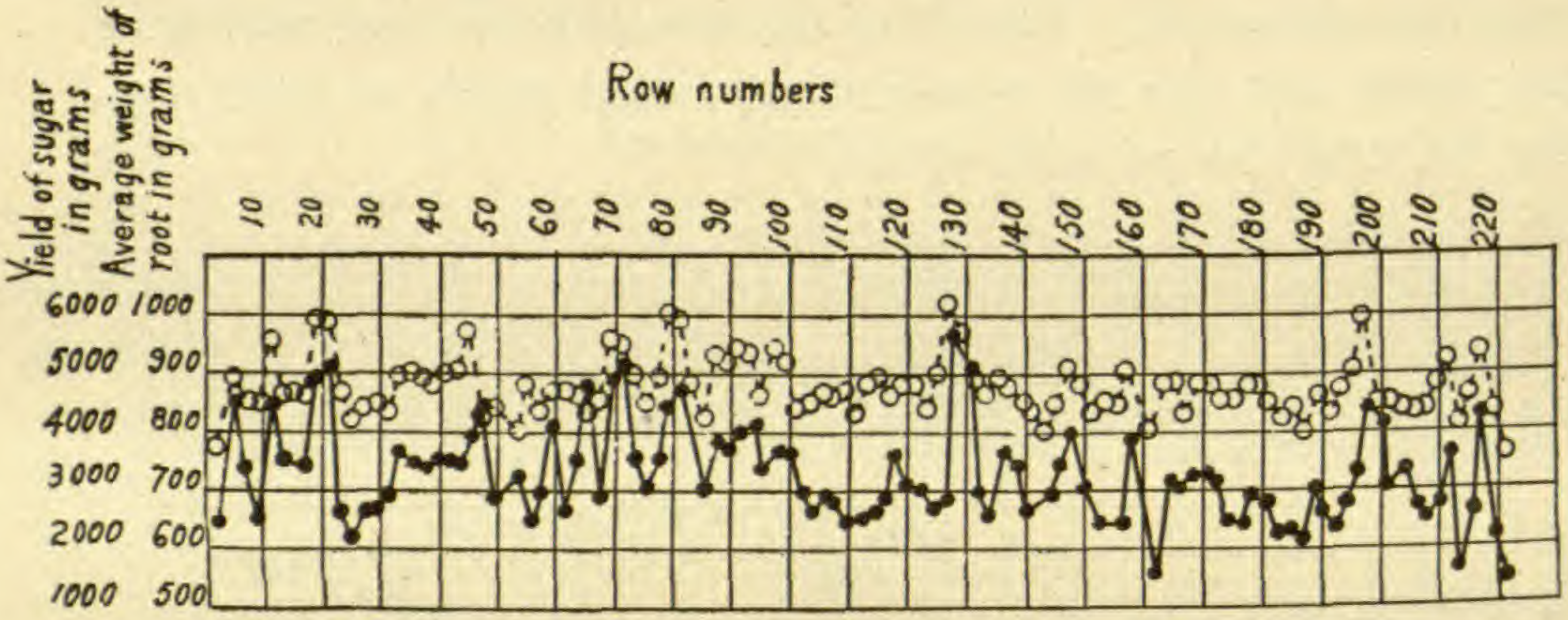

FIG. 46.—Effect of soil irregularities on relationship between average root weight and yield of sugar in consecutive check rows, Madison, 1912; solid line indicates average weight of root per row; broken line, yield of sugar.

negatively correlated with average weight of root. Both yield and percentage, therefore, should be considered in making selections. Obviously, the best single character is yield of extractable sugar, but even with its use a lower limit should be set for percentage. The extra cost of determining coefficients of purity used in calculating extractable sugar, however, would probably condemn the use of this method for commercial purposes.

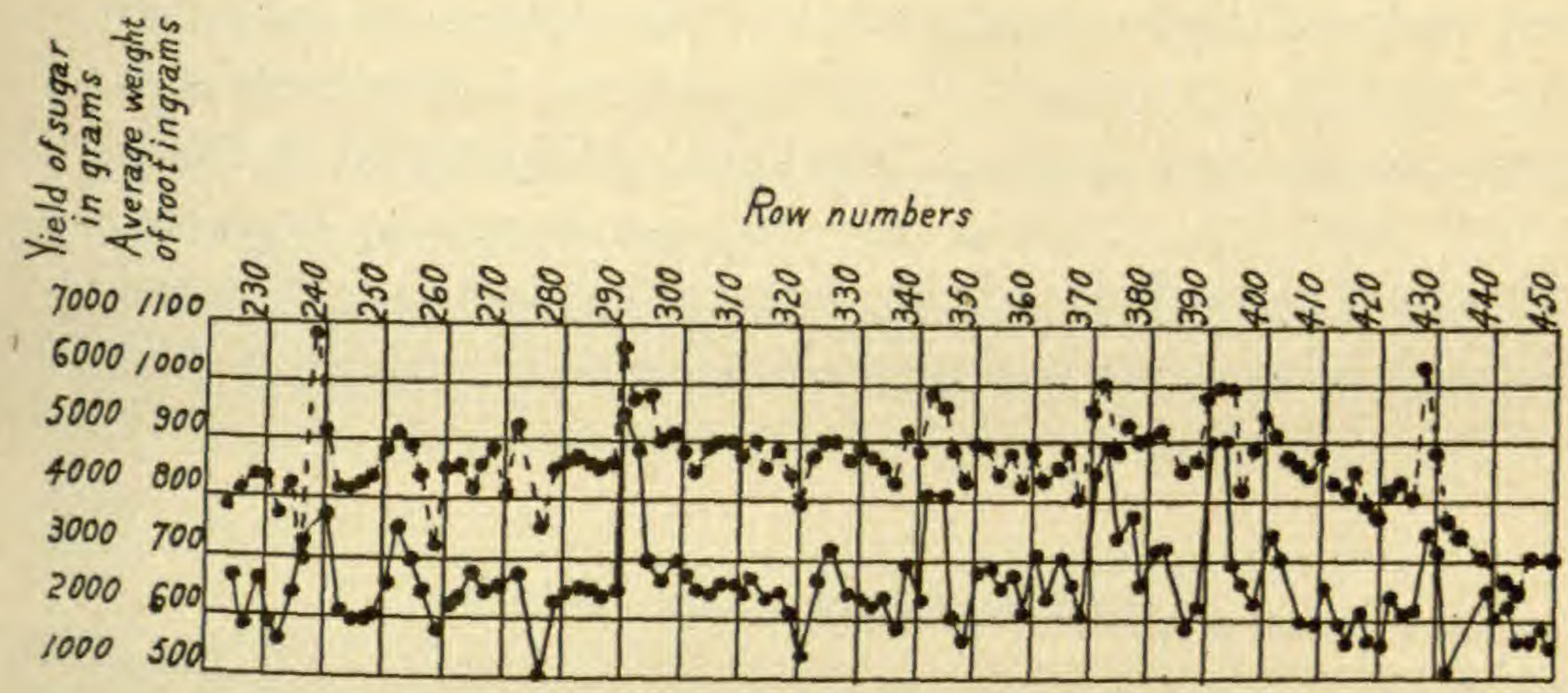

FIG. 47.—Effect of soil irregularities on relationship between average root weight and yield of sugar in consecutive check rows, Madison, 1912; solid line indicates average weight of root per row; broken line, yield of sugar.

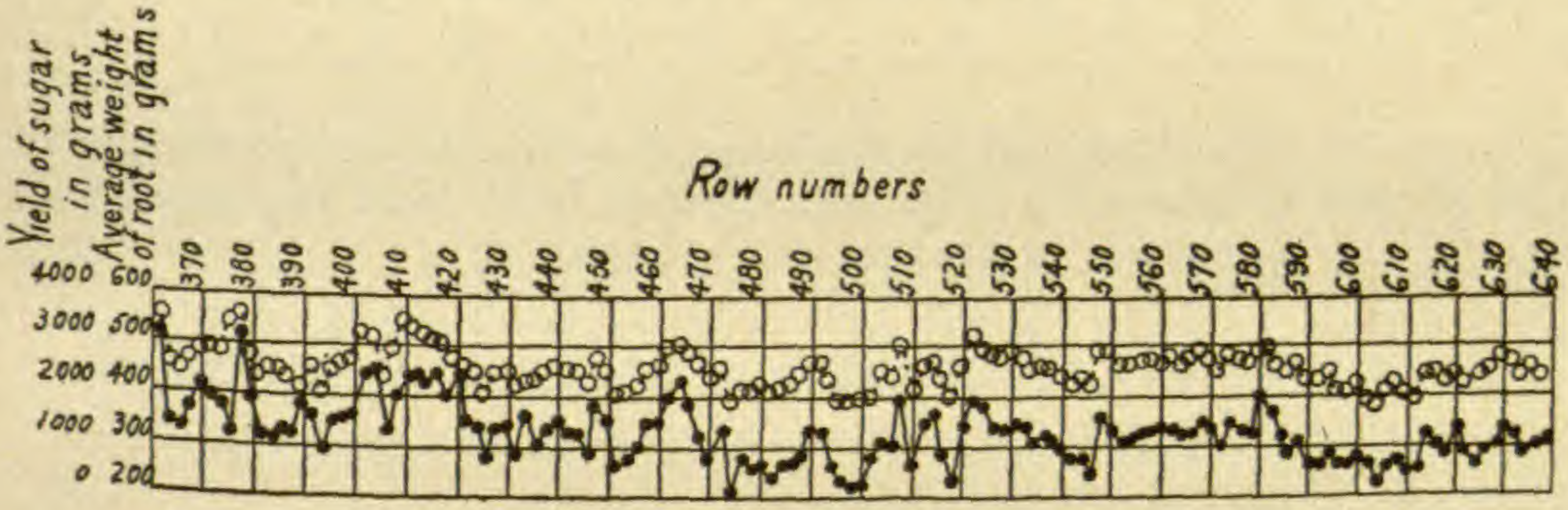

FIG. 48.—Effect of soil irregularities on relationship between average root weight and yield of sugar in consecutive check rows, Madison, 1913; solid line indicates average weight of root per row; broken line, yield of sugar.

Discussion of results

The results of the foregoing experiments may appear, at first sight, to disprove the possibilities of beet improvement, but this is only apparent. The fact of past improvement is beyond question, as the average percentage of sugar, average size of root, and yield of sugar per acre have been increased greatly. Moreover, there is no valid reason for assuming that these characters have reached their maximum development. The differences exhibited by the varieties Morrison's Kleinwanzleben, Kleinwanzleben's Original, and Madison Original Selections, as illustrated in fig. 1, show that sugar-beet varieties may still be altered by breeding. For the sake of discussion it will be assumed that beets are improved

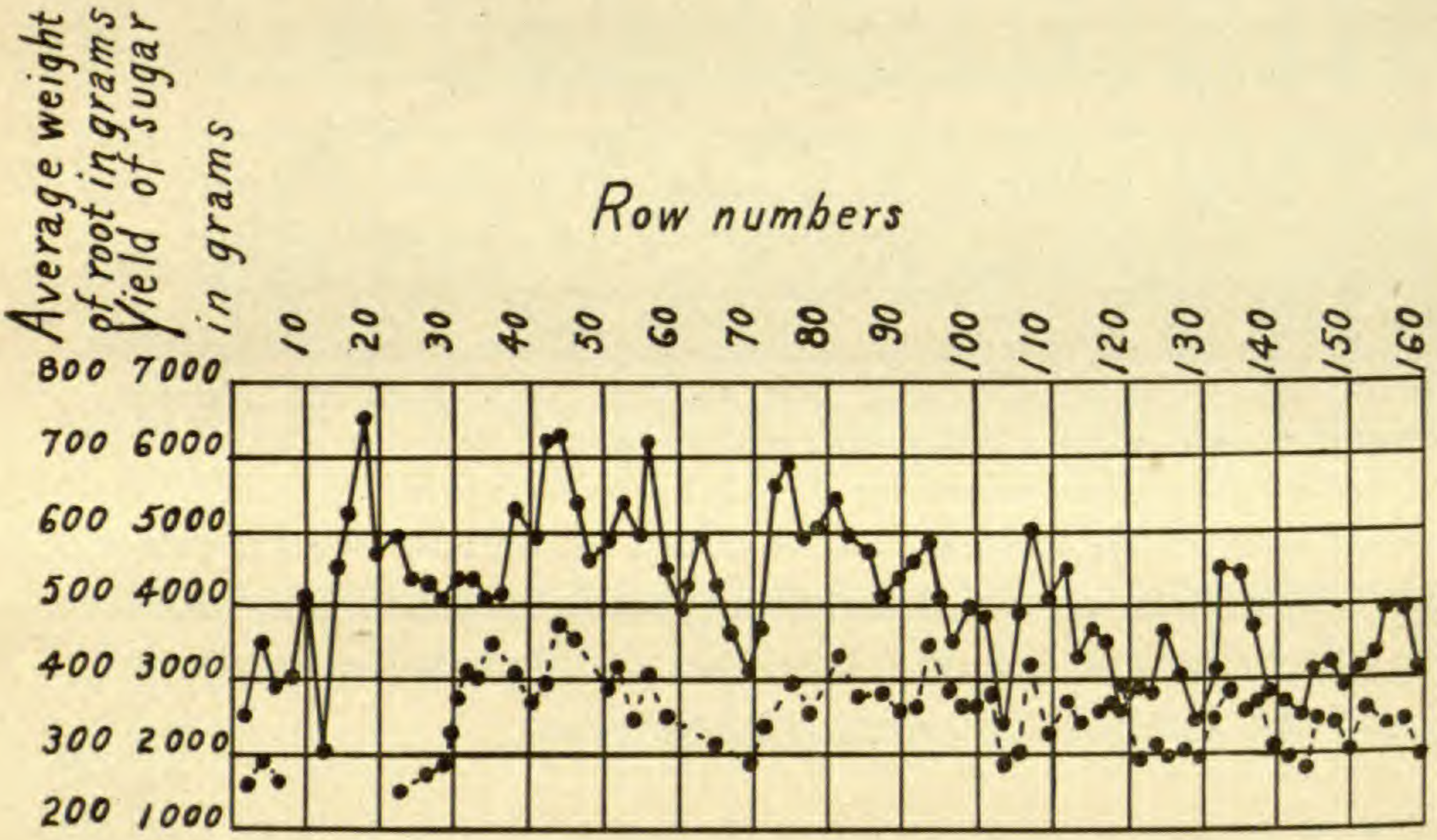

FIG. 49.—Effect of soil irregularities on relationship between average root weight and yield of sugar in consecutive check rows, Madison, 1914; solid line indicates average weight of root per row; broken line, yield of sugar.

either (1) by a gradual accumulation of small variations through the process of continuous selection, or (2) by the isolation and multiplication of an occasional mutation.

Continuous selection is based upon the theory that "like begets like," or the tendency of superior individuals to transmit their qualities to their progeny. As shown in the foregoing tables and graphs, the best roots transmit no better qualities than do mediocre roots. Their differences are mere fluctuations, therefore, and have no influence on beet improvement. This has no bearing, however, upon the value of continuous selection when applied to real differences, as the possibilities of improvement through a gradual accumulation of small real differences is still an open question. Since aside from the progeny test no method of distinguishing real differences between beet roots has been in vogue, the selection of choice roots by chemical and physical means probably has played no part in sugar-beet improvement except where an occasional root has mutated and thus given rise to a superior physiological species.

The real differences between sugar-beet families are usually very small, as may be noted from the difference in size of corresponding variations of individual check and progeny rows. In fact, their real differences are greatly exceeded by their fluctuations.

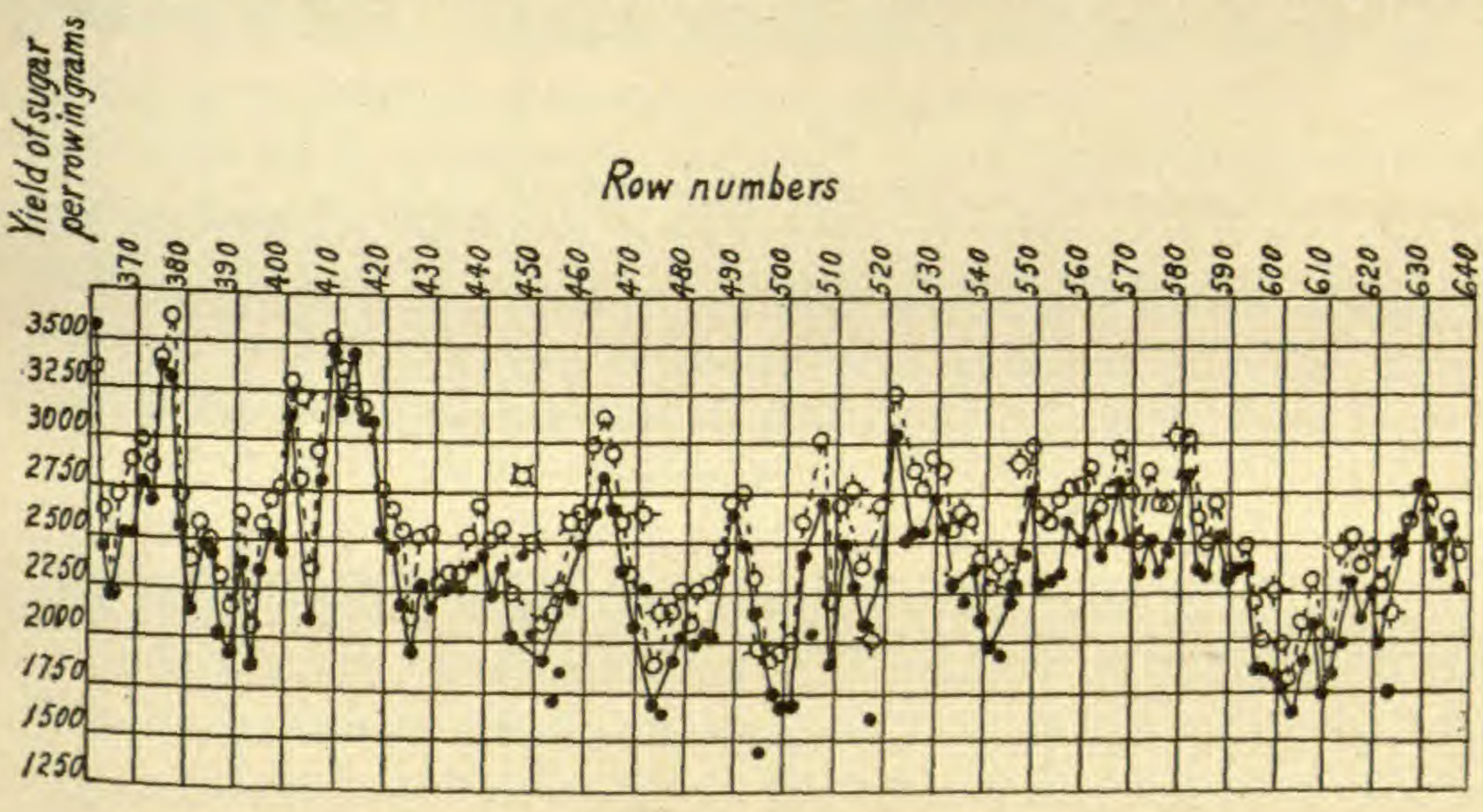

FIG. 50.—Relationship between actual yield and corrected yield of sugar in consecutive check rows, Madison, 1913; solid line indicates actual yield; broken line, corrected yield.

Both the best and the poorest families transmit average qualities. Hence continuous selection does not seem to be an efficient means of improvement. Moreover, it is difficult to conceive how it could have played any important part in sugar-beet improvement in the past.

The isolation of mutations probably offers more promising opportunities for improving beets than continuous selection. Our records show no evidence of mutations, but the numbers are too small to disprove their occasional occurrence. Moreover, no table presented contains data for more than two consecutive generations, which is insufficient to determine with certainty either the fact or the frequency of their occurrence.

The mutations sought by the sugar-beet breeder are not necessarily morphologically distinguishable from other individuals of the variety. They are plants which have undergone constitutional alteration and hence possess new potential limits of physiological elasticity which they transmit to their progeny.

The valuable plants of this class are those which transmit a higher sugar-producing capacity to their progeny than possessed by the variety regardless of their own qualities. In fact, we are hardly justified in assuming that the mutants themselves possess conspicuously high qualities. They are more likely to lie near the mean of the variety than at either limit of its range. This is illustrated for a particular case in fig. 51.

The frequency polygon *A* shows the actual distribution of 3784 beets for percentage of sugar, while A^1 shows a similar distribution of the hypothetical progeny of a supposed mutant. Their means, represented by the vertical lines *M* and M^1, are 17.67 and 18.67 respectively, and hence differ by 1 per cent sugar. . The bulk of the mutant's progeny in polygon A^1 lie between classes 17–21, which is about 22 times as great as the proportion lying above 21. Since the records of the progeny are an expression of the potentialities of the mutant, the mutant itself is 22 times as likely to lie between 17–21 in the original population of polygon *A* as above this group.

The possibilities of finding a mutant by taking a limited number of roots from the material represented in polygon *A* are somewhat greater in the classes above 21 than between 17–21. There are in polygon *A* about 100 times as many individuals in the classes 17–21 as above 21. If the mutant were equally likely to appear in either group, the chances of finding it by taking an equal number of roots from each group would be 0.01 as great in classes 17–21 as in the population above 21. However, we found that the chances of the mutant's lying in group 17–21 are 22 times as great as in the other group, therefore the possibilities of finding it by drawing an equal number of roots from each group are not as 0.01:1, but as 0.22:1, or about 1:4.5. If the whole polygon *A* were divided into two groups, so that all the roots containing 21 per cent sugar or less were to lie in one group and all the remaining roots in the other, the chances of finding the mutant by taking an equal number of roots from the respective groups would be as 1:6.

The ratios 1:4.5 and 1:6 are small and hardly warrant the expenditure of large sums of money for chemically selecting

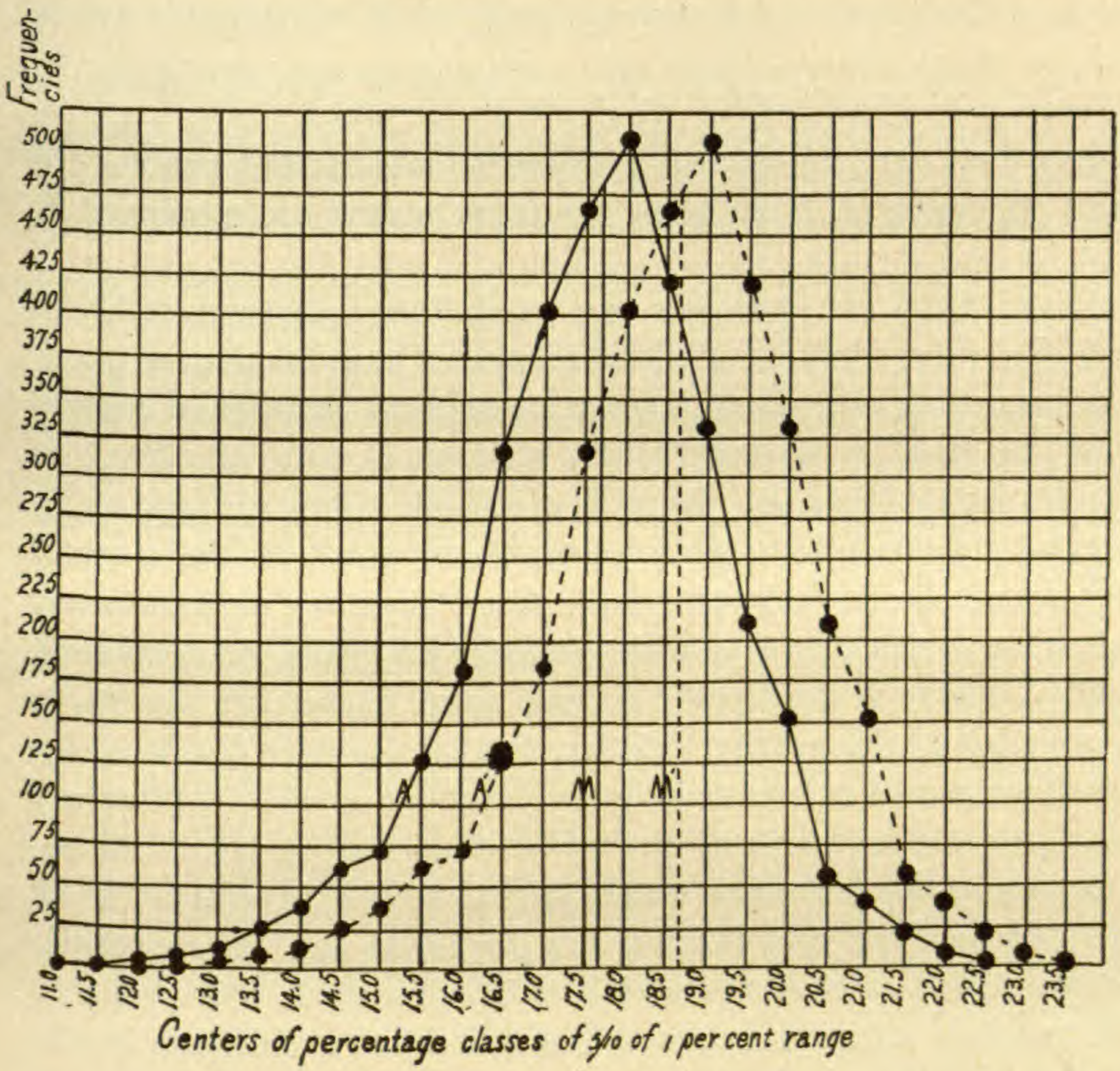

FIG. 51.—Most probable location of mutants

individual roots of high sugar content to increase the probability of finding mutants. Tests of 6–10 times the usual number of roots taken at random would be more economical and fully as effective.

As continuous selection has resulted in failure and only the mutation theory remains as a promising means of sugar-beet improvement, some very decided changes should be made in methods. First of all, more efficient methods of making tests should be devised in order to reduce the effects of soil irregularities and distinguish real differences. This can best be accomplished by a more frequent use of checks and replications. Replications, however, require a liberal seed supply. Although a single plant ordinarily produces a limited quantity of seed, there are means of increasing the normal yield considerably. A root divided into halves and planted at the customary distances will produce nearly twice as much seed as a whole root. Moreover, seed yield is greatly influenced by soil fertility. A rich soil stimulates under a proper temperature the production of flowering shoots and enables the plant to mature a large crop. If these means are employed to increase the average seed production and only the heaviest seed-yielding plants are used for the preliminary tests, a sufficient quantity of seed may be obtained to make a large number of plantings from each individual. When a sufficient number of replications are made to distinguish real differences, only the few best families should be saved. As roots of a single family are apparently of equal breeding value, it is advisable to plant all the roots of each family in an isolated group, where distance or intervening obstructions will prevent their crossing with plants of a different family. There is apparently no gain from harvesting the plants of each family separately. Moreover, the combined yields of the individual plants of a family are necessary to make a satisfactory test of the relative merits of the families the following year. Thereafter, no further selection would appear to be necessary. If all but 3 or 4 of the best families are discarded and the remainder mixed and prevented from crossing with poorer stock, they probably will maintain their vigor and are not likely to deteriorate in the absence of further selection.

The possibility of further improvement apparently lies in the isolation of more valuable mutants, but whether the original stock or the new variety should be used as a source of material can be determined only when it is known in which the mutants will occur with the greater frequency.

SUMMARY OF BIOMETRICAL CONSTANTS COMPILED FROM TABLES II–XI, XIV–XXV, AND XXVII–XXXII

No. of table	Characters	Mean	Standard deviation	Coefficient of correlation
II	Percentage of sugar in root in 1905	19.23±0.042	0.977±0.029	
	Yield of seed per beet in ounces in 1906	8.94±0.179	4.199±0.126	−0.027 ±0.039
III	Percentage of sugar in root in 1906	20.77±0.065	1.296±0.046	
	Yield of seed per beet in ounces in 1907	11.87±0.267	5.357±0.189	0.074 ±0.050
IV	Percentage of sugar in root in 1908	23.60±0.056	1.021±0.039	
	Yield of seed per beet in ounces in 1909	9.68±0.305	5.547±0.216	−0.157 ±0.054
V	Percentage of sugar in root in 1909	20.18±0.052	1.63 ±0.036	
	Yield of seed per beet in ounces in 1910	3.94±0.139	4.36 ±0.099	0.098 ±0.032
VI	Percentage of sugar in root in 1910	18.72±0.031	1.16 ±0.022	
	Yield of seed per beet in grams in 1911	215.90±3.115	117.29 ±2.202	0.049 ±0.026
VII	Quantity of sugar per root in ounces in 1905	2.64±0.037	0.87 ±0.026	
	Yield of seed per beet in ounces in 1906	8.86±0.176	4.14 ±0.124	−0.082 ±0.026
VIII	Quantity of sugar per root in ounces in 1906	3.89±0.086	1.725±0.060	
	Yield of seed per beet in ounces in 1907	11.78±0.268	5.407±0.190	0.081 ±0.049
IX	Weight of root in ounces in 1905	13.94±0.202	4.748±0.143	
	Yield of seed per beet in ounces in 1906	8.94±0.179	4.200±0.126	−0.056 ±0.042
X	Weight of root in ounces in 1906	19.42±0.470	9.480±0.330	
	Yield of seed per beet in ounces in 1907	11.82±0.265	5.341±0.186	0.024 ±0.050
XI	Weight of root in ounces in 1910	18.96±0.151	5.78 ±0.106	
	Yield of seed per root in grams in 1911	216.75±3.086	118.44 ±2.182	0.071 ±0.026
XIV	Percentage of sugar in mother roots in 1908	20.53±0.032	0.472±0.023	
	Average percentage of sugar in progeny in 1910	13.60±0.068	0.998±0.048	−0.149 ±0.067
XV	Percentage of sugar in mother roots in 1912	16.31±0.081	1.065±0.058	
	Value of progeny rows for percentage of sugar in 1914 expressed in percentage of their checks	96.46±0.114	4.499±0.243	−0.040 ±0.076
XVI	Percentage of sugar in mother roots in 1912	16.31±0.081	1.065±0.058	
	Average percentage of sugar in progeny in 1914	13.61±0.074	0.972±0.052	−0.090 ±0.076
XVII	Ounces of sugar in mother root in 1908	2.44±0.029	0.53 ±0.021	
	Ounces of sugar per beet in progeny in 1910	3.70±0.038	0.68 ±0.027	0.032 ±0.050
XVIII	Ounces of sugar in mother root in 1906	3.28±0.107	1.21 ±0.076	
	Ounces of sugar per beet in progeny in 1908	2.43±0.032	0.36 ±0.023	−0.100 ±0.088
XIX	Ounces of sugar in mother root in 1907	4.67±0.063	1.413±0.044	
	Ounces of sugar per beet in progeny in 1909	4.01±0.040	0.897±0.028	0.104 ±0.044
XX	Weight of mother root in ounces in 1908	10.31±0.121	2.19 ±0.086	
	Ounces of sugar per root in progeny in 1910	3.74±0.041	0.75 ±0.029	0.092 ±0.055
XXI	Weight of mother root in ounces in 1906	16.22±0.548	6.19 ±0.388	
	Ounces of sugar per root in progeny in 1908	2.41±0.032	0.36 ±0.023	−0.115 ±0.087
XXII	Weight of mother root in ounces in 1906	16.22±0.548	6.19 ±0.387	
	Average percentage of sugar in progeny in 1908	21.73±0.074	0.84 ±0.053	0.168 ±0.086

SUMMARY OF BIOMETRICAL CONSTANTS COMPILED FROM TABLES II–XI, XIV–XXV, AND XXVII–XXXII—*Continued*

No. of table	Characters	Mean	Standard deviation	Coefficient of correlation
XXIII	Weight of mother root in ounces in 1908	10.29±0.121	2.19 ±0.086	
	Average percentage of sugar in progeny in 1910	18.50±0.047	0.84 ±0.033	0.055 ±0.054
XXIV	Weight of mother root in ounces in 1908	10.25±0.120	2.15 ±0.085	
	Average weight of progeny roots in ounces in 1910	20.61±0.242	4.33 ±0.171	0.023 ±0.056
XXV	Weight of mother root in ounces in 1906	15.74±0.565	6.33 ±0.400	
	Average weight of progeny roots in ounces in 1908	11.28±0.142	1.59 ±0.100	−0.095 ±0.089
XXVII	Average percentage of sugar in beet families in 1912	14.07±0.063	0.826±0.045	
	Average percentage of sugar in beet families in 1914	13.61±0.074	0.972±0.052	−0.229 ±0.072
XXVIII	Value of beet families for percentage of sugar in 1912 expressed in percentage of their checks	96.01±0.329	4.313±0.233	
	Value of beet families for percentage of sugar in 1914 expressed in percentage of their checks	96.35±0.330	4.315±0.233	0.089 ±0.076
XXIX	Ounces of sugar per root in beet families in 1908	2.33±0.014	0.24 ±0.010	
	Ounces of sugar per root in beet families in 1910	3.69±0.042	0.70 ±0.030	0.031 ±0.060
XXX	Average weight of root in ounces in beet families in 1906	18.8 ±0.391	4.30 ±0.277	
	Average percentage of sugar in beet families in 1908	21.7 ±0.077	0.85 ±0.055	0.056 ±0.091
XXXI	Average weight of root in ounces in beet families in 1908	10.75±0.070	1.15 ±0.049	
	Average percentage of sugar in beet families in 1910	18.52±0.054	0.89 ±0.038	0.054 ±0.060
XXXII	Average weight of root in ounces in beet families in 1908	10.74±0.067	1.139±0.047	
	Average weight of root in ounces in beet families in 1910	20.57±0.261	4.45 ±0.185	−0.0003±0.059

Summary

1. Differences in the size and sugar content of individual beet roots show no evidence of inheritance. They are fluctuations, therefore, and apparently play no part in beet improvement.

2. No correlation was discoverable between percentage or quantity of sugar in sugar-beet roots of ordinary sizes and their yield of seed, nor between their yield of seed and the average percentage of sugar in their progeny.

3. The fluctuations of beet families planted in progeny rows in alternation with check rows exceeded their real differences, but real differences were distinguishable by the use of a large number of replications (cf. fig. 1).

4. Areas of beets in an apparently uniform field of small dimensions showed a difference of 2 per cent sugar.

5. Percentage of sugar and yield of sugar of sugar-beet rows vary independently. Progeny rows should be graded on both percentage and yield of sugar, therefore, or on yield of extractable sugar.

6. The average weight of root per row increases with yield of sugar and decreases with percentage of sugar.

7. The discontinuance of selection for one generation caused no deterioration in percentage of sugar. In fact, there was some apparent gain.

8. No improvement in yield or percentage of sugar was obtained by continuous selection. Both the good and the poor families transmitted average qualities.

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POLLEN STERILITY IN RELATION TO THE GEOGRAPHICAL DISTRIBUTION OF SOME ONAGRACEAE[1]

CARL C. FORSAITH

(WITH PLATES XIX–XXI AND ONE FIGURE)

Before the development of methods of research in biology, the question of the origin of species was not debatable, since it was taken for granted that all had arisen as a result of special creation. LINNAEUS, however, in 1757 was convinced that this doctrine needed modification. His departure from the accepted hypothesis was brought about by the appearance of an intermediate form of *Tragopogon* resulting from a cross of *T. pratensis* and *T. porrifolius*, to which he gave the significant species name *T. hybridus*. Too much emphasis cannot be laid upon this fact, since it paved the way for the later and more complete conception of the fundamental laws of evolution.

From that time the occurrence of hybrid forms was recognized now and then, until the reality of their existence became universally admitted. During the later investigations of these plants, it became evident that hybridism was very closely correlated with that of a more or less sterile quality of the generative organs, in proof of which there has arisen a prodigious amount of literature. These numerous citations may well be considered as having established the general biological principle of sexual impotency resulting from earlier parental crosses. Since this form of degeneration is observed more readily in the microspores than in the megaspores, it naturally follows that the presence of a large and varying amount of infertile pollen may be considered as indicative of previous interbreeding of plants where such conditions obtain. Although this relation of pollen abortion to hybridism has become quite widely accepted, there appears to be still some doubt as to the validity of the general principle. Consequently, it may be advisable to mention a limited number of references in its support.

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[1] Contributions from the laboratories of Plant Morphology of Harvard University No. 81.

In 1832 DUTROCHET (10) noticed the abortion of pollen grains in hybrids of *Prunus*. This relation, without doubt, received even greater rcognition under DARWIN'S influence in 1859. The appearance of his *Origin of species* (5), in which he mentions the sterility of the offspring from hybrid parents, did much toward establishing this fact. The impaired fertility of garden varieties of plants likewise is valuable proof of this principle. The long-continued improvement of horticultural species by crossing has led to much investigation in this connection, and as a result many cases are on record where forms thus produced have become nearly or quite completely sterile. As an example of this form of sterility, conditions found in a garden hybrid of *Fuchsia* may be mentioned. This genus is well known to cross readily, and the resultant impotency of the sexual elements was observed by SMITH (22) in 1876. In his article he states that the pollen in garden fuchsias "is always variable." In a hybrid from *Fuchsia splendens*×*procumbens* he noticed that only about one-third of the pollen cells were triangular in form, and the remaining two-thirds were of a "peculiar shape" which (in view of our later studies) may be interpreted as an indication of a tendency to abortion in the anther. During the same year ANDERSON (1) states that only a few seeds in this species were potent, and a condition similar to this was cited by MEEHAN (21) in relation to cultivated varieties of *Viola* in 1889.

Since this article deals more especially with pollen conditions in the genus *Epilobium*, reference is made to HAUSSKNECHT'S (17) monograph of the genus, in which he mentions several hybrid forms. In his discussion of these crosses, he speaks of pollen sterility in relation to hybridization as follows: "Das beste Merkmal gewähren dann in diesem Falle die Pollenkörner, welche ich bei primären Bastarden in der kleinen und geschlossen bleibenden Anthere stets völlig verschrumpft vorfand. . . . ." Statements similar to this were made by GUIGNARD (16) two years later in regard to hybridization in *Cistus, Begonia, Mespilus*, and *Crataegus*, the pollen of which he describes thus: "Les grains de pollen fertiles représentent environs le quart du nombre total, le rest est formé de grains de dimensions moindres, presque vides de protoplasma, ou n'ayant qu'un noyau au moment de la maturité de l'anthère." In his later discussion of a special case, he concludes as follows: "Ce resultat m'apparâit donc fournir un argument important en faveur de la nature hybride de la plante en question."

The advent of the mutation theory of DEVRIES added a new factor to this discussion, the results of which have appeared in numerous publications. A brief mention of a very few of these results in regard to the correlation of hybridization and sexual impotency will suffice. DEVRIES (7) speaks of reduced fertility in crosses of *Oenothera biennis*×*muricata* and *Cytisus laburnum*× *purpureus;* BEER (4) showed that all the pollen of *Oenothera longifolia* did not reach maturity, as there was not sufficient nutritive material to carry all the grains to completion; TISCHLER (23) described a *Bryonia* hybrid resulting from a cross of *Bryonia alba* and *B. dioica* thus: "Die Pollenkörner sind sehr haufig (bei GREGORY fast immer) degeneriert, und von sehr ungleicher Grösse"; and GATES (12, 13, 14) describes the cytological development in relation to pollen degeneracy in some hybrid species of *Oenothera.*

BATESON (3) has been very influential in establishing the principle of sterility as a result of hybridism. Numerous references to his own observations add much weight to the value of the probability that previous crossing is indicated by an important condition of the microspores. This proof is augmented likewise by many citations of the results obtained by other investigators of this phenomenon. Conclusions similar to these were obtained by JEFFREY (18, 19, 20) as a result of studies of a large number of the vascular cryptogams, conifers, and angiosperms. The results of his researches may be summarized briefly as follows. In the more constant and distinct species, the pollen grains are generally equal in size, full of protoplasm, and show no indications of an arrested development. On the other hand, those forms which show a large degree of fluctuation and contain known hybrids, generally present a varying amount of impotent pollen cells. These opposing conditions are strictly in accord with the general principle just mentioned. Consequently, JEFFREY was led to infer that those species which are characteristically constant and exhibit perfect pollen should be considered as pure. In contrast to this type of spore development, he regarded those forms which are habitually variable and which present impotent microspores as hybrid segregates, or hybrid species.

In view of this great quantity of evidence in favor of the coexistence of hybridism and sterility, together with a habitual lack of contradictory proof, it seems safe to assume that these conditions illustrate a general biological correlation. Consequently, whenever there appears a large percentage of impotent pollen on the anther, the natural conclusion is that this sterile state has been brought about by previous ancestral crossing.

These facts naturally lead to the question of the stability of hybrids in nature, which the morphological evidence of pollen sterility reveals as such. This question can likewise be answered in the same manner as the first, namely, by reference to cited examples. BATESON (3) in his discussion of this subject mentions a number of instances where hybrids have remained constant, instead of following the usual Mendelian principle. In regard to this phenomena he states as follows: "Literature of hybridization and heredity abounds with examples of hybrids which are said to have bred true, or, as we should say, without segregation"; DE VRIES (7) in a similar discussion of *Oenothera biennis*×*muricata* mentions constant hybrids which resemble one parent more than the other; and DAVIS (6) in speaking of crosses of *Oenothera biennia* and *O. grandiflora* says that hybrids were produced which resembled *O. Lamarckiana* to such a degree as to justify the consideration as a working hypothesis that *O. Lamarckiana* arose as a hybrid, and (to quote more directly) "it will be apparent to the reader that if the evidence should finally indicate *Lamarckiana* to be of hybrid origin, as a number of writers have already suggested, many of the so-called mutants are likely to be interpreted as segregates splitting off according to Mendelian expectations." Thus it is apparent that there are numerous quite constant hybrids which may be considered as segregated species.

Having discussed the morphological indications of the relation of pollen sterility to that of hybridization, and the proof of the stability of hybrids thus formed, it is evident that these two so well established principles must have some bearing upon the origin of certain races of plants. At present there are two conceptions in regard to the evolution of species. There is that of DARWIN (5) which states that new forms represent a gradual transition of the existing structure through a slow process of a natural selection of minor variations. In opposition to this doctrine, there is the DeVriesian hypothesis of mutation, which explains the origin of new forms by the sudden acquisition or loss of characters (known as germinal variations), which are inheritable and breed true to type. The strongest argument against this latter conception is presented by the fact that the so-called mutants, especially those of *Oenothera*, show all the morphological evidences of hybrid origin. Those who support this view of evolution, however, state that the sterility of the pollen is as much an evidence of mutation as hybridization, but they fail to show the distinct difference between so-called mutants and those forms which may represent segregates derived from hybrid ancestors.

The argument that there is no tangible evidence of a gradual evolution is always advanced as a refutation against the Darwinian doctrine. As BARTLETT (2) states, "the few who still hold that the selection of continuous variations would suffice to bring about specific differentiation can bring forward little or no evidence to support their view. The evidence all points to the utmost fixity of organisms, aside from mutations." This point of view is based upon an apparent lack of transitional forms which are, in fact, characteristically absent in so far as the exterior structure is concerned. This conception owes its origin to the paucity of fossil plants, and the highly altered state of the external features of the remains which have been preserved as carbonized or petrified material and impressions. On the other hand, the internal organization of the woody stem shows abundant evidence of a gradual transition, in striking contrast to a characteristic lack of proof of the sudden appearance and later utmost fixity of anatomical structures. Although the general principles of plant morphology, that is, retention, reversion, and recapitulation, have become firmly established, and although their value in solving evolutionary problems is widely recognized, it may be advisable to mention a few examples which will show their application to the doctrine of a slow and continuous development.

Studies in this direction have shown that there has been a gradual transition of the pitted tracheid from that form characteristically present in the lowest of the vascular cryptogams, to that type normally found in the higher plants. The vessel also shows evidences of a continuous and uninterrupted evolution from that form found in the Gnetales to the compositaceous type of conducting elements. In the case of the wood ray, all the evidence points to this type of development. In the lowest forms of the vascular plants the ray is represented by a slight transformation of the tracheary elements, which gradually give place to the uniseriate parenchymatous plate, characteristic of the conifers. In the Gnetales and the lower angiosperms, this modification of the fibrous material occurs as a band of intermingled ray parenchyma and lignified fibers (the aggregate ray). By a gradual transition of the included tracheids or libriform fibers to storage cells (the first stage of which is seen in the septate condition of the fibers) the broad compound ray is formed. The wood ray reaches its highest development in the more advanced angiosperms, where the aggregate form has become spread out into scattered plates known as diffuse rays. Similar evidences of a gradual transition are seen in the development and distribution of wood parenchyma. This proof of a slow and unbroken evolution of these several woody elements is based, not only upon a study of fossil and existent forms, but also upon a comparison of the relative relation of progressive and conservative regions of the plant.

The value of this evidence of a gradual transition of woody structures cannot be overestimated, since it represents facts supporting general principles and distinctive of all the vascular plants from the lowest to the highest orders. Consequently, any factors of so universal occurrence as the development of the woody stem, conditions as equally characteristic of ancient as of modern forms, cannot logically be considered as "little or no evidence" in favor of a dilatory and uninterrupted change to a different type of structure. Furthermore, when the amount of proof exhibited by a study of all the main groups of the higher plants is weighed against the quantitative examples of so-called mutations, its numerical predominance is strikingly in favor of the Darwinian rather than the DeVriesian hypothesis of evolution.

In contrast to this slow transformation in plants, there are larger and more inconstant variations which are brought about as a result of hybridization. These differences, thus formed, may follow a strict Mendelian ratio, or become segregates which breed true to type. This manner of fluctuation is so well known, however, that any further discussion of it is manifestly unnecessary.

Turning again to the consideration of the probable origin of species by mutation, as advanced by those who are in favor of this view, it is at least significant that by far the greater majority of the so-called species thus produced have descended from ancestry whose genetical purity is questionable. The genus *Oenothera* has been mentioned so frequently in this connection that it naturally deserves special consideration. This genus has long been known to hybridize freely, and also to have produced constant generations from crosses as a result of a segregation of characters. To place so much weight upon such precarious evidence as a distinction between segregates and so-called mutants, therefore, appears from the morphological standpoint, at least, to be unjustifiable.

Since the question of mutation so intimately concerns the Onagraceae, it seems advisable to consider the probable occurrence of hybrids in a few genera other than *Oenothera*, as indicated by the morphological evidence of pollen sterility. In this connection the following species will be discussed in respect to the probable relation of pollen sterility to hybridism and geographical distribution, namely: *Epilobium angustifolium* L., *E. latifolium* L., and *E. Dodonaei* Vill. of the subgenus *Chamaenerion; Epilobium hirsutum* L., *E. luteum* Pursh, and *E. obcordatum* Gray of the section *Lysimachion; Clarkia pulchella* Pursh, *C. rhomboidea* Dougl., and *C. Xanthina* Gray; *Oenothera serrulata* Nutt.; *Gongylocarpus fruticulosus* Benth. and *G. rubicaulis* Cham. and Schl.; *Diplandra lepezioides* Hook. and Arn.; and *Zauschneria californica* Presl.

The material for investigation was selected from herbarium specimens, representing widely separated stations throughout the greater part of the ranges of these species. Mature buds were chosen and treated with alcohol and NaOH, which caused them to swell to their original size. The pollen thus treated was examined microscopically, and the relative amount of abortive grains (those which were shrunken, small, or empty) was determined by a more or less careful count of several fields of view. The difference between the fertile and the sterile microspores is perfectly distinct. The fertile ones appear as fully developed uniform spores filled with protoplasm; while those which have become degenerate are small, shrunken, and empty. Those buds, however, which showed less than 5 per cent of infertile pollen were considered as representative of genetically pure descent. This figure was chosen by DORSEY (**9**) after a careful investigation of *Vitis*. In this genus species of unquestionable ancestry were shown by a careful count never to contain more than this ratio as a result of physiological conditions.

From this general discussion of the relation of pollen sterility and variation in respect to hybrid formation, attention may be turned profitably to a more detailed discussion of the *Chamaenerion* group of *Epilobium*. It is of interest to note that DEVRIES (**8**) has stated that the pollen in *Epilobium* is "wholly fertile," but the writer's observations have led to different conclusions in regard to microspore conditions in this genus. *Epilobium angustifolium* L., with few exceptions, presents good pollen grains in the anthers chosen from plants which were collected in the more southerly part of its range. This condition is significant, since this region represents that part of its zone where it is outside the influence of *E. latifolium* L. This uniformly pure condition of the male generative cells is, without doubt, due to the fact that in the more southern stations *E. angustifolium* is practically a monotypic species, owing to the absence of an ally with which it is able to cross. On the other hand, where the "great willow herb" grows within the range of *E. latifolium*, the pollen shows a tendency to abortion in a varying degree.

A still more comprehensive understanding of the apparent relation of pollen sterility to geographical distribution can be obtained by reference to figs. 1–8, text fig. 1, and list I. Fig. 1 shows the pollen of *E. angustifolium* L. as it appears in plants from Auburn, New Hampshire. It will be observed that all the grains are of uniform size and full of protoplasm. Fig. 2, representing a much enlarged view of pollen from the same region, shows clearly that there are no defective grains, but that all are equally well developed. Fig. 3 illustrates similarly perfectly developed microspores from the anther of a "fireweed" collected near Pocono Plateau, Pennsylvania, and the uniform structure of the pollen from a West Virginia specimen is pictured in fig. 4. The two abortive grains among the perfectly formed ones in fig. 4, without doubt, have resulted from physiological conditions. In contrast to this perfect development of the pollen cells in these more southern representatives, fig. 5 represents the formation characteristic of the more northern regions where it grows within the range of *E. latifolium*. The pollen for this and the following illustration was chosen from a plant collected on Mount Albert in Gaspé

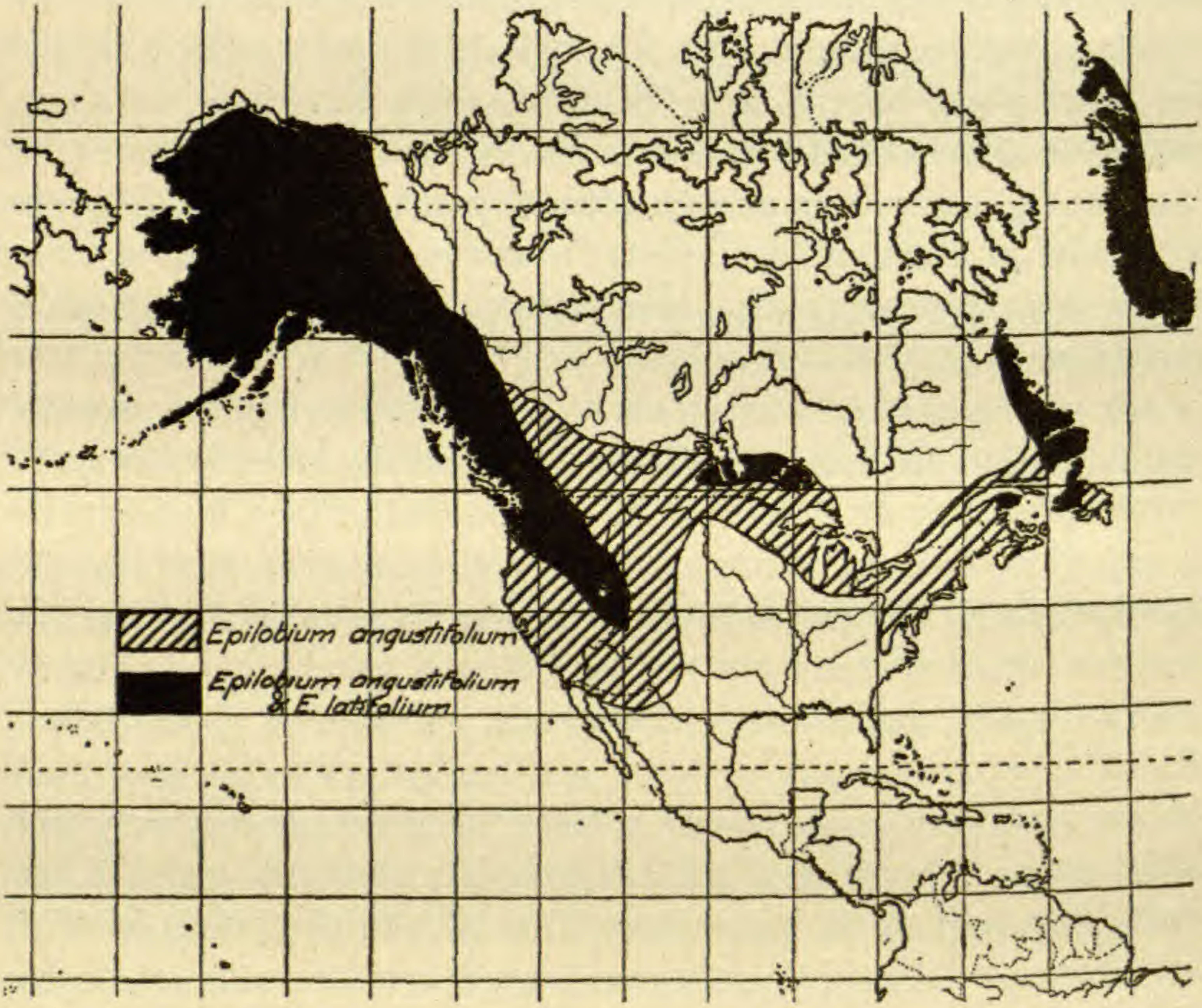

FIG. 1.—Relative distribution of *Epilobium angustifolium* L. and *E. latifolium* L.; solid black indicates region where the two species are coexistent; hatching indicates territory where *E. angustifolium* extends beyond range of *E. latifolium*.

County, Quebec. Here, it will be observed, nearly one-third of the microspores are destitute of protoplasm, and all the grains, with a single exception, are much reduced in size. In fig. 6 similar indications of hybridism appear. In one case all the grains in a single tetrad, still remaining intact, have become defective. That like influences of degeneration may have caused many of the microspores of a Himalayan representative to stop growth in the early stages of development may be seen by reference to fig. 8. Several of the grains are perfectly developed, but a close observation will show also many small sterile pollen cells among those which have reached maturity.

A comparison of text fig. 1 and list I will show that there is a quite general tendency to abortion in that part of its range where *E. angustifolium* coincides with that of its nearest ally, *E. latifolium.* Defective microspores as an indication of hybridization are indicated by an asterisk in the list. In text fig. 1 those regions where these two plants are coexistent are represented in solid black. In contrast to this, those where *E. angustifolium* occurs alone are represented by hatching.

In view of the general principle of the correlation of pollen sterility and hybridism, previously mentioned, these facts are significant. From these studies it is apparent that when *E. angustifolium* grows within the range of its nearest ally, crosses take place which result in hybrid offspring. This relation is strikingly evident from the morphological standpoint at least, when one takes into account the presence of defective microspores in specimens chosen from that part of its habitat coterminous with that of *E. latifolium.* In contrast to this condition there is the more constant development of the pollen in buds selected from the more southern stations where *E. angustifolium* is practically monotypic.

LIST I

STATIONS OF *Epilobium angustifolium*, SHOWING RELATION OF PERFECT AND DEFECTIVE POLLEN TO LOCALITIES; DEFECTIVE POLLEN INDICATED BY AN ASTERISK

*Blanc Sablon River; Grand Falls, Newfoundland; East Avalon Peninsula, Newfoundland; *North of St. Paul's Bay, Cow Head, W. Newfoundland; *Curling, Bay of Islands, Newfoundland; *St. George's Pond, near Bay of

Islands, Newfoundland; Grindstone Island, Magdalen Islands, Quebec; *Seven Islands, Saguenay County, Quebec; *Lake Edward, Quebec; *Rivière du Loup, Timiscouata County, Quebec; *Mt. Albert, Gaspé County, Quebec; Table-topped Mountain, Gaspé County, Quebec; Little Métis, Quebec; Prince Edward Island; Kings Port, N.S.; Dead River, Somerset County, Me.; Fort Fairfield, Aroostook County, Me.; Jaffrey, N.H.; Mt. Washington, N.H.; Auburn, N.H. (figs. 1, 2); Mechanicsville, Vt.; Willoughby Notch, Vt.; Ashburnham, Mass.; Newton, Mass.; Southington, Conn.; Pocono Plateau, Pa. (fig. 3); Nescopec, Luzerne County, Pa.; Blister Swamp, Randolph County, W.Va. (fig. 4); *Mungo Park, Nipigon Lake, Ont.; *Brown County, Wis.; Keweenaw County, Mich.; Banff Clearing, Alberta, Can.; Center City, Minn.; New Mexico; *Bridge Park, Carbon County, Wyo.; *Artist's Glen, Colo.; *Weber River, Wahsatch Mountains, Utah; *Revelstocke, Selkirk, Can.; Carbonate Landing, B.C. (50° 30″ n. lat.); *Crane Lake, Assiniboia, Can.; *Emerald Lake, B.C.; Renfrew, Vancouver Island, Can.; *Silver City, Owyhee County, Idaho; Boise, Idaho; *Canyon County, Idaho; Clear Creek Canyon, Ormsby County, Nev.; Gunnerson Watershed, W. Cent. Colo.; Saturna Island, Humboldt Bay, Ore. Boundary; Clark Springs, Spokane, Wash.; Egbert Spring, Douglas County, Wash.; Swauk River, Kittitas County, Wash.; *Spalding, Ore.; Ten Sleep Lakes, Big Horn County, Wyo.; *Wahlamet, Douglas County, Ore.; Cold Water Canyon, San Bernardino County, Cal.; Waitesburg, Wash.; *Mt. Rainier, Wash.; Cascade Mountains (49° n. lat.); *Dutch Harbor, Unalaska, Aleutian Islands; *Makushin Bay, Unalaska; *Nazan Bay, Atka, Aleutian Islands; *Lake Lindman, Yukon River; *Disko, Greenland; *East Greenland; *Nain, Labrador coast; *Roma, Labrador coast; *Okak, Labrador; Hopedale, Labrador; Grand Lake, Newfoundland; *Newfoundland coast; *Table-topped Mountain, Quebec (fig. 7); *Mt. Albert, Gaspé County, Quebec (figs. 5, 6); *Turin, Marquette County, Mich.; Pagosa Springs, S. Colo.; San Francisco Mountains, Ariz.; Socorro County, N.M.; *Yellow Stone National Park; *Roger's Pass, Selkirk (51° 30″ n. lat.); Nagai Island, Schumagin, Alaska; Tulare County, Cal.; *Bighorn Mountain, Wyo.; *Nashes Fork, Albany County, Wyo.; *Yukon, Can.; Anvik, Alaska; *Pluma, S.D.; Paradisino, Italy; *Lucetheshend, Surrey, England; *Sapporo, Japan; *La Buillard, Vosges Mountains, France; Louette St. Pierre, Belgium; Hupeh Province, Central China; *Rimkin, Tibet (fig. 8); Manchuria; Kurrum Valley, Afghanistan; *Island in Okhotsk Sea, N. Pacific; *Quichjock, Lapland; Hakone, Japan; *Reykjahled, N. Iceland; *Deuthfoss, Iceland; Harx, Germany.

Epilobium latifolium L. shows a similar condition of pollen sterility as a result of past crossing of its progenitors. Out of 32 plants examined, only 4 were found to contain structurally perfect cells, in contrast to 28 which showed abnormally developed microspores, ranging from 10 to nearly 100 per cent in some cases. It is not surprising that this species should show a larger degree of abortion than the allied member of the subgenus *Chamaenerion*, since its entire range coincides with that of *E. angustifolium* as represented by the solid black portion of text fig. 1. List II shows the relative amount of sterile and perfect specimens of pollen. Fig. 9 illustrates pollen conditions in *E. latifolium* L. collected in Newfoundland. Reference to this figure will show that nearly all of the grains (those which are small, empty, and shriveled) are degenerate. Fig. 10 pictures the microspore formation in a plant from La Plata Canyon in Colorado. It will be observed that all the grains, with a single exception, are shrunken, and the fully formed one is abnormal in having more than the usual number of germination pores. Evidences of hybridism in an Alaskan *E. latifolium* are indicated in fig. 11. In this illustration the infertile grains appear as shrunken cells which are destitute of protoplasm. It is of importance to note in this connection that there is a general tendency in *E. latifolium* to abortion, and this condition is still more suggestive when it is observed that this plant, throughout its entire range, is coexistent with *E. angustifolium*.

LIST II

STATIONS OF *Epilobium latifolium*, SHOWING RELATION OF PERFECT AND DEFECTIVE POLLEN TO LOCALITIES; DEFECTIVE POLLEN INDICATED BY AN ASTERISK

*Disko, Greenland; *Godthaab, Greenland; *Tessiastuk, Greenland; *Hamilton Inlet, Indian Harbor, Greenland; *Roma, Labrador coast; *Valley of Exploits River, Newfoundland; Bay of Islands, Newfoundland; *Rope Cove, Newfoundland (fig. 9); *Grand Cascapedia River, Quebec; *St. John River, Gaspé County, Quebec; *Dartmouth River, Gaspé County, Quebec; *La Plata Canyon, Colo. (fig. 10); *Gunnerson Watershed, W. Cent. Colo.; *Graymount, Colo.; *Clear Creek, Colo.; *Buffalo River, Wyo.; *Mary Baker Lake, Mont.; *Fort St. Michael, Alaska; *Mt. Rainier, Wash.; *Seattle Wash.; *Shunagan Feld, Alaska; Lake Indian, Upper Yukon; *Chugackik Bay, Alaska; *Yakutat Bay, Alaska (fig. 11); *Selkirk, 118° 20′ n. long. and 51° 45′ n. lat.; *Juneau, Alaska; *Cold Foot, Yukon River, Alaska; *Cape Nome, Alaska; *Nazan Bay, Atka, Aleutian Islands; Akutan, Aleutian Islands; Glacier River, Unalaska, Aleutian Islands; *Mountains of Colorado.

It has already been pointed out that those species in which hybridism is habitual generally show a well marked variation. In this connection it may be well to mention the genera *Rubus*, *Rosa*, and *Crataegus*, whose fluctuation is very pronounced, in proof of which the lack of agreement in regard to their classification among systematic botanists is significant. This type of variability likewise is very noticeable in a study of any large amount of herbarium material of *Epilobium*, such as was observed by the writer in his examination of specimens at the Gray Herbarium. It is equally important in this connection to state that HAUSSKNECHT (17) mentions 14 *formae* of *E. angustifolium*, 10 of *E. latifolum*, and 6 of *E. Dodonaei*. Thus it would appear that this subgenus presents capricious conditions of change similar to the genera just mentioned, which possess known hybrids and likewise present morphological evidences of pollen sterility as an indication of past interbreeding.

Epilobium Dodonaei Vill. is apparently subject to similar sterile conditions, since 2 out of the 3 specimens obtainable showed abortive pollen. This feature was also observed by Miss HOLDEN in Cambridge, England, as a result of an investigation of microspore conditions in relation to hybridism in this species and in *E. angustifolium* from Europe. The probability that crosses of this nature have taken place is strengthened by the fact that a hybrid from *E. Dodonaei*×*E. spicatum* is noted by HAUSSKNECHT as described by HENNIGER.

Before turning to other species of the Onagraceae it seems advisable to summarize briefly the behavior of the species already discussed. It is apparent that the section *Chamaenerion* presents features which are characteristic of genera subject to hybridization in nature, as indicated both by sterility of the pollen and by a well marked variation. It is also evident that there is a distinct relation between this morphological proof of intercrossing and the geographical distribution of the species. In those regions where *E. angustifolium* has an opportunity to cross with its close allies, the anatomical proof appears conclusive that such crosses have taken place. Evidences of this phenomenon are still further strengthened by the fact that whenever this plant occurs sufficiently far south for crosses with other species of the subgenus *Chamaene-*

rion to be impossible, its microspores are usually perfectly developed and there appears little indication of abortion.

Hybrids have long been recognized in the subgenus *Lysimachion*, of which many are mentioned by HAUSSKNECHT, the names and parentage of which can be learned by reference to his monograph of the genus. Since this subgenus contains so many species, extending over a wide and continuous territory, a study of the pollen conditions in connection with their geographical distribution is impossible. It is significant, nevertheless, that the 3 species examined (*Epilobium hirsutum* L., *E. luteum* Pursh, and *E. obcordatum* Gray) showed evidences of pollen sterility. Similar microspore conditions were observed by JEFFREY (19) in a hybrid derivative of *E. hirsutum*.

Since the pollen in all of these species remains in the tetrad formation, it furnishes an excellent example of the relation of abortion to the number of grains derived from the mother cell. In a recent discussion of this subject DEVRIES suggested that, since three of the megaspores in the angiosperms are sterile, we might be led to infer that all the higher plants are hybrids, "at least on the material side." It is evident, however, that this condition has no bearing upon the question, since this type of degeneration represents a constant feature, and one which is in no way affected by intercrossing. In contrast to this form of spore abortion, that mode normally exhibited by *Epilobium hirsutum* and *E. obcordatum* may be mentioned. In anthers which show no evidences of abortion, the 4 microspores in the tetrad develop equally, and all are full and fertile at maturity. Conditions analogous to these were found to obtain in *Zauschneria californica* Presl., as indicated in figs. 17 and 18. Those plants, nevertheless, which show indications of being segregates from hybrid ancestors, possessed degenerate pollen grains, but these defective spores bore no constant relation to the original number in the tetrad. Fig. 12 shows the pollen formation in an apparent hybrid derivative of *Epilobium obcordatum* Gray. The spores at the left of the figure are quite fully developed in respect to the number in the tetrad. A single exception appears in the lower of the two groups, where a single grain has failed to reach maturity. The remaining clusters, it will be observed, present more evidences of deterioration, and a further study of the illustration will show also that a large and variable number of the microspores have failed to attain normal development. Thus it is apparent that the process of degeneration in hybrid forms is in no way related to the processes of abortion of megaspores in the mother cell. The evidently pure species of the subgenus *Lysimachion* and the monotypic *Zauschneria* present uniformly perfect development of all the spores in the tetrad. In anthers showing morphological proof of earlier contamination, however, the number of abortive grains is in no way related to the number of microspores in the mother cell, since all the 5 possibilities of degeneration obtain.

The pollen conditions in *Clarkia* are not especially different from those already described for *Epilobium*. Of 16 specimens of *Clarkia pulchella* Pursh which were examined, 8 were found to contain nearly uniformly potent pollen cells. The remaining 8, however, showed distinct evidences of hybridism, as revealed by a large percentage of infertile pollen. *Clarkia rhomboidea* Dougl. showed the results of degenerating influences in a far less degree, and only 1 of 10 buds investigated presented indications of sterility. *Clarkia Xanthina* Gray exhibited features similar to those already mentioned in the discussion of spore conditions in *C. pulchella*. One-half of the buds studied presented defective microspores. Fig. 13 shows the type of pollen presented by plants possessing no morphological evidences of spore degeneration. In contrast to this uniform pattern, however, fig. 14 pictures the spore conditions in plants which have apparently been derived from hybrid ancestors. It will be observed that about half of the grains are empty and shriveled, and are strikingly unlike the fertile cells illustrated in the figure. As the character of pollen sterility in *Clarkia* differs in one of the essential features relative to *Epilobium*, it will not be discussed at length. Furthermore, since the several species occupy the same region, it is but natural to assume that they are more or less inclined to intercross freely. The offspring from these unions, accordingly, show a large and varying amount of defective microspores. There is, on the other hand, a perfect development of the pollen where such crosses have not taken place.

A consideration of pollen conditions in a few of the Onagraceae would be far from complete without a brief mention of some species of *Oenothera*. For this reason the distinct *Oenothera serrulata* Nutt. was chosen. This form is considered by some to be sufficiently well defined to be regarded as a separate genus, *Meriolix*. Of 22 specimens which were examined in the usual way, all were found to contain more than 50 per cent of abortive pollen grains. The defective grains, as can be seen by reference to fig. 15, are strikingly distinct when contrasted with the few fertile microspores. Likewise, the great variability in this genus, as well as an abundance of natural hybrids, are so well known and so universally accepted, that any further discussion of these phenomena in relation to sterility in hybrids would be superfluous. It is significant, nevertheless, that this species should show so large a percentage of defective pollen, and, as such, presents a condition strictly in accord with the general principle previously mentioned.

In contrast to the degenerate pollen development in *Oenothera*, conditions in the anthers of *Gongylocarpus fruticulosus* Benth. and *G. rubricaulis* Cham. and Schl. should be mentioned. Both of these species show uniformly well developed microspores; there occur no indications of abortion in any of the grains. Similar spore formation was observed in *Diplandra lopezoides* Hook. and Arn., as illustrated in fig. 16. This shows all the cells to be equal in size and of uniform development. This naturally leads to the conclusion that the plants here mentioned have come from genetically pure ancestors. Although the genus *Gonyglocarpus* is considered by ENGLER and PRANTL (11) to be monotypic, 2 species were listed at the Gray Herbarium. The presence of two species, nevertheless, is unimportant from the standpoint of hybridism. *G. fruticulosus* is found in California, and *G. rubricaulis* grows near Vera Cruz, Mexico. The relative remoteness of these stations naturally precludes the impossibility of interfertilization, thus insuring in each a pure line of descent. The anatomical evidence of uncrossed species likewise supports this conclusion. All the pollen grains examined showed no indication of degeneration, but all were full of protoplasm and of equal magnitude.

Conditions of spore formation very similar to those described for the 2 species of *Gongylocarpus* were observed in *Zauschneria californica* Presl. It was possible to obtain only 4 specimens, but those presented microspore development such as might readily be expected in a monotypic genus. All the pollen cells were equal in size and fertility, as indicated by figs. 17 and 18. Fig. 17 represents a low magnification of the pollen of *Zauschneria*, and it will readily be observed that all the spores are perfectly developed. Fig. 18, a higher magnification, shows this fact even more conclusively. It is apparent also that all the spores in each tetrad reach maturity, and in no case do they show degeneration such as is habitual in 3 of the megaspores of the mother cell.

In respect to spore formation, this genus shows features parallel to those presented by the essentially monotypic *Gongylocarpus* and the geographically limited *Epilobium angustifolium*. The presence of entirely fertile pollen in these species is very significant. It is evident, of course, that hybridism is impossible. On this account all the specimens under consideration may be regarded as representative of pure lines of descent. That this purity of lineage is closely related to a perfect development of the pollen cells is strikingly evident from studies in this direction. The monotypic *Zauschneria* and the essentially monotypic species just cited generally present microspores which are perfect in every respect. Opposed to this type of spore formation, however, species possessing known or apparent hybrids generally contain pollen which is prone to abortion in varying degrees. Consequently, these 2 types of kindred phenomena show in a very conclusive manner the validity of the assumption that sterile pollen is reliable proof of hybrid lineage.

In the earlier paragraphs of this paper, the writer has attempted to show that there are certain general biological principles which have become firmly established. Naturally these principles have grown slowly, and have become far-reaching only in direct proportion to the amount of investigation carried on in relation to their application. Among the many laws thus formulated, that in regard to sexual impotency of hybrids is of the utmost importance in this discussion. The study of this problem, however, leads to a consideration of the probable origin of species. As has already been stated, there are 2 distinct views in reference to this question. On the one hand, there is the idea of a gradual and continuous development; and on the other hand, the sudden and intermittent acquisition or loss of certain characters. These variations are said to breed true to type, and to form new and distinct species. The first of these conceptions receives very strong support from the characteristically gradual development of the woody elements in the lignified stem. These organs, as revealed by anatomical investigations, show a slow and continuous transition from the organization normally present in the lowest of the vascular cryptogams, to the form typical of the higher angiosperms. This support of the Darwinian hypothesis is still further strengthened by a characteristic lack of contradictory proof in the form of an intermittent course of development. Examples in favor of the opposing view represent isolated cases in certain groups of plants, of which the genus *Oenothera* has furnished the largest percentage. As has already been stated, there is a fundamental objection to the doctrine of mutation, especially in connection with *Oenothera*. This genus is well known to hybridize freely, and consequently the lineage of its so-called mutants must invariably be open to question.

This part of the discussion naturally leads into even narrower limits, that is, a recognition of hybrids in nature. In this connection there exists a general morphological proof of hybrid derivation. This condition is indicated by a distinct although variable percentage of abortive pollen grains in an anther. It should also be mentioned in this connection that most of the so-called mutants show this very evidence of hybrid descent.

It can readily be seen that the geographical distribution of a plant might have a direct influence on hybridization, and such has proved to be the case. *Epilobium,* especially the section *Chamaenerion,* furnishes an excellent example in this connection. *E. angustifolium* from those regions where it is essentially monotypic (the southern part of its range in the United States) presented generally perfect pollen. In contrast to this state, in specimens from the northern zone (where it is in contact with the allied species *E. latifolium*) the pollen showed a strong tendency to abortion in varying degrees. Conditions similar to those were observed also in *E. latifolium*, and more generally in the subgenus *Lysimachion*, where there was quite habitual tendency to abortion of the microspores. This situation is due, without doubt, to the more constant presence of species between which crosses are possible.

In contrast to this condition, there is that presented by the 2 essentially monotypic species of *Gongylocarpus*, the geographically limited *Epilobium angustifolium*, and the strictly monotypic *Zauschneria californica*. In these plants there appears no evidence of pollen degeneracy, which (in view of the general principle of sterility in hybrids) is precisely the condition one would expect. It is, nevertheless, just as significant that these species which are unable to cross should present uniformly well developed pollen grains. This generally fertile state is in striking contrast to a habitual degenerate condition where there is frequent interfertilization. Thus it is apparent that reduced fecundity is valuable evidence of hybrid derivation, while a uniform development of the microspores is, other things being equal, as equally indicative of uncontaminated parentage.

From these studies of the Onagraceae in relation to geographical distribution, it is evident that impotent microspores are generally present in plants growing sufficiently near closely allied species to be the result of intercrossing. Opposed to this type of spore formation, there is a habitually perfect condition of the pollen chosen from plants growing in more isolated localities, and from monotypic species where interbreeding is manifestly impossible. Thus it is evident, from the morphological standpoint, that the Onagraceae intercross quite freely, and consequently there must be present many natural hybrids which greatly complicate genetical studies of species in this family.

Conclusions

1. It is apparent that abortive pollen, in a large though varying degree, is an indication of hybrid derivation.

2. There are many recognized hybrid segregates which have remained constant and true to type.

3. The relation of the geographical distribution in the subgenus *Chamaenerion* of *Epilobium* to hybridism is very pronounced. Whenever there is no possibility of intercrossing, the pollen is usually perfect. In contrast to this, there is a more general tendency to abortion in those regions where interbreeding is possible.

4. *Epilobium hirsutum*, *E. luteum*, *E. obcordatum*, and *Zauschneria californica* show conclusively that there is no relation between the number of microspores which may abort in apparent hybrid derivatives and the original number in the tetrad.

5. The essentially monotypic *Gongylocarpus*, the geographically limited *Epilobium angustifolium*, and the strictly monotypic *Zauschneria* present perfect pollen, a condition strikingly in accord with the general principle of hybrid sterility.

6. It is evident, on morphological grounds at least, that natural species crossing is a general feature among the Onagraceae, where such is possible in respect to geographical distribution and other factors.

In conclusion, the writer wishes to express his sincere thanks to Professors B. L. Robinson and M. L. Fernald for permission to obtain specimens from the Gray Herbarium; and to Miss Day, Librarian at the Gray Herbarium, for aid in securing literature. These investigations were carried on in the Laboratories of Plant Morphology of Harvard University, under the direction of Professor E. C. Jeffrey, and to him I am greatly indebted for advice.

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EXPLANATION OF PLATES XIX–XXI

FIG. 1.—*Epilobium angustifolium* L., showing entirely fertile pollen; ×75.

FIG. 2.—*E. angustifolium* L., showing perfectly developed pollen grains; ×125.

FIG. 3.—*E. angustifolium* L., showing no indication of abortion due to hybridism; ×150.

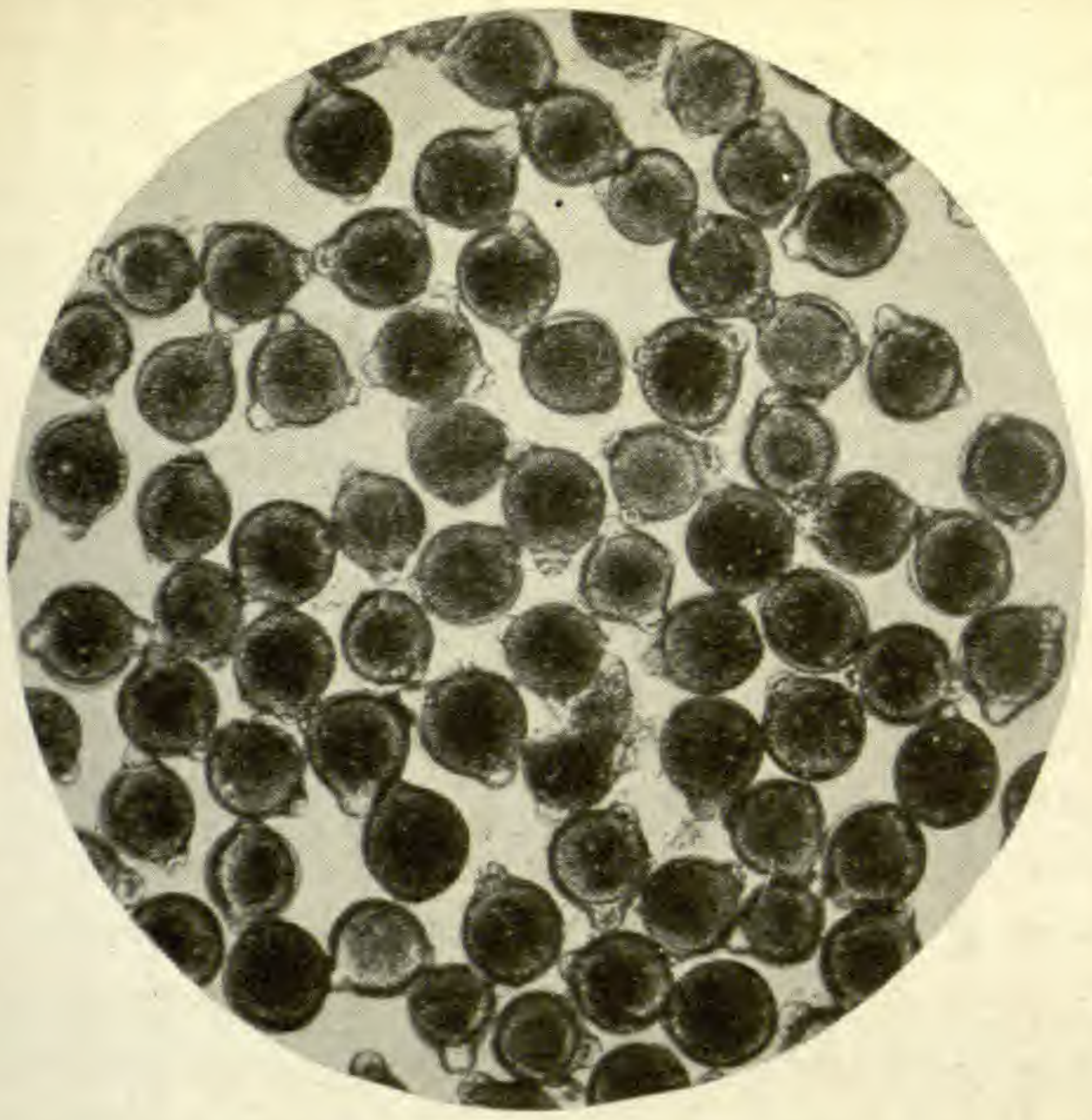
FIG. 4.—*E. angustifolium* L., showing fertile pollen; ×140.

FIG. 5.—*E. angustifolium* L., showing abortive pollen; ×125.

FIG. 6.—*E. angustifolium* L., showing fertile and abortive pollen, and a single tetrad in which all the grains are defective; ×125.

FIG. 7.—*E. angustifolium* L., showing indications of hybridism, as shown by the degenerate pollen grains; ×100.

FIG. 8.—*E. angustifolium* L., showing fertile and infertile microspores; ×200.



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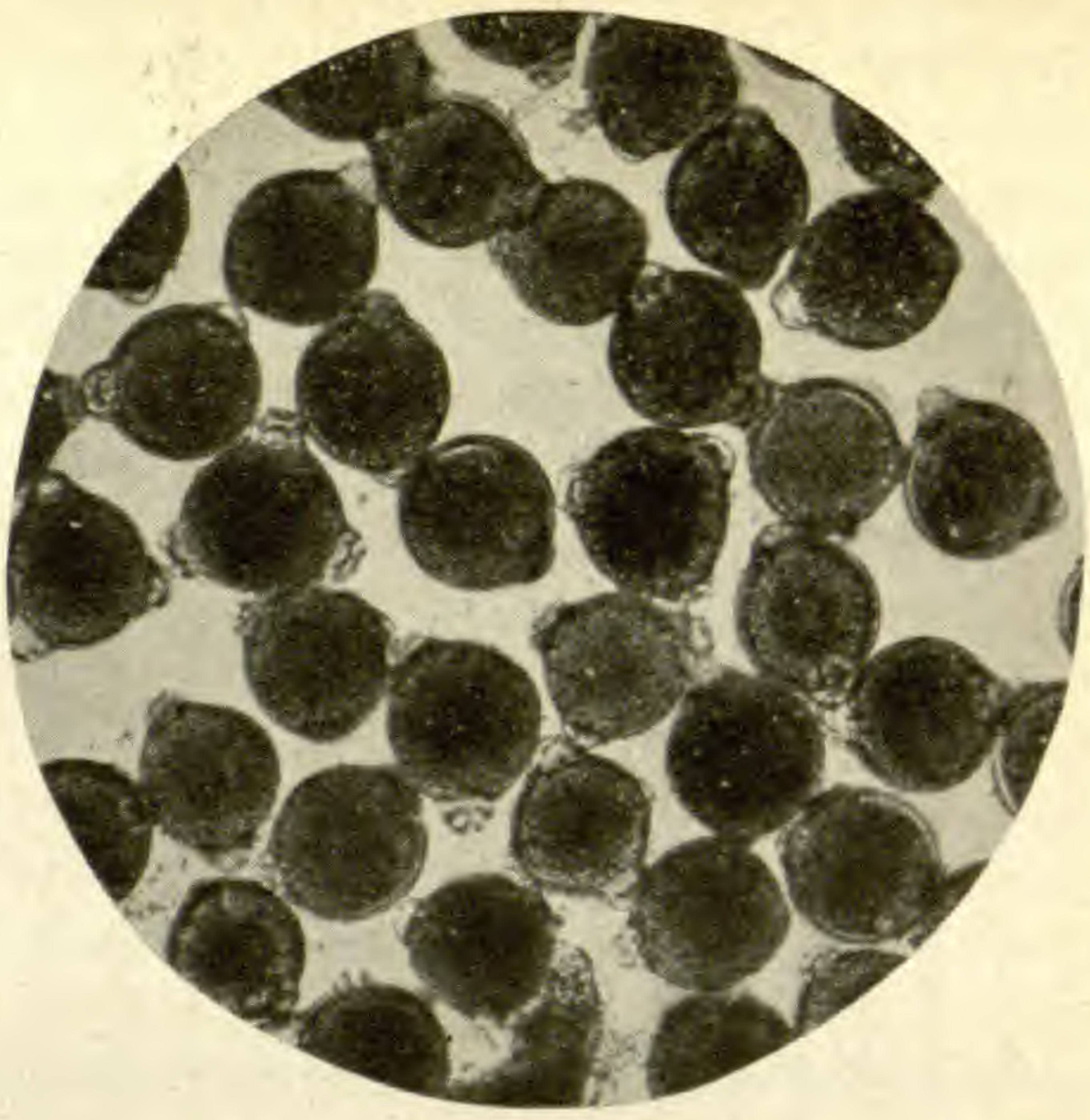

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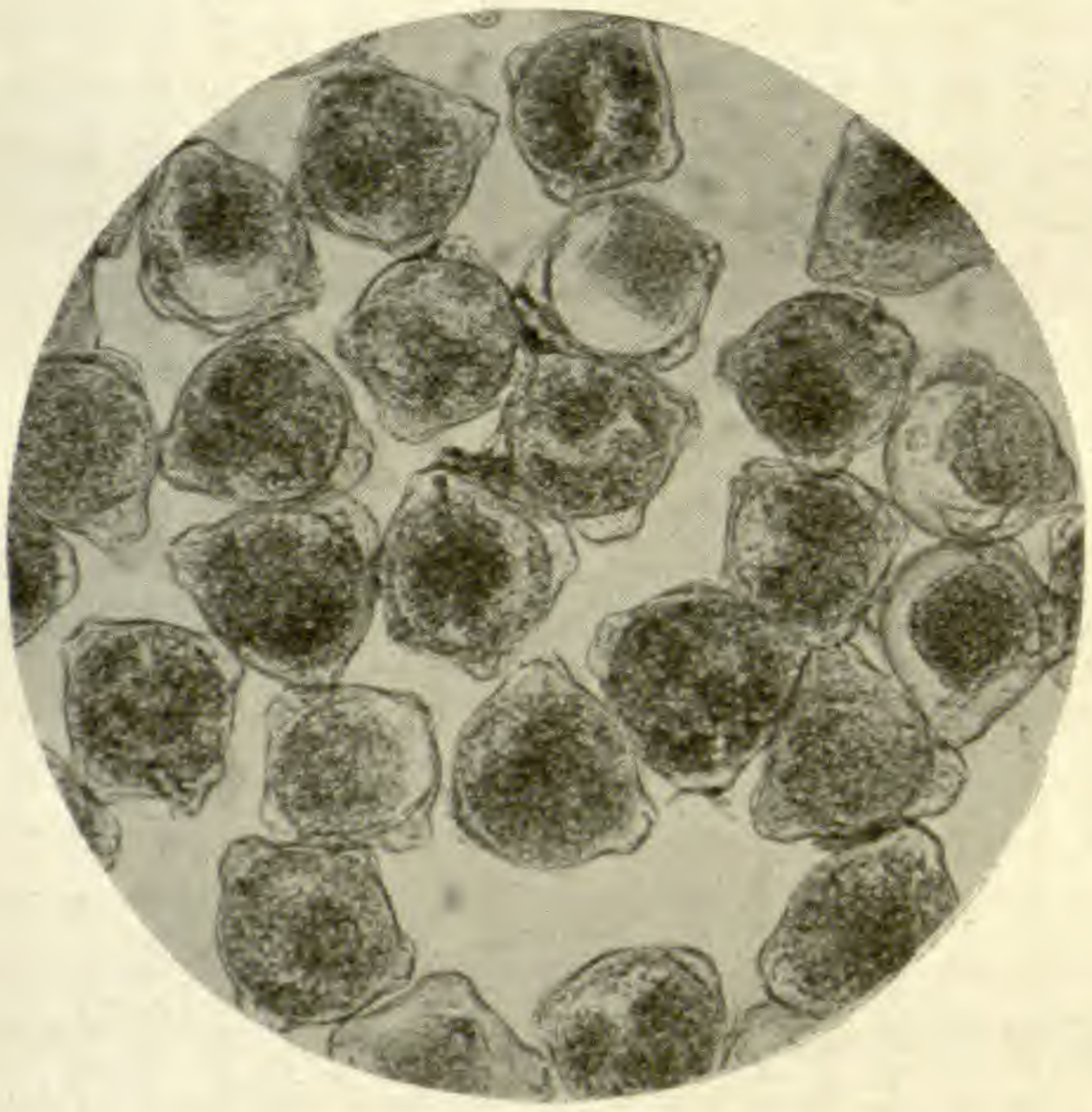

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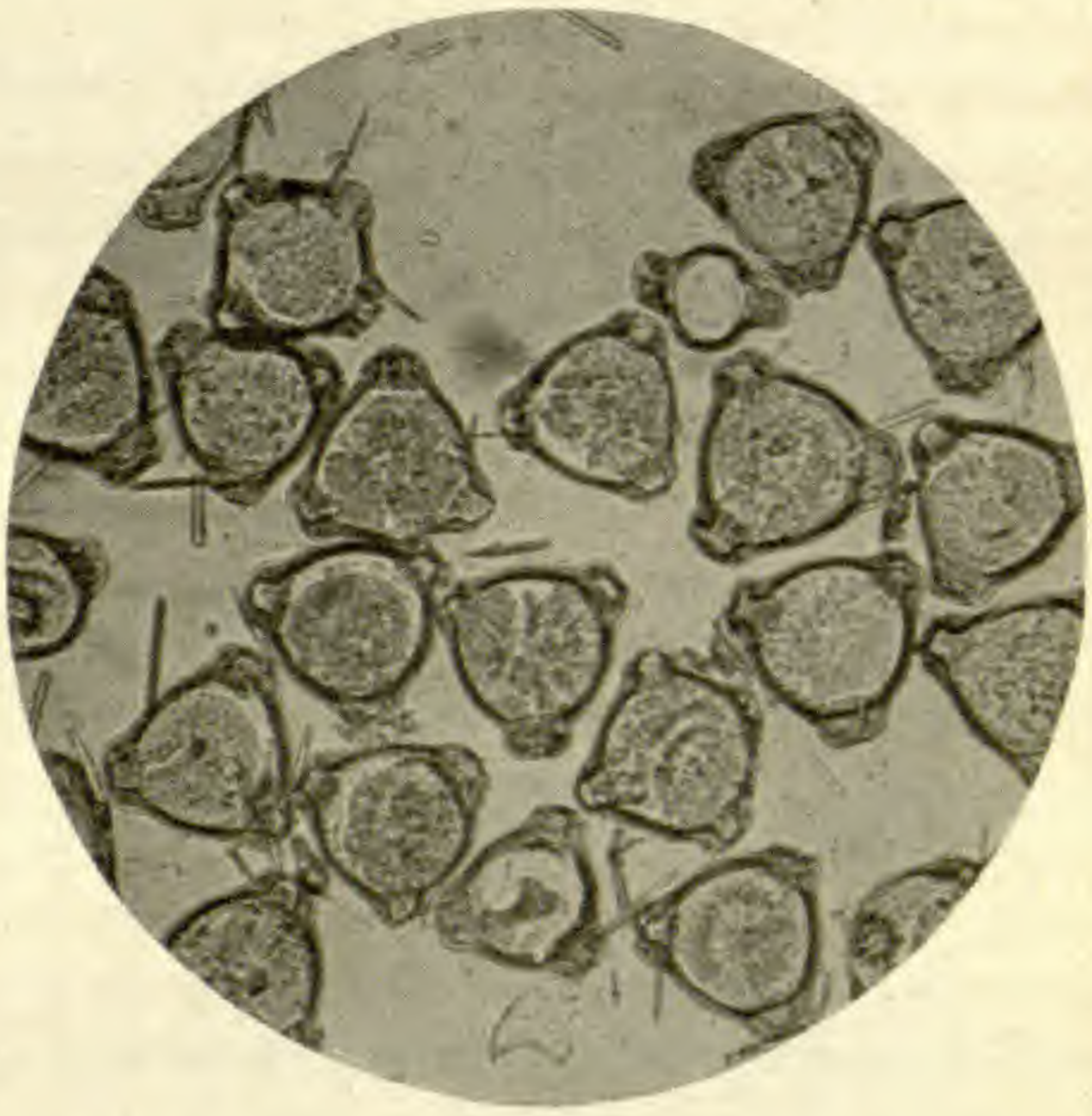

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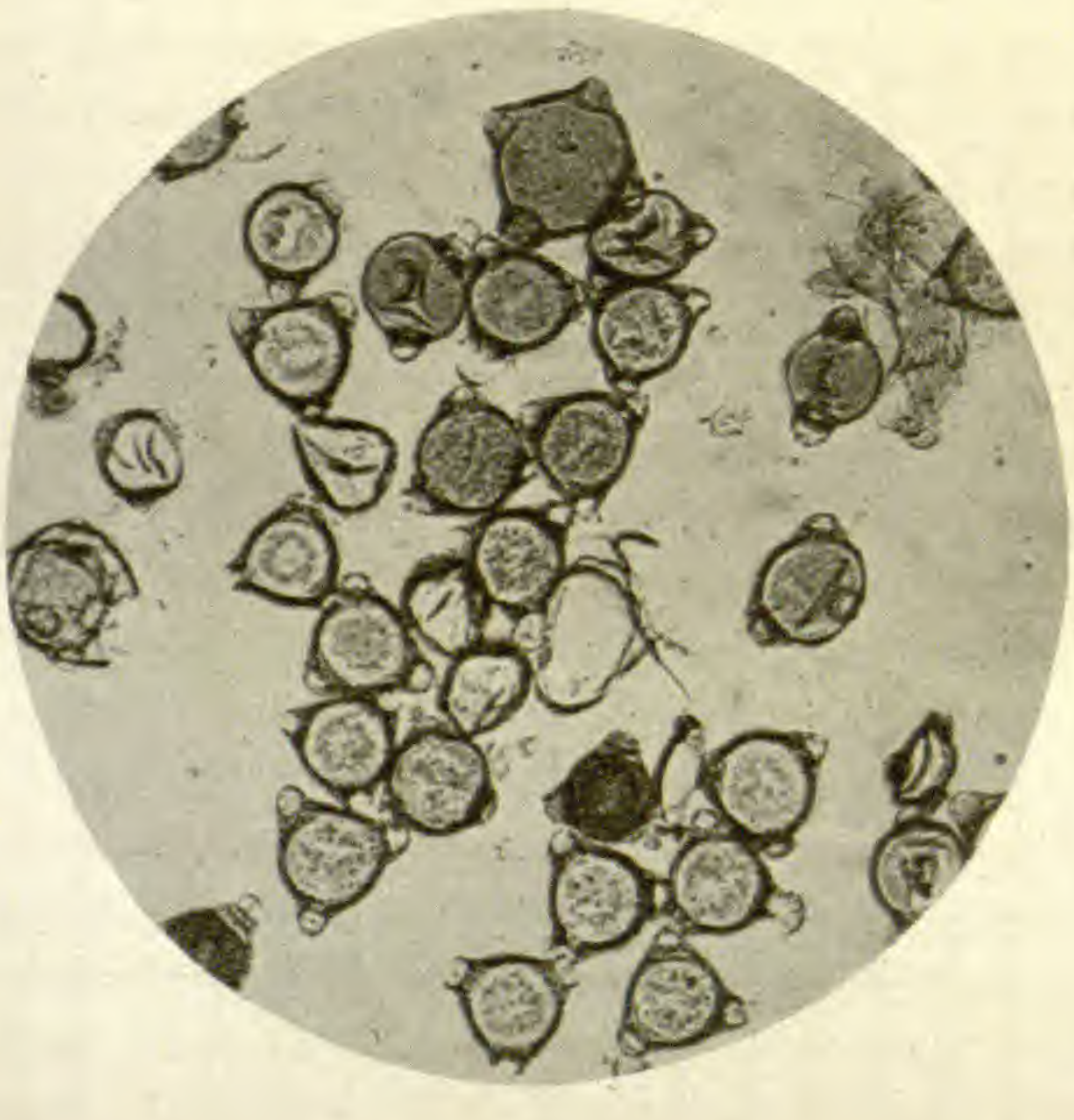

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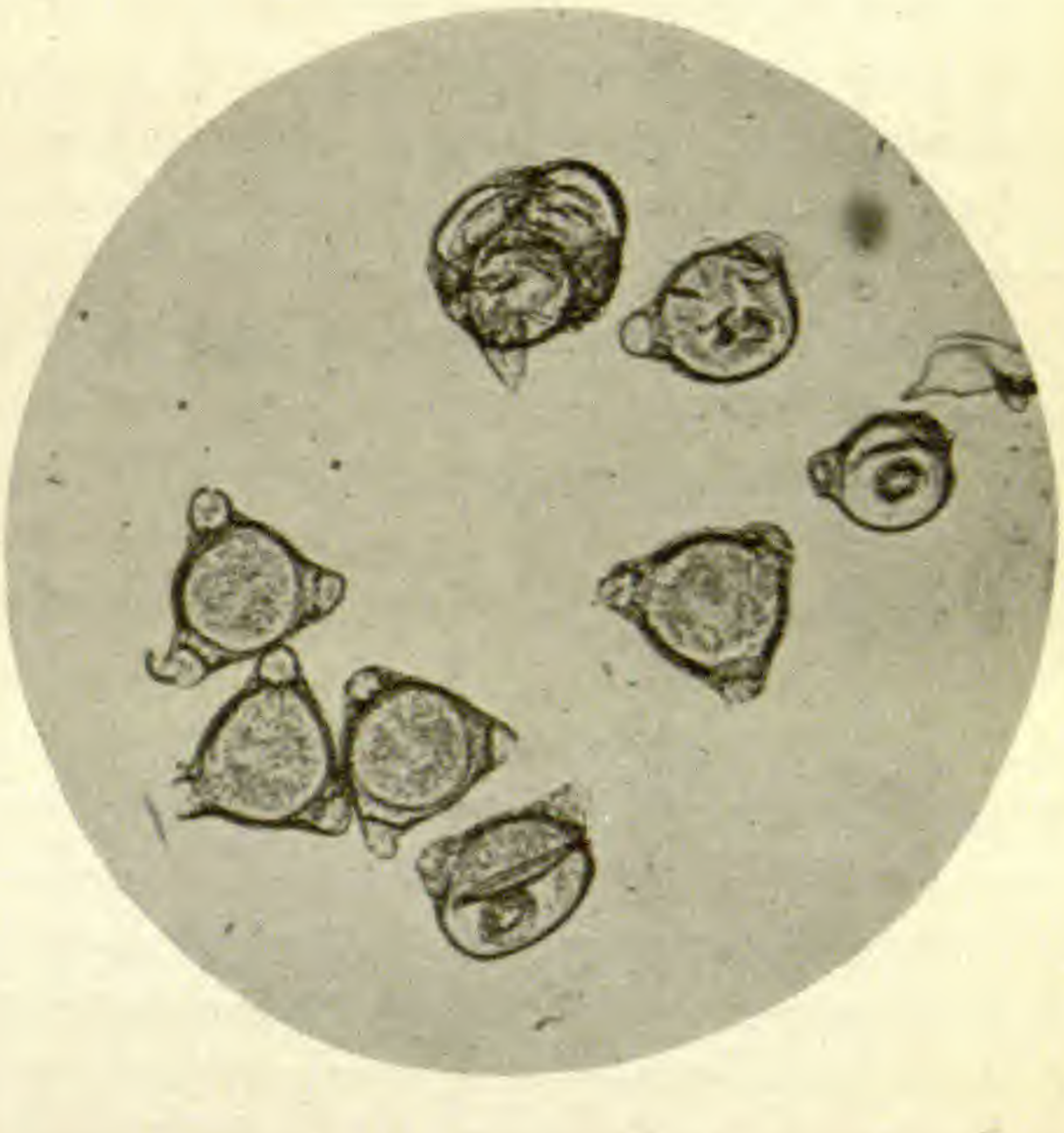

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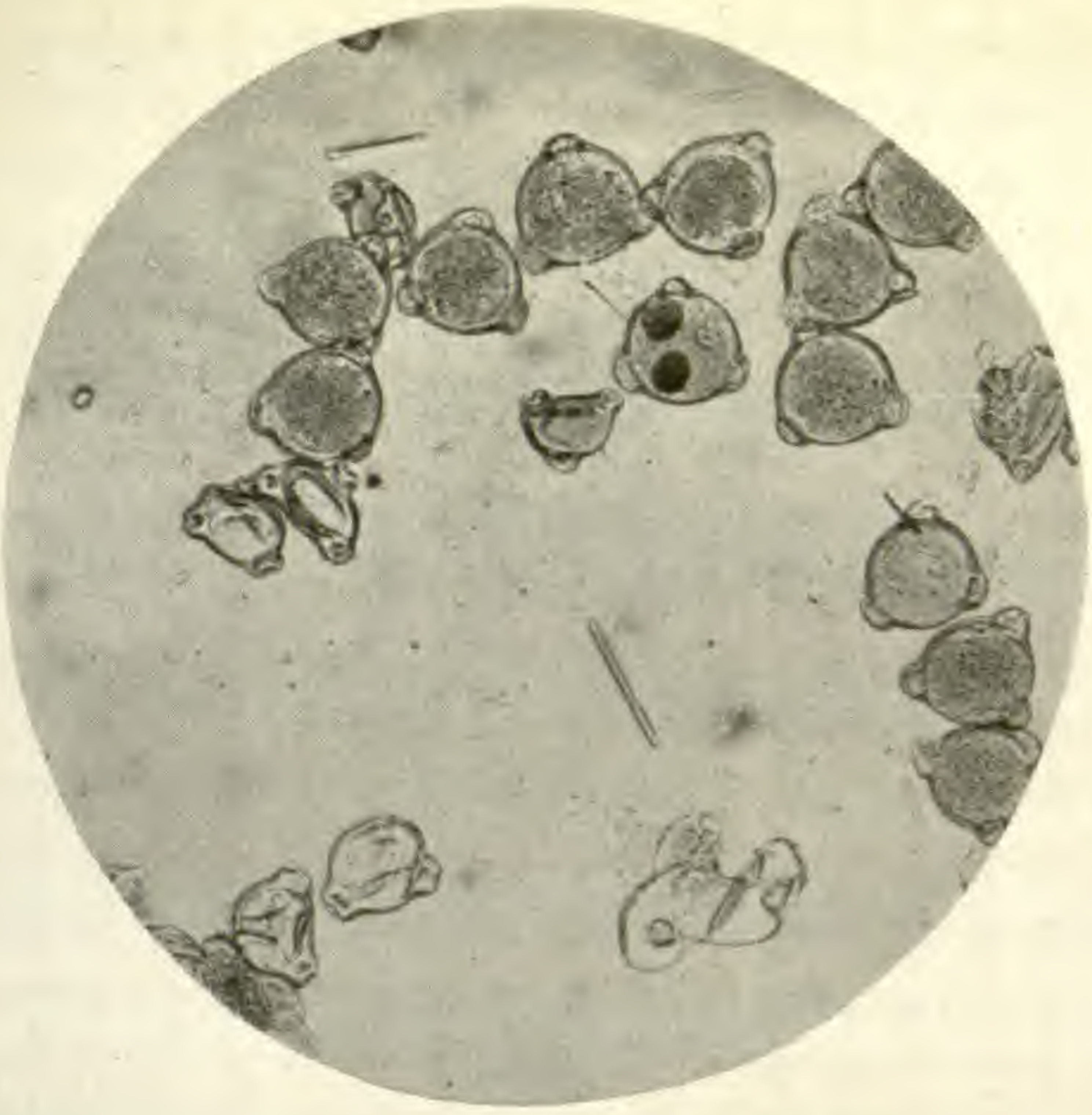
FORSAITH on POLLEN STERILITY



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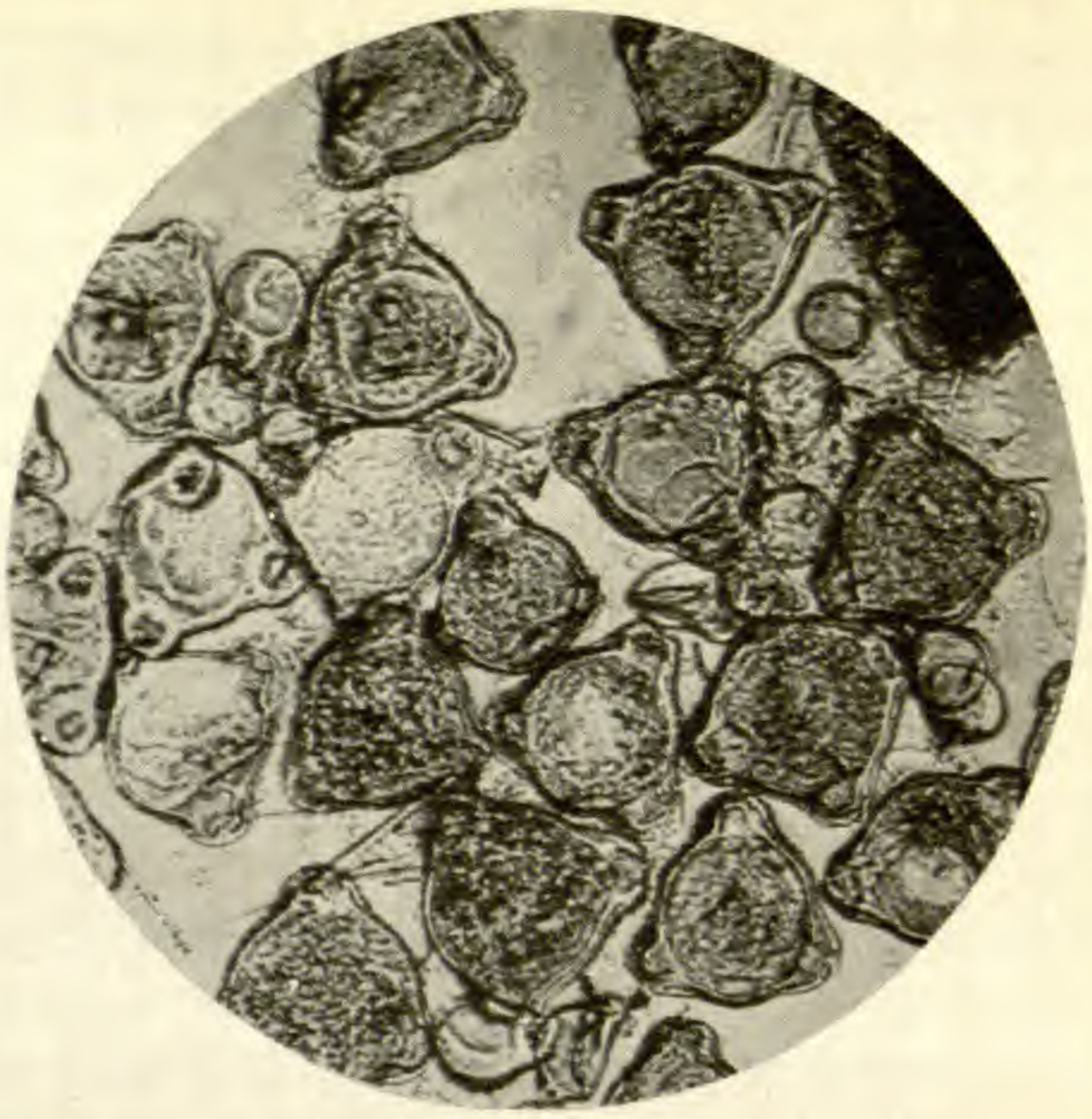

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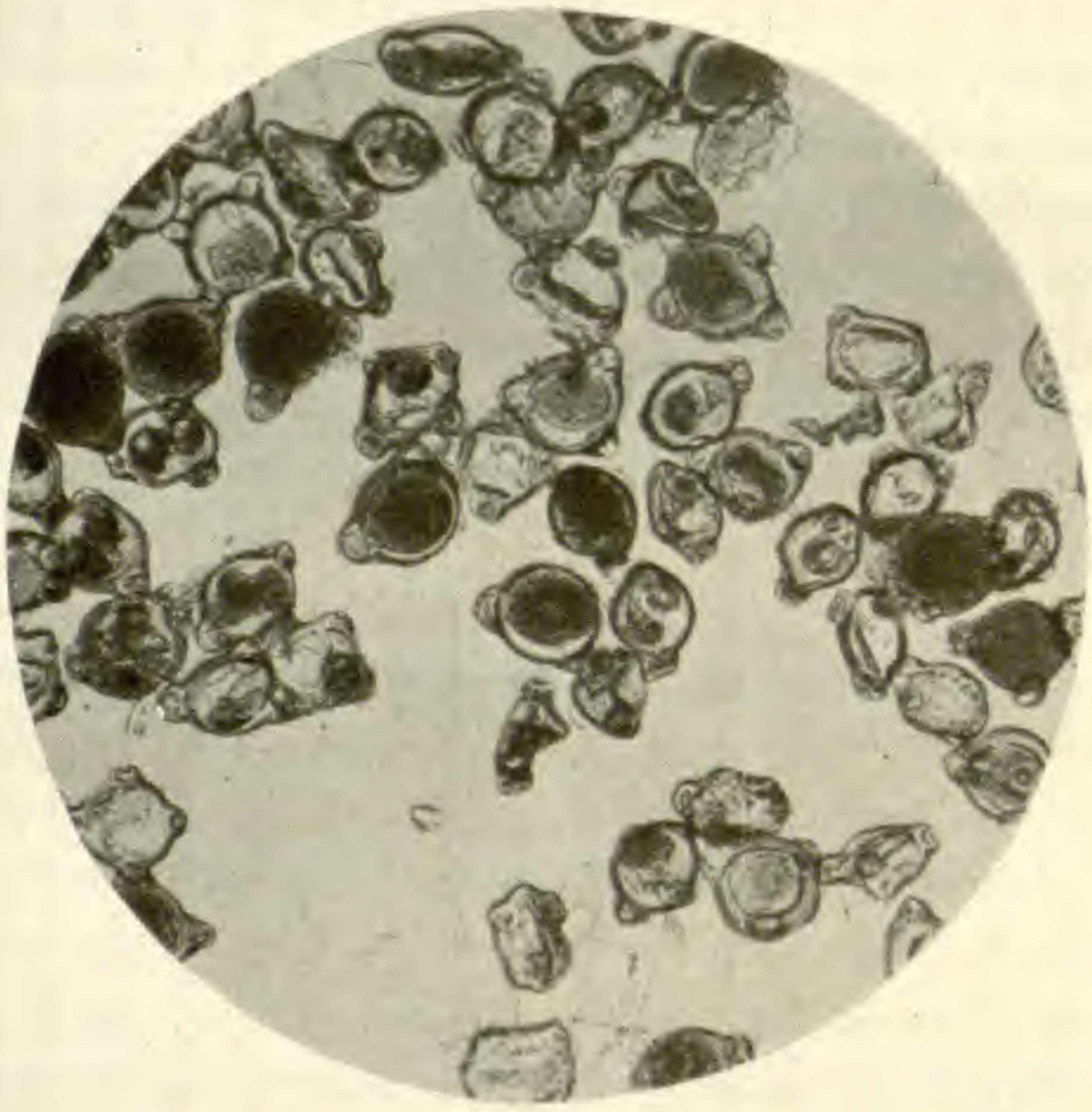

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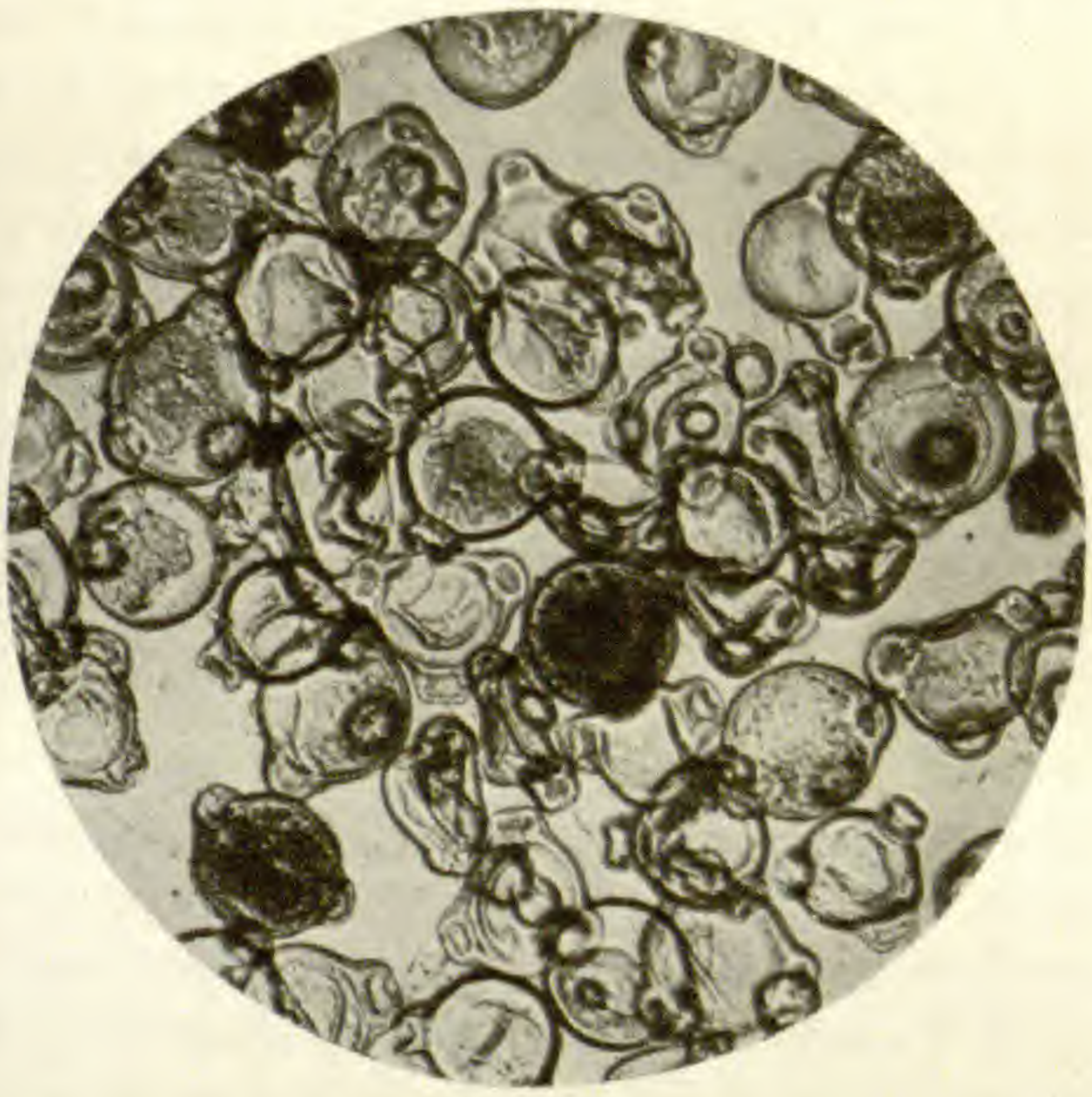

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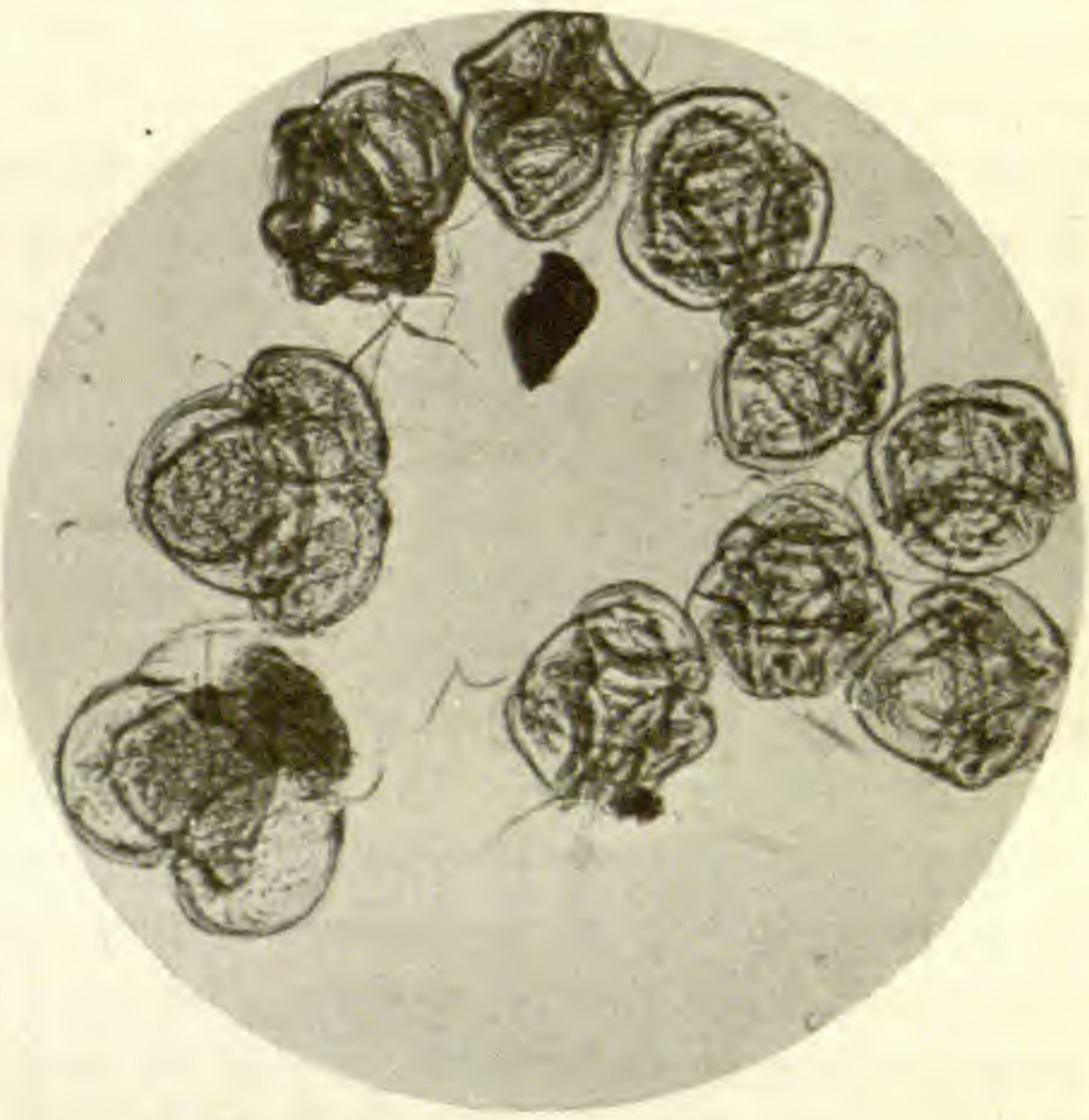

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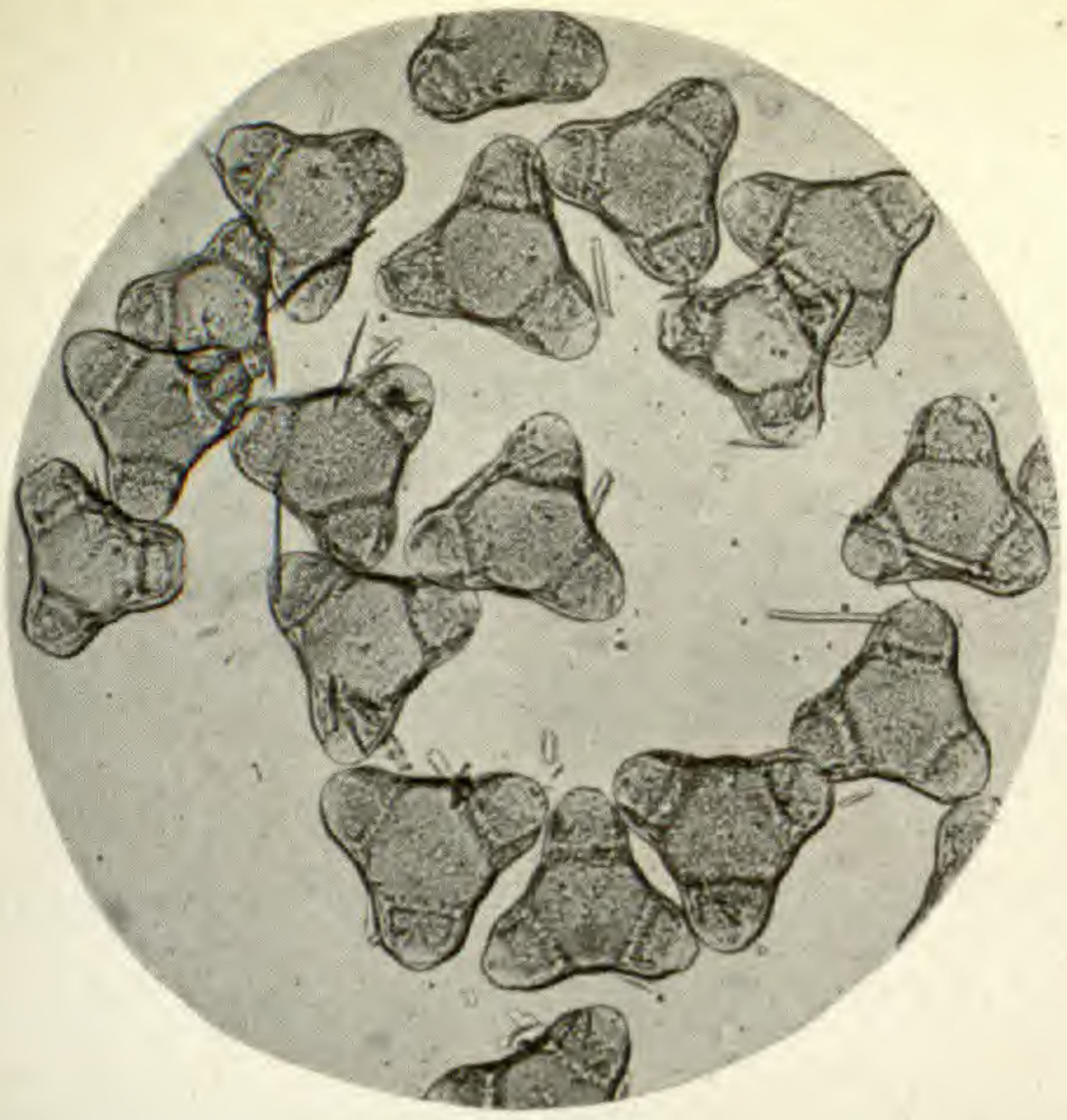

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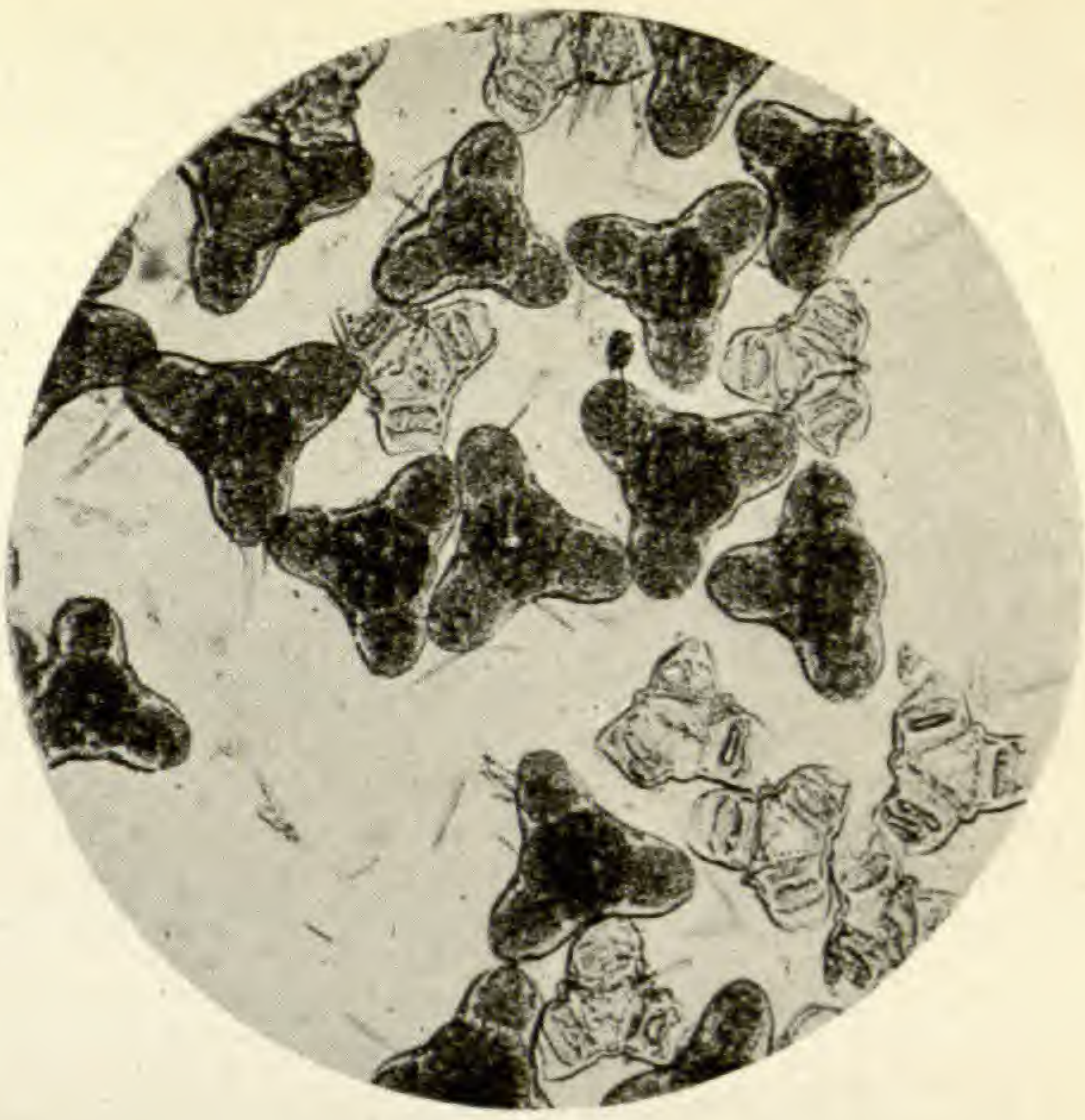

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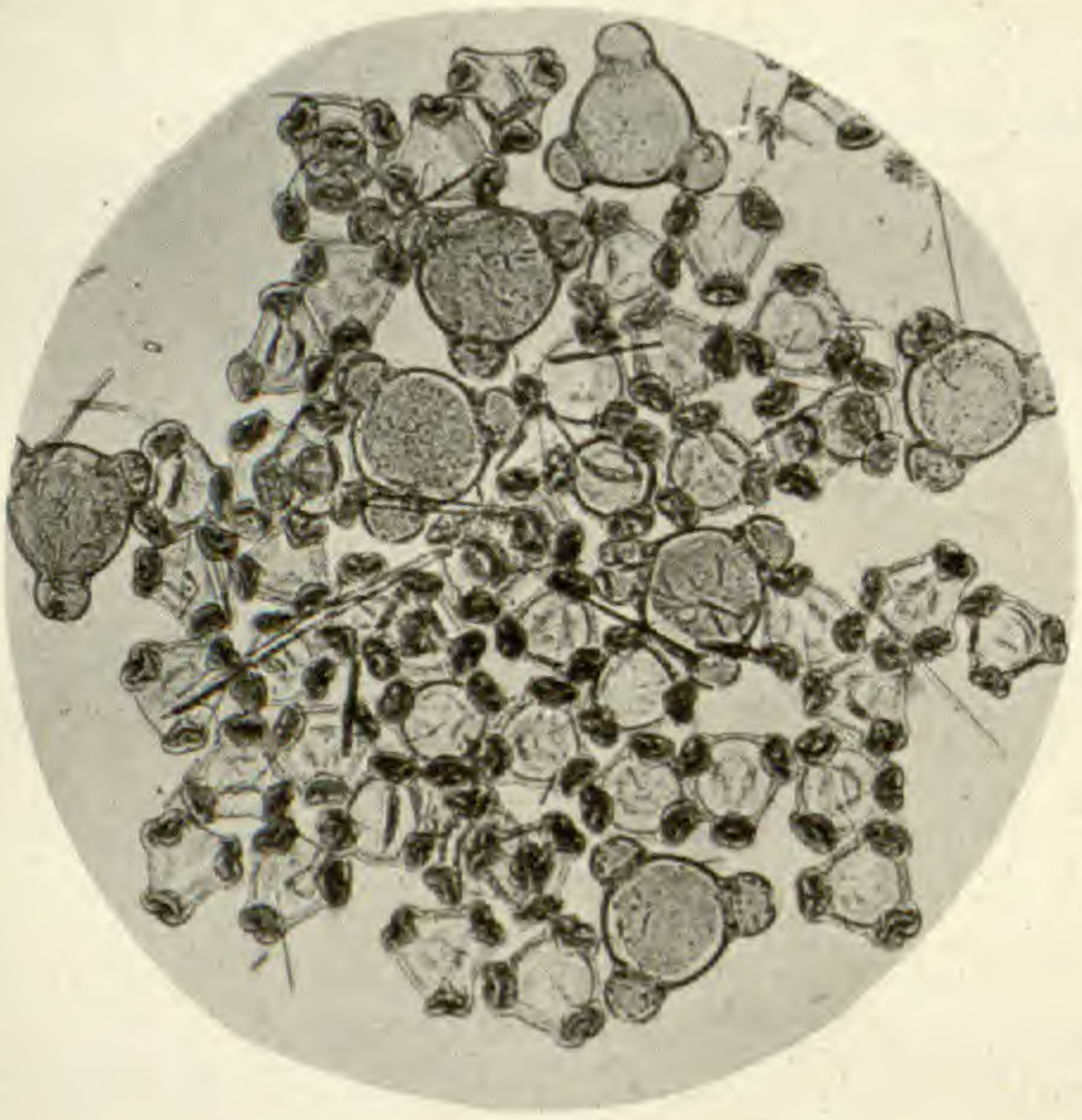

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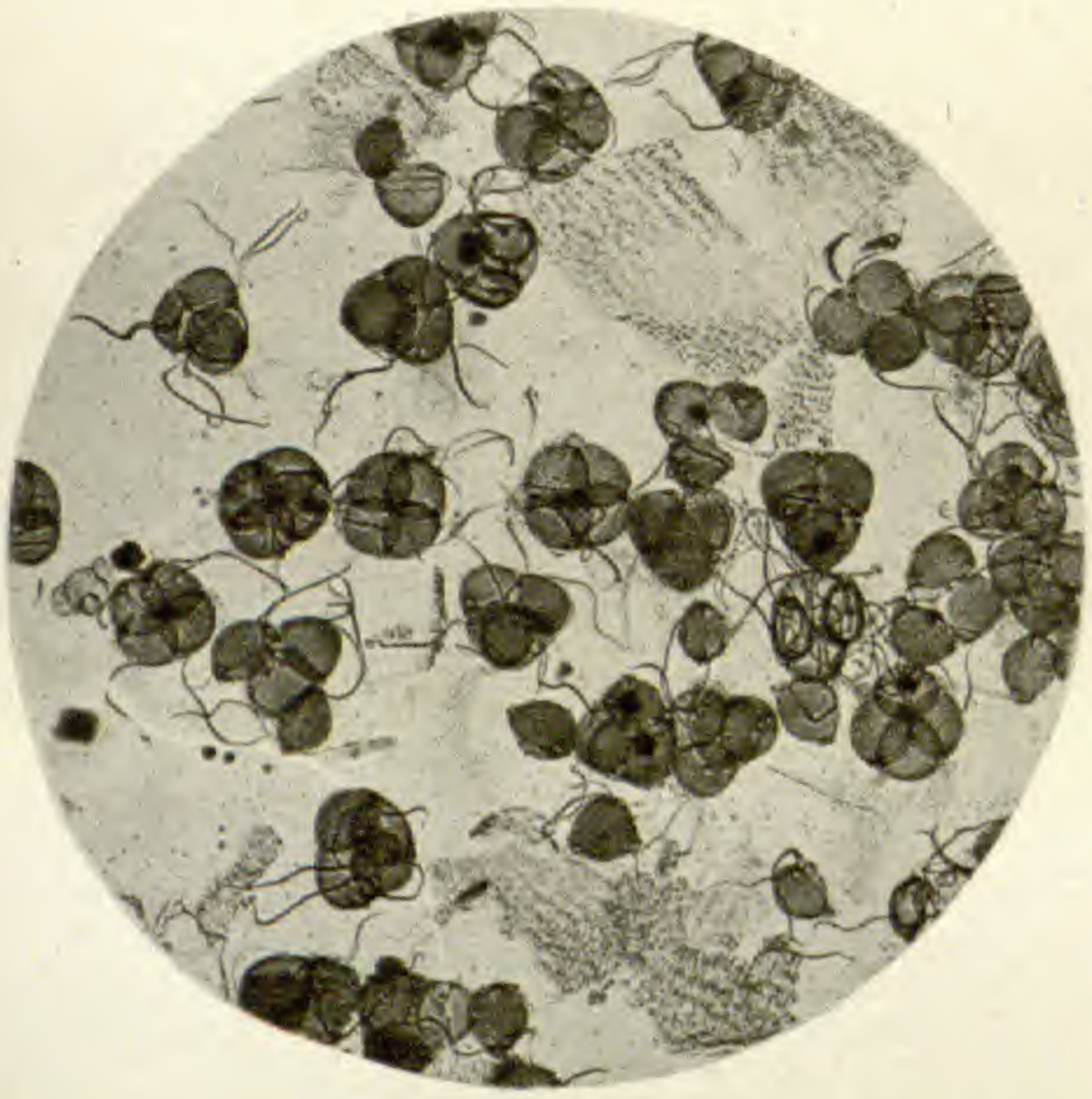

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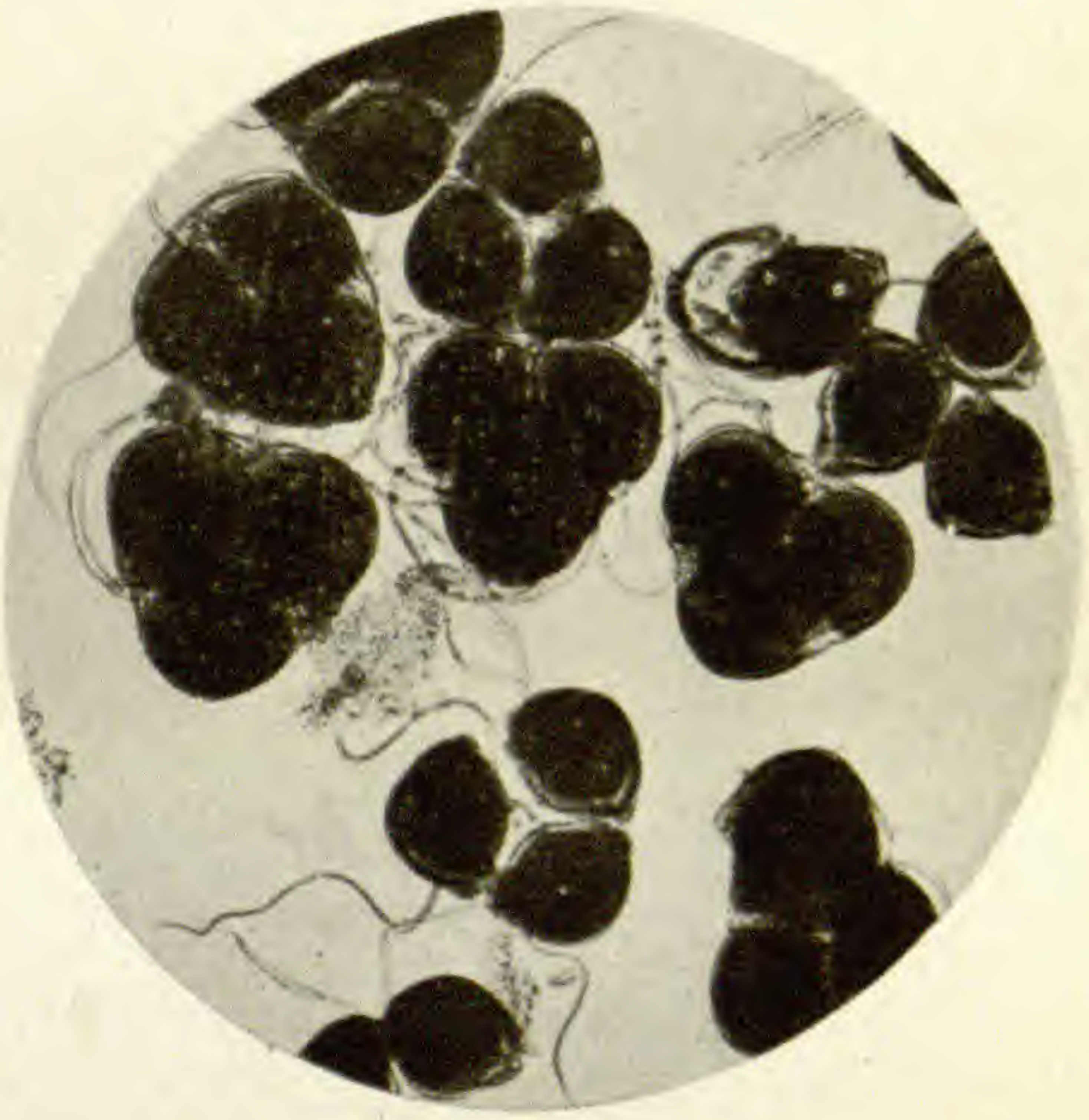

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FORSAITH on POLLEN STERILITY

FIG. 9.—*E. latifolium* L., showing evidences of abortion in a large percentage of the grains; ×75.

FIG. 10.—*E. latifolium* L., showing defective pollen; ×150.

FIG. 11.—*E. latifolium* L., showing perfect and imperfect pollen; ×125.

FIG. 12.—*E. obcordatum* Gray, showing fertile and abortive pollen grains in relation to tetrad; ×125.

FIG. 13.—*Clarkia Xanthina* Gray, showing uniformly developed pollen grains; ×125.

FIG. 14.—*C. Xanthina* Gray, showing fertile and infertile pollen; ×125.

FIG. 15.—*Oenothera serrulata* Nutt., showing relative size of fertile and degenerate pollen; ×125.

FIG. 16.—*Diplandra lopezoides* Hook. and Arn., showing functional microspores; ×200.

FIG. 17.—*Zauschneria californica* Presl., showing no indication of abortion in any of tetrads; ×50.

FIG. 18.—*Z. californica* Presl., showing tetrads in which all grains are uniformly developed; ×125.

THE EPIDERMAL CELLS OF ROOTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 221

EDITH ADELAIDE ROBERTS

(WITH SEVENTEEN FIGURES)

Introduction

The epidermal cells of roots have received more or less attention, especially those epidermal cells which form root hairs. The lines along which investigations have been conducted may be grouped readily under investigations made when the root is in an air medium and those made when the root is in a liquid medium. The factors in the air medium which have been discussed are moisture, light, temperature, contact, length of cells, mode of succession, position of nucleus, osmotic pressure, membranes, and food supply. The factors in the liquid medium discussed are calcium nitrate, potassium nitrate, salt, and bog conditions.

MOISTURE.—PERSECKE (**16**), working with *Zea mais* and *Pisum sativum*, states that the root hair development depends upon the amount of air and water in the interstices of the soil. SCHWARZ (**20**), using the same forms, comes to the conclusion that there is a minimum of moisture at which the hair formation begins, an optimum in which the best development is obtained, and a maximum where the hair development nearly or entirely ceases. PFEFFER (**18**) and others attribute more importance to moisture than to light as a factor in hair development. The conclusion from these observations is that moisture is a factor in the determination of the formation or non-formation of an epidermal cell of a root into an outgrowth called a root hair, but how or why this is so receives no consideration, nor do the limits of the amount of moisture necessary to become a limiting factor.

LIGHT.—The effect of light upon cells in general was investigated by KRAUS (**14**), who found that darkness increased the length of cells. DEVAUX (**2**) found the same and that this favored the development of root hairs on corn. On the other hand, EWART (5), in the formation of root hairs on the root tendrils of *Vanilla*, found darkness accelerating and light retarding their formation. In this work, however, the moisture factor was not eliminated. PETHYBRIDGE (17) found that light retarded the formation of hairs upon oat and corn roots grown in water cultures. SCHWARZ (20) found that light and darkness had no effect on the root hair formation. SNOW (21) states that the effect of light and darkness, if any, is indirect. Light, then, as a factor has been experimented with to some extent with varying results, possibly owing to the fact that it has been associated with other factors.

TEMPERATURE.—No work on temperature as a single factor has been carried on. SCHWARZ (20) found that a temperature of 27–28°C. did not overcome the inhibitory effect of water as the roots grew smooth. SNOW (21), working with high temperatures plus moisture, found a decreased hair production brought about by the increasing elongation of the internal cells.

CONTACT.—The effect of contact has received some attention. SCHWARZ (20) observed that water roots upon entering the substratum develop hairs, and that when the soil is saturated the hairs on corn seedlings disappear, although soil particles are still present, and suggests that it may be due to chemical stimuli or retardation of growth, since it could not be due to contact. PFEFFER (18) denies that contact is a factor, for he found that on climbing roots hairs were produced on the side near the support where there is the greater moisture. SNOW (21) grew corn seedlings between plates and found no hairs on the sides touching the plates, while there were hairs on the other two sides; but again moisture may be the limiting factor and not contact.

JUEL (11) associated short cells and root hairs, as also did VAN TIEGHEM (25) and KRAEMER (13), and he finds that short cells remain short if they do not form root hairs. LEAVITT (15) finds two types of potential root hair cells. The first is that in which any cell of the outer layer may acquire the character of a root hair (trichome) by putting out a hair. This is characteristic of the dicotyledons, of some divisions of the monocotyledons, and of the Filicales. The second type is that in which they originate as specialized elements. These cells differ in the formation of their cell plate, the wall lies somewhat diagonally, and the cell differs from the other epidermal cells in shape, size, and content. This type he finds in the Schizaeaceae, *Equisetum*, *Azolla*, *Lycopodium*, *Phylloglossum*, *Isoetes*, *Selaginella*, and in Nymphaeaceae. Snow (21) finds that no definite length can be given as the limit for the formation for hair development; that in some roots the average length of piliferous cells is less than that of the smooth cells, but that the differential elongation of the epidermal and cortical cells is important, and that hair formation depends upon their ratio, that is, between the capacity of the epidermal cells to elongate and their ability to do so.

Bardell (1) comes to the same conclusion as Snow. A few of the measurements given by Bardell are shown in table I.

TABLE I

Plant	Length cortical cell (mm.)	Haired epidermal cell (mm.)	Hairless epidermal cell (mm.)
Pisum sativum......	231	161 (−71)	191 (−40)
“ “	179	139 (−40)	172 (− 7)
Zea mais...........	69	66 (− 3)	77 (+ 8)
“ “	69	69 =	77 (+ 8)
“ “	40	55 (+15)	55 (+15)
“ “	36	0	33 (3)
“ “	124	58	62

It is difficult to draw the conclusion which is given, for in the one case a difference of 40 mm. in length between epidermal and cortical cells calls forth a root hair; while in another the same difference accounts for the absence of a root hair, and measurements equal to, or greater than, or less than, seem to account for haired or unhaired epidermal cells.

Succession.—Schwarz (20) found that hairs were always produced in acropetal succession, while Devaux (2) asserted that new hairs could appear among the old ones; whereas Haberlandt (8) agrees with Schwarz (20), emphasizing the fact that no new hairs ever arise among existing ones.

Position of the nucleus.—Haberlandt (8) noted the position of the nucleus in *Brassica alba* to be at the tip. In *Pisum* *sativum* he finds that the protrusion of the root hair takes place opposite the nucleus. KÜSTER (12) takes exception to this. In the figures in SNOW'S (21) work the nucleus has no definite position.

OSMOTIC PRESSURE.—PFEFFER (18) found the osmotic pressure in corn root hairs to be greater than that of the cortical cells. STIEHR (23) found that root hairs on seedlings of *Spergula* which had grown in moist air when put in a 1 per cent magnesium solution burst at once, the nucleus being thrown out, and that always a greater percentage of the younger hairs burst. GANONG (7) found that the root hairs of *Salicornia* withstood 90 per cent salt water, *Suaeda maritima* 60–70 per cent, and *Atriplex patulum* 40 per cent. DRABBLE and LAKE (3) found that in mesophyll cells in plants growing in the same condition the osmotic pressure of the cell sap is generally the same, and in the plants of any area the osmotic pressure varies with the physiological scarcity of water. FITTING (6), using cells from the leaf, found that species showing high pressures in dry desert conditions show much lower pressures in moist situations, and concluded that certain plants adjust their osmotic pressure to the medium. ECKERSON (4) found that the root hairs were plasmolyzed by sucrose, varying from 0.20 μ to 0.30 μ. STANGE (22) found that in water cultures with nutrient solutions of high concentrations the osmotic pressures of roots are much higher than others; bean and pea in moist soil have pressures of 6.25N KNO_3 when growing in concentrated medium.

MEMBRANES.—SCHWARZ (20) found that the membrane of the root hair of *Taxus baccata* was of two parts: an inner layer which stains blue with chlorzinc iodide, and an outer layer which stains yellow brown. This outer layer is a mucilage which is hard in dry soil, and as moisture increases strongly swells and finally goes into solution. The mucilage layer gives no color with iodine and sulphuric acid, but stains red in an alcoholic solution of acid carmine. HESSE (9) measured the thickness of the membranes of the root hairs of many plants. He found that the thickness varied greatly with the family, but is nearly uniform within the family; that in any plant the thickness of any root hair membrane varies with the medium; and that in *Rosa canina* growing in a dry situation the root hair has a stiff pointed apex and that the membrane is of lignin, but that in moist air no lignin is found.

LIQUID MEDIUM.—*Calcium nitrate.*—SCHWARZ (**20**) found that 15 per cent of calcium nitrate inhibited hair production. KRAUS (**14**) found roots richly haired in calcium nitrate.

Potassium nitrate—BARDELL (**1**) found that the root hairs on *Zea mais*, *Triticum vulgare*, *Avena sativa*, and *Tradescantia* sp. tend to decrease as the fractional solutions of KNO_3 increase in strength from 0.01 normal to 0.09.

Salt.—HILL (**10**) found that in *Salicornia* and *Suaeda* the root hairs can regulate their osmotic pressure in proportion to the osmotic pressure of the soil water.

Bogs.—TRANSEAU (**24**), working on bog plants, found that *Larix* roots when not surrounded by water develop root hairs abundantly. RIGG (**19**) used the development of the root hairs of *Tradescantia* as an indicator of the presence of toxins.

FOOD SUPPLY.—SCHWARZ (**20**) found that in reducing the food supply by removing portions of the endosperm the length of zone of root hairs decreased. SNOW (**21**) experimented with *Helianthus* and found that those with the most cotyledon remaining had the best formation of root hairs.

Investigation

This investigation was prompted by the fact that the presence or absence of root hairs is so often used as an indicator of the effect of changed external conditions. That the effect of the varying conditions might be known, it seemed well to try to determine the varying factors within the epidermal cell of the root, as well as the effect of the varying factors without and their reciprocal relations.

In order to picture as definitely as possible all the forces which might be affecting the epidermal cell, we may consider the average epidermal cell as having the form similar to fig. 1. Here it is seen that on four sides (*2, 3, 4, 5*) the epidermal cell adjoins another epidermal cell, while on the inner side (*6*) it faces the wall of a cortical cell, and on side *1* it is exposed directly to the varying external conditions.

In fig. 2, which is a median section of fig. 1, the following factors need to be considered: (1) *air medium*, (*a*) the physical character of walls *1, 2, 4, 6;* (*b*) the chemical nature of walls *1, 2, 4, 6;* (*c*) the osmotic pressure on both sides of walls *2, 4, 6*, and inside *1;* (2) *liquid medium*, (*a*) the physical character of walls *1, 2, 4, 6;* (*b*) the chemical nature of walls *1, 2, 4, 6;* (*c*) the osmotic pressure on both sides of walls *1, 2, 4, 6;* (3) *length of cells*.

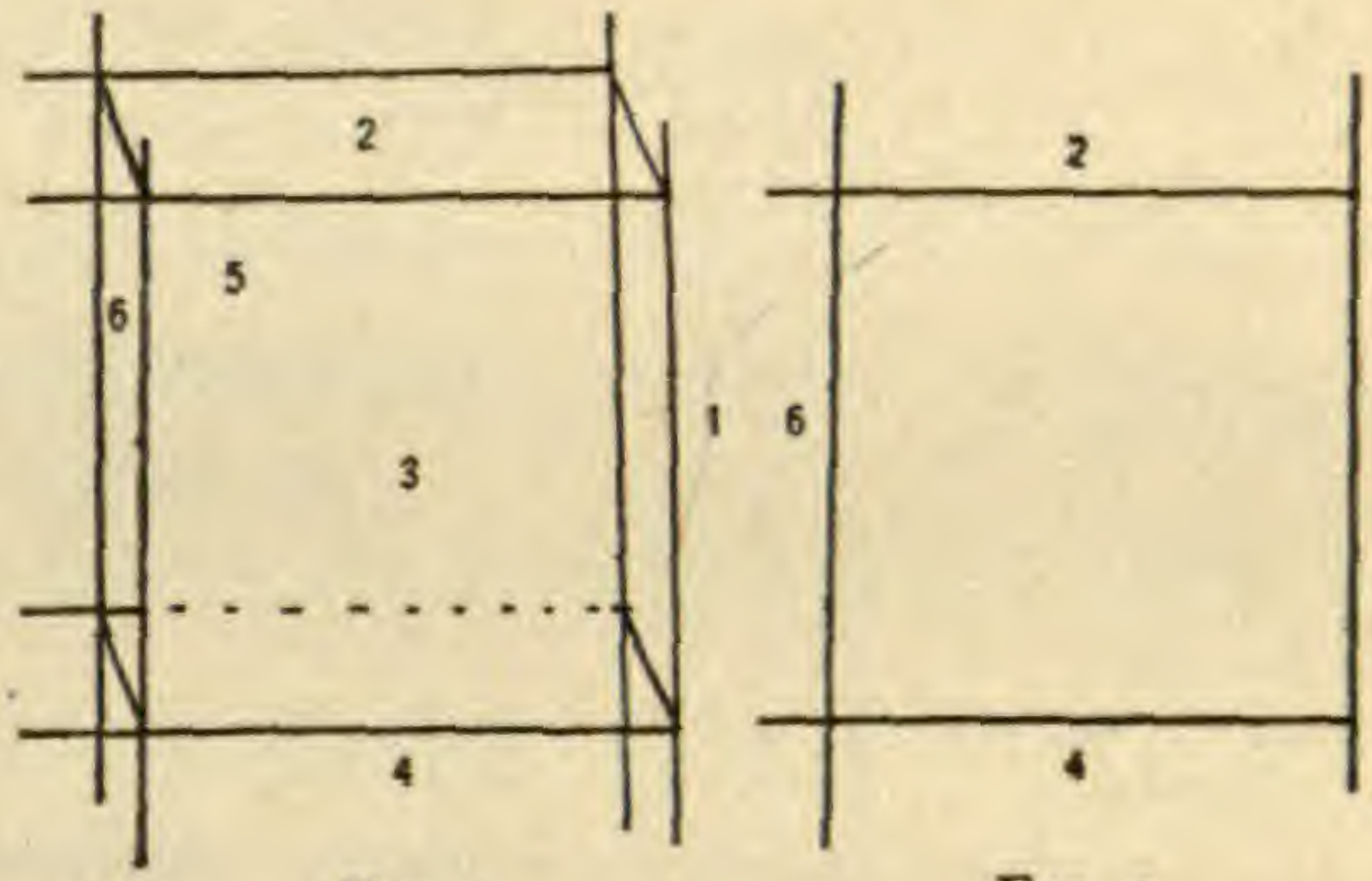

FIG. 1 FIG. 2

FIGS. 1, 2.—Fig. 1, form of an epidermal cell; fig. 2, median section of fig. 1.

TABLE II

Condition	Epidermal cell in mm.	Adjoining cortical cell in mm.
Haired..............	35	30
"	45	30
"	37	37
"	60	50
"	44	40
"	37	55
"	40	50
"	20	10
"	40	40
"	60	60
Unhaired............	40	80
"	45–40	80
"	40	40
"	20	20
"	12	20
"	32	42

A few measurements were made of the length of walls of cortical cells in comparison with the length of adjoining haired and unhaired epidermal cells. These were taken upon the same corn root, the haired epidermal cells being those just above water and the unhaired just below water, with the results shown in table II. These measurements, although few, indicate that there is no definite relation between the length of the cortical cell and the epidermal cell which decides whether the epidermal cell shall become extended into an outgrowth or not. Fig. 3 shows a haired cell longer than the adjoining cortical cell, and fig. 4 shows two short epidermal cells adjoining one cortical cell. These measurements are corroborated by observations made throughout the work, many forms and all possible variation in length of cortical and epidermal cells being found.

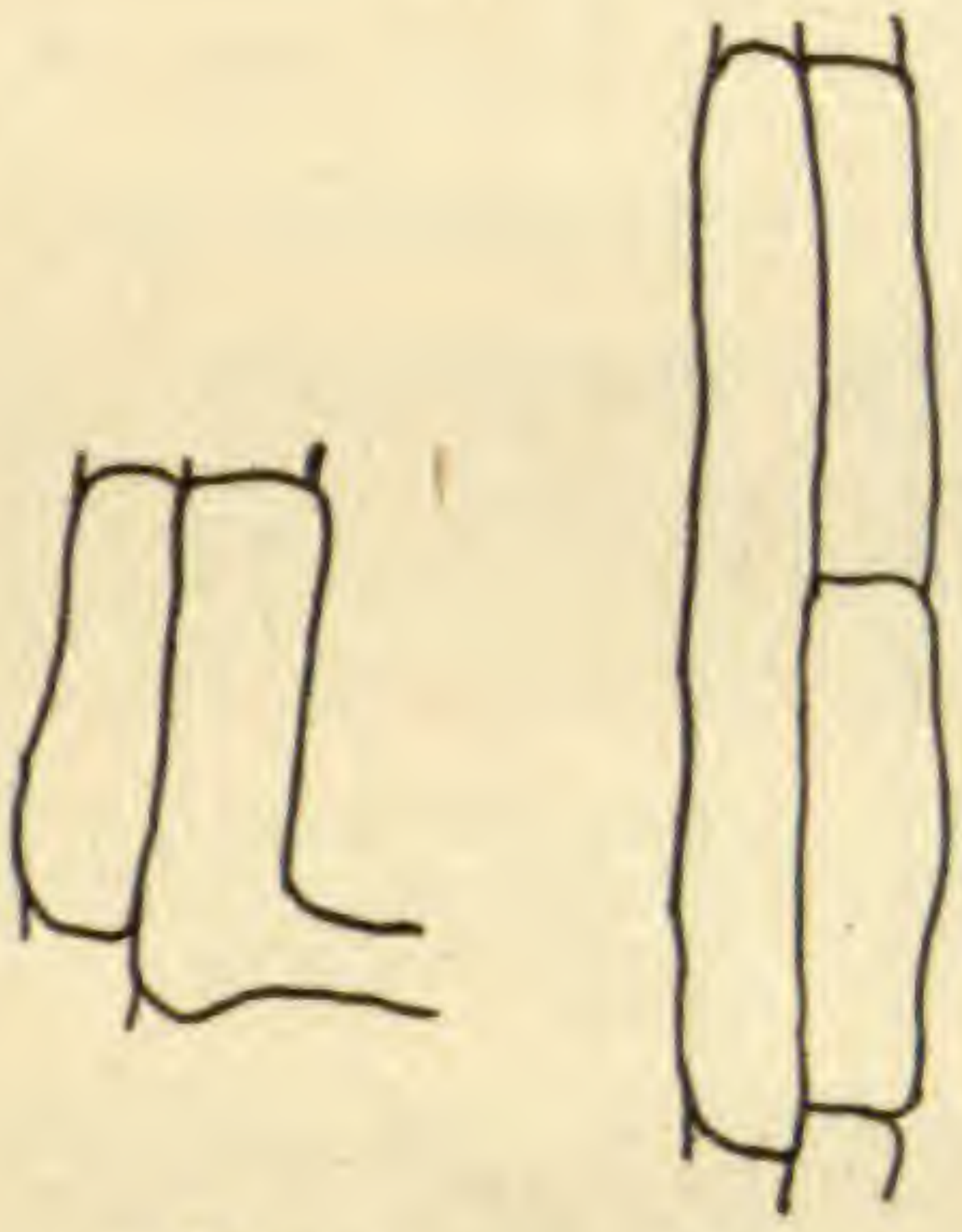
FIG. 3 FIG. 4

FIGS. 3, 4.—Fig. 3, haired cell longer than adjoining cortical cell; fig. 4, two short epidermal cells adjoining one cortical cell.

Position of nucleus.—The position of the nucleus has been found to have no relation whatever to the hair or to its initial formation. When the wall first swells, the entire wall bulges; later there is a greater swelling in one portion of it, the nucleus may not be near that portion; if the nucleus is near that portion, it may not follow the growth of the hair and be found near the tip of the hair, but remain in the base of the hair or in another part of the cell. There appears to be no relation between the position of the nucleus and the formation of the hair.

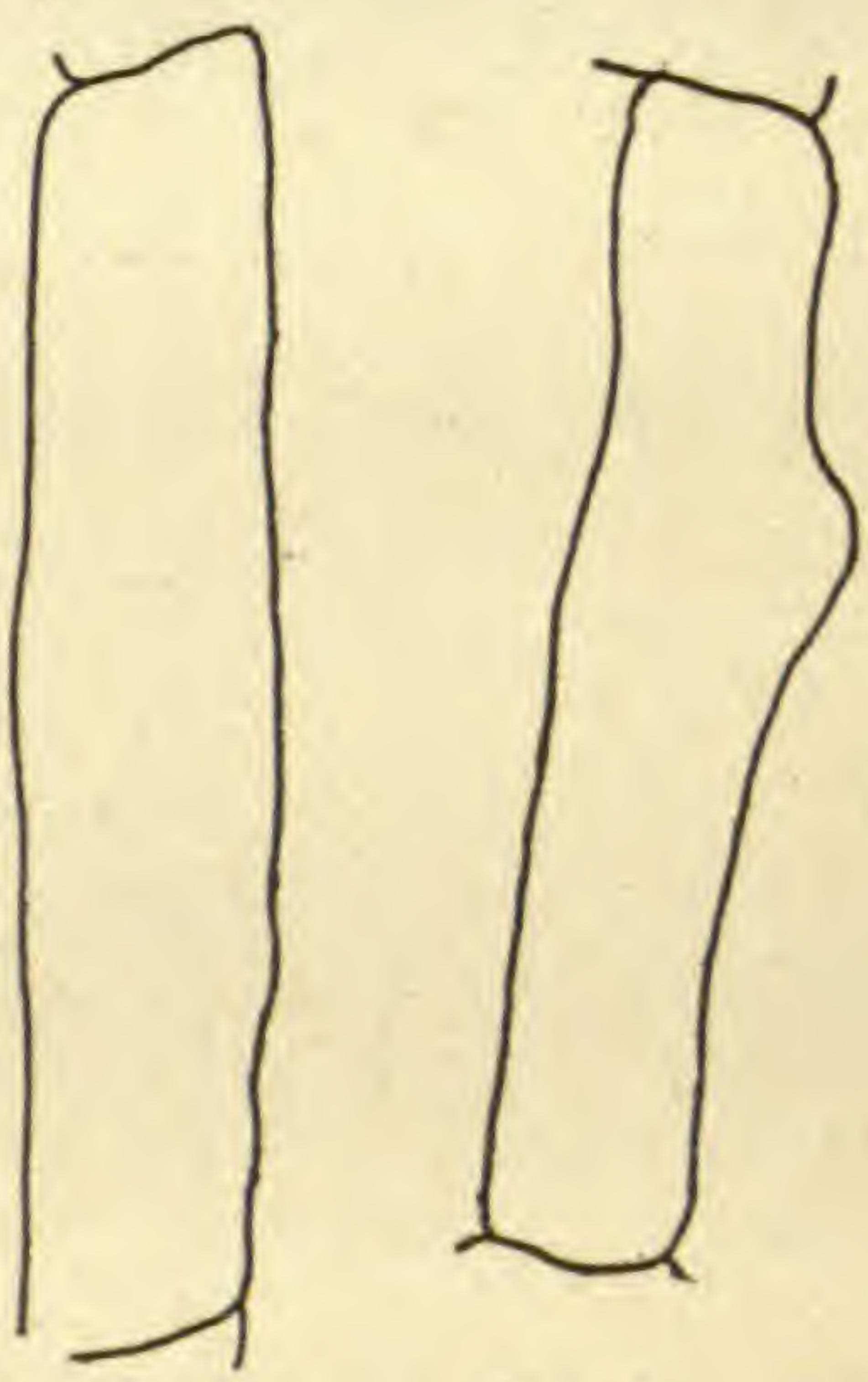
FIG. 5 FIG. 6

FIGS. 5, 6.—Fig. 5, bulging of entire wall; fig. 6, showing a narrowing of the swollen portion.

I. IN AIR MEDIUM

a) *The physical character of walls 1, 2, 4, 6*.—The first evidence of the formation of a root hair is the bulging of the entire wall *1*, as shown in fig. 5; then a portion of the wall stretches more than the rest and there is a narrowing of the swollen portion, as in fig. 6; but it may be noted that the base of a root hair is always greater in diameter than the rest of the hair, as in fig. 7. Seedlings of endive and lettuce demonstrate this fact. Branching of the hairs is common, indicating a difference in the physical character of the wall *1*. Sometimes the branch will be of less diameter than the main hair, as in fig. 8; and again branches will be of equal diameter, as shown in fig. 9.

The stretched condition of the wall is evidenced by the fact that in some root hairs which are grown in moist air the membranes burst when the hairs are placed in water, or in a solution which has an osmotic pressure lower than that of the root hair cell, as shown in fig. 10; whereas plasmolysis occurs when the root hairs are placed in a solution of higher osmotic strength (fig. 11). The root hair remains normal when placed in a solution of an osmotic pressure equal to that of the cell content, as in fig. 12.

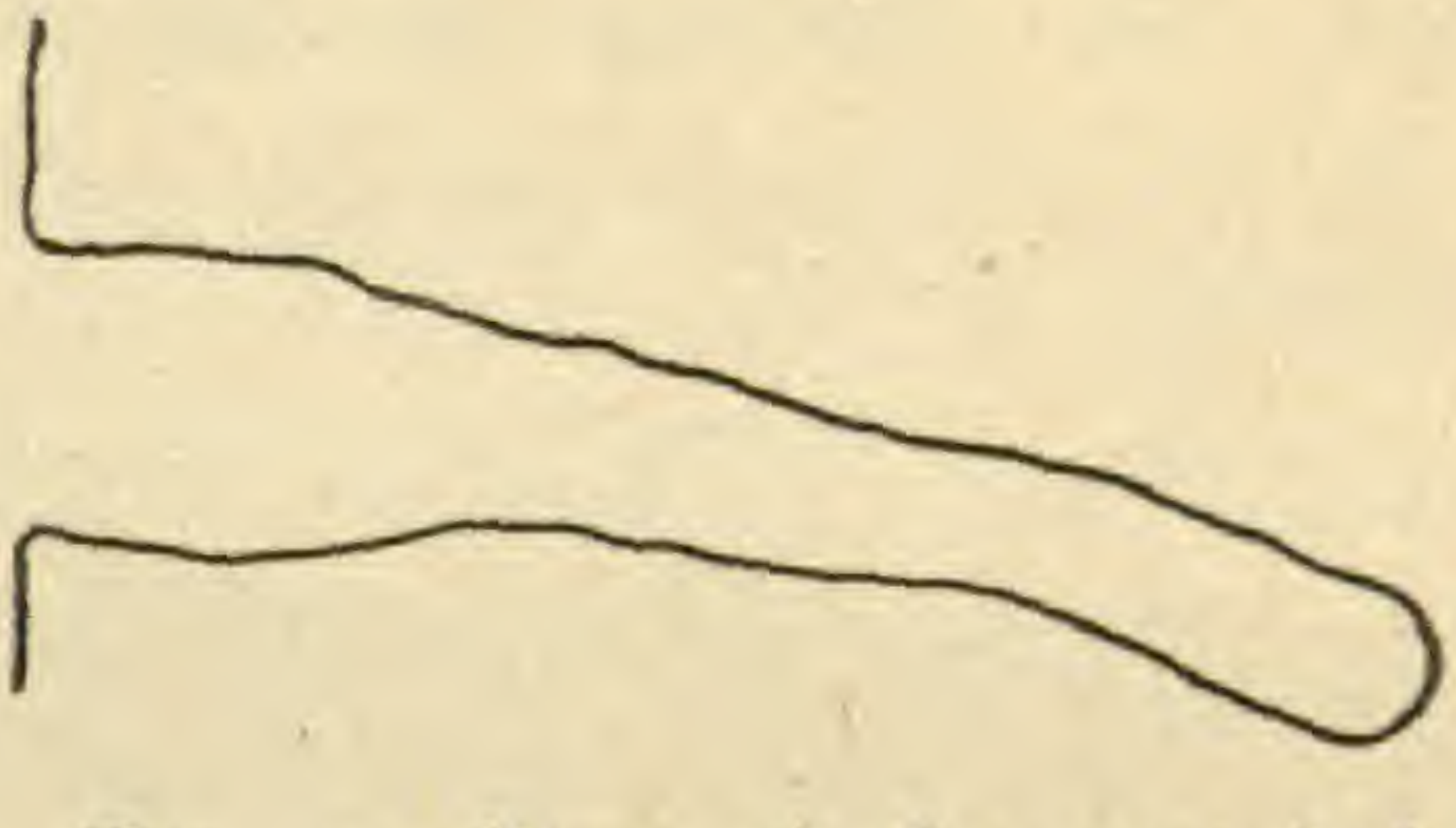

FIG. 7.—Base of the root hair greater in diameter than the rest of the hair.

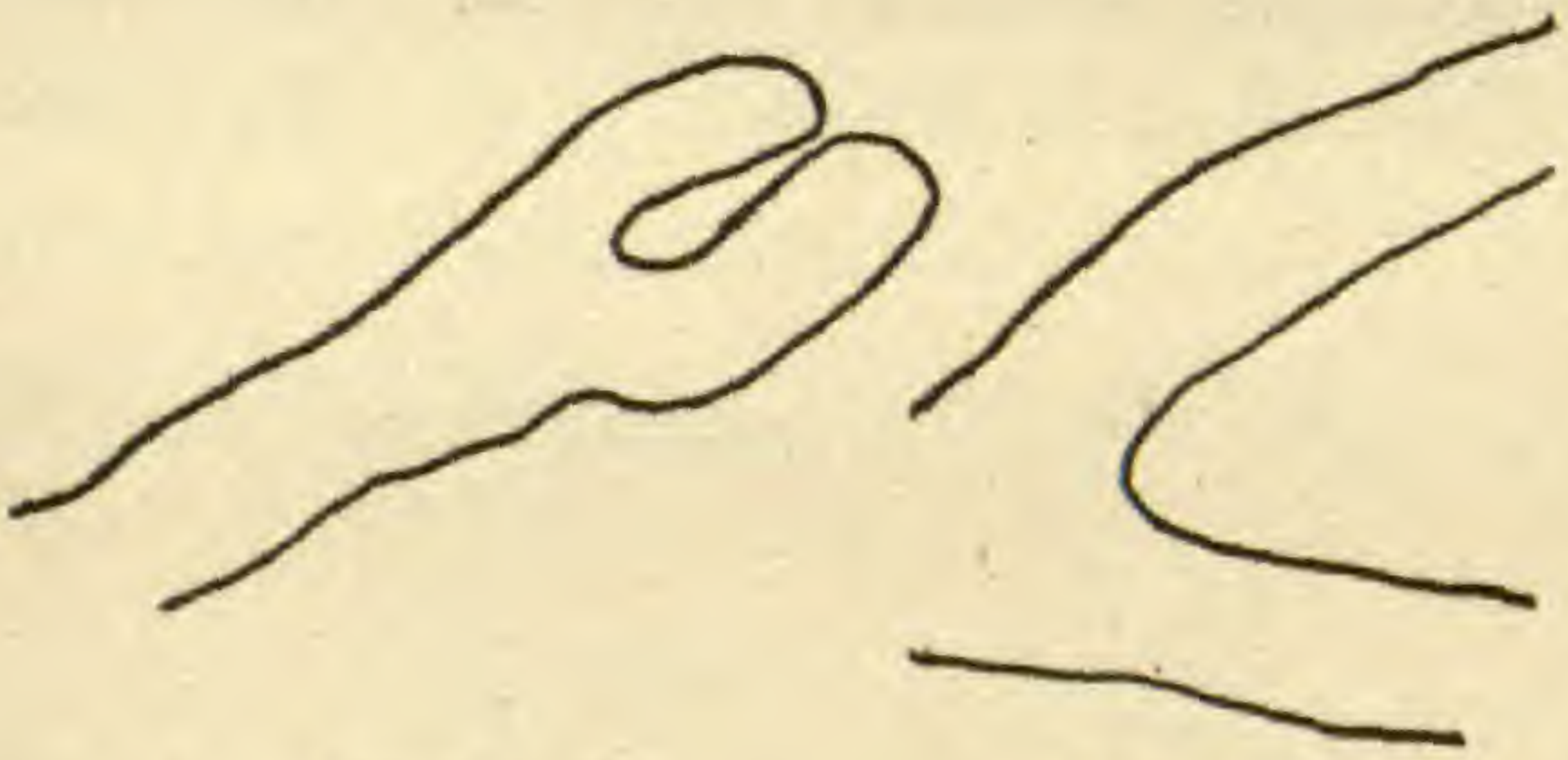

FIG. 8 FIG. 9

FIGS. 8, 9.—Fig. 8, branch of the root hair of less diameter than the main hair; fig. 9, branches of equal diameter.

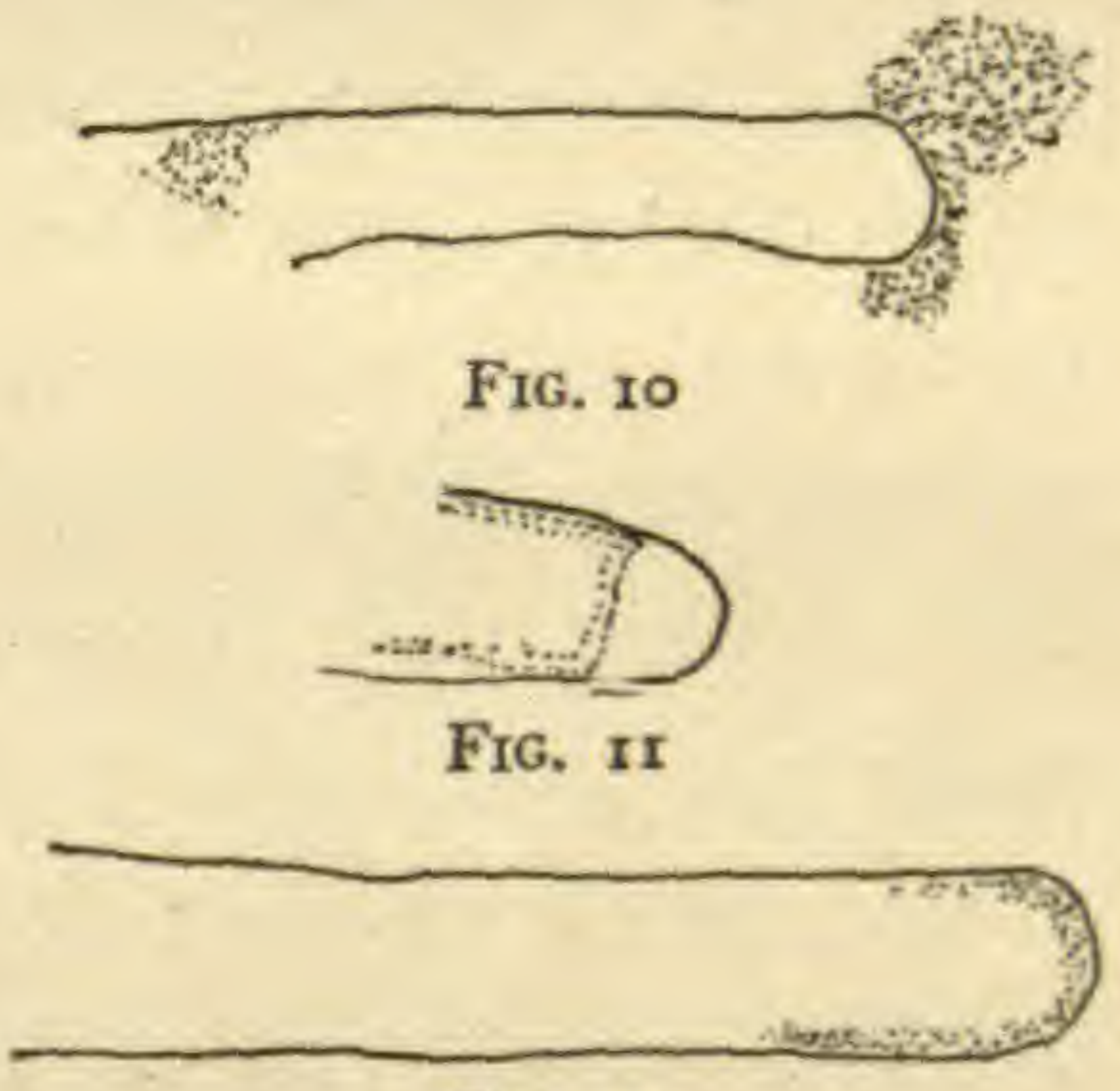

FIG. 10

FIG. 11

FIG. 12

FIGS. 10–12.—Fig. 10, root hair grown in moist air and placed in water, membrane burst and content escaped; fig. 11, root hair which has been in a solution of an osmotic pressure higher than that of the root hair; fig. 12, root hair placed in a solution of an osmotic pressure equal to that of the cell content.

The following forms when grown in moist air and then mounted in distilled water were found to burst immediately: alfalfa, barley, cabbage, mustard, *Nasturtium*, radish, *Xanthium*, and wheat. These when mounted in a solution of sucrose of an osmotic pressure equal to the osmotic pressure of the cell in question remain unbroken, whereas plasmolysis occurs if a solution of higher osmotic pressure is used. The cells burst at the tip and a part of the content of the cell is ejected. The nucleus is thrown out if it happens to be at the tip of the root hair, but in case it is in or near the base of the cell it remains within the root hair. The break in the wall is immediately closed as the membrane springs back, and it is hard to detect the point of rupture except for the position of the escaped protoplasmic contents. The younger cells are more likely to burst than are the older ones. Fig. 13 (*a*) shows a radish root hair mounted in 0.24M sucrose solution; fig. 14 (*b*) is the same cell after being put in a 0.40M sucrose solution. It is to be noted that the walls corresponding to *2*, *4*, *6* are only very slightly changed, and wall *1* has decreased, thus showing that even in the same cell the physical condition of wall *1* is unlike that of walls *2*, *3*, *6*. In cells which do not burst when mounted in water, the surface of wall *1* is increased, as evidenced by a swollen tip which may or may not assume odd shapes. This indicates that wall *1* of these plants has a greater resistance, thereby indicating a variation in the epidermal cells of different plants, as well as a difference in wall *1* from walls *2*, *4*, *6*.

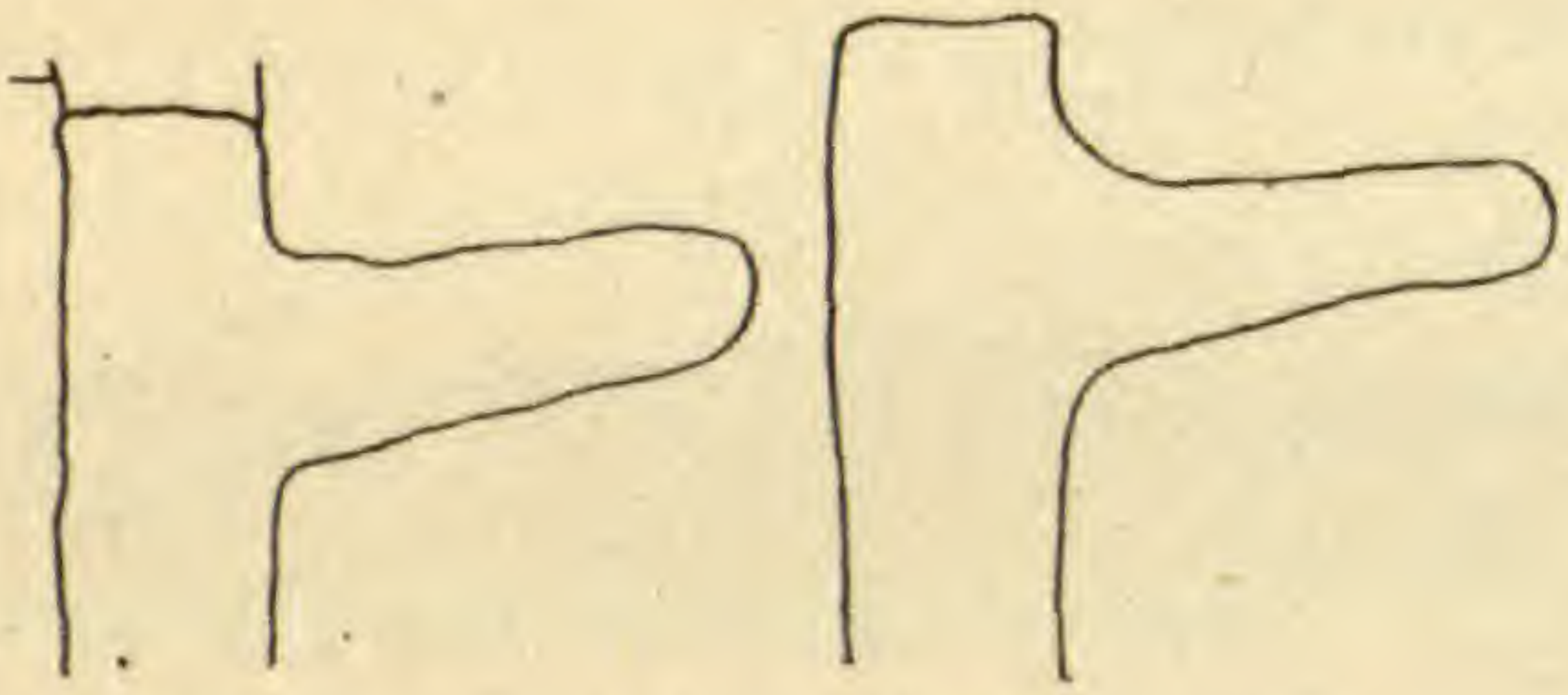

FIG. 13 FIG. 14

FIGS. 13, 14.—Fig. 13, root hair mounted in 0.24M sucrose; fig. 14, root hair after being placed in 0.40M sucrose.

It is frequently seen in many forms, for example, alfalfa, cabbage, and *Verbascum*, that short root hairs are mingled with long ones, giving the appearance of younger and older ones being together (fig. 15). The difference may be one of time of formation or of a variability in the growth of wall *1;* at least in these forms the length of the hairs is not a grading one as seen in the forms usually figured.

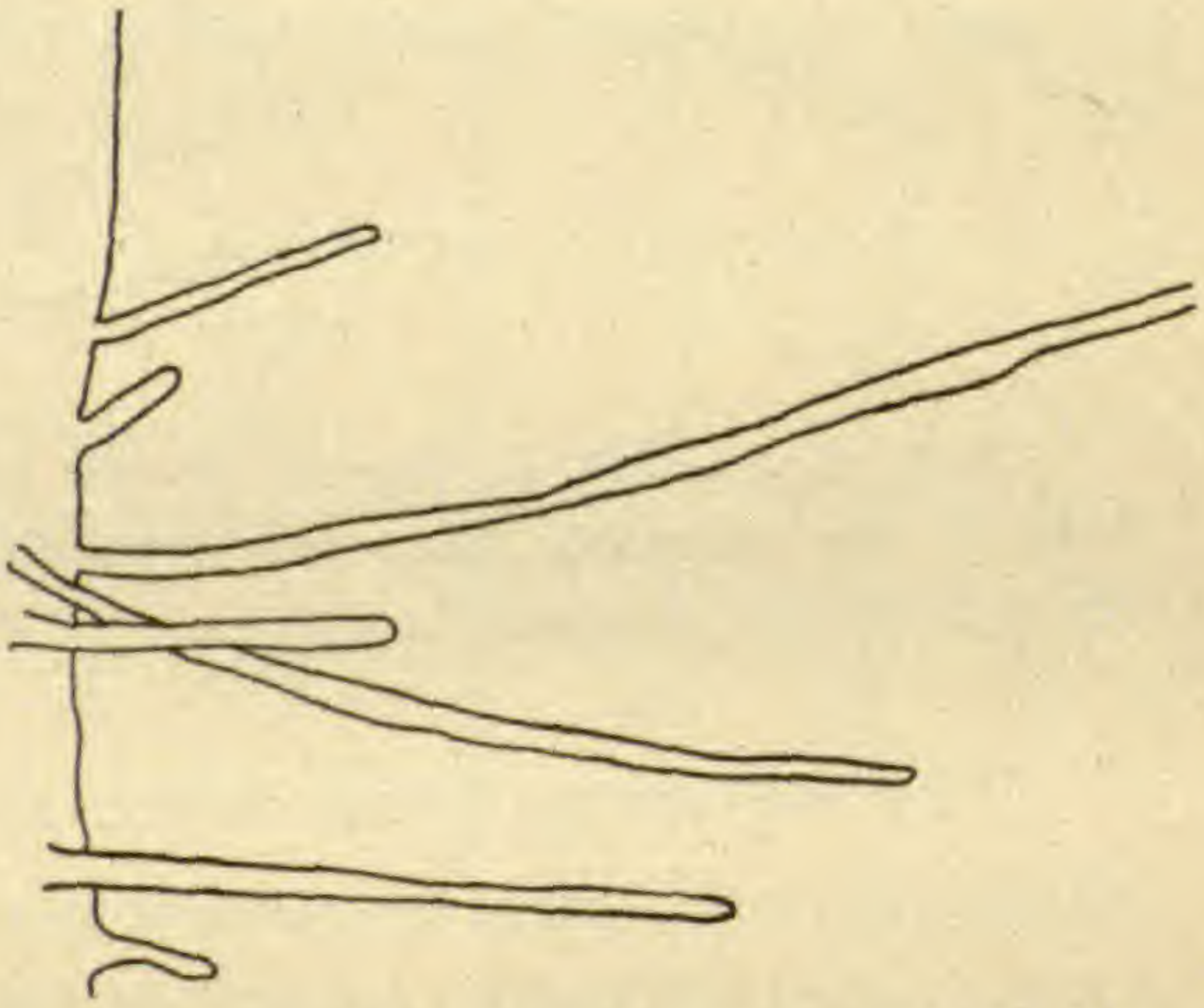

FIG. 15.—Short root hairs mingled with long ones.

b) *The chemical nature of walls 1, 2, 4, 6.*—It will be seen from table III that a majority of the forms investigated have a wall made up of two parts, an inner membrane which gives the cellulose reaction with 75 per cent H_2SO_4 and IKI, and an outer membrane which stains red with Ruthenium red, denoting pectin. That this membrane is calcium pectate is shown by the fact that on the addition of ammonium oxalate the membrane breaks down and calcium oxalate crystals are formed. It will be seen that some forms have a third membrane at the tip of the root hair. This membrane stains with all the callose stains, blue with aniline blue and resorsin blue, red with corallin soda. It will be noted that corn is the only form examined which has one membrane and that of cellulose.

TABLE III

PLANT	CHEMICAL NATURE OF ROOT HAIR MEMBRANES		
	Inner	Outer	At tip
Alfalfa..........	Thin cellulose	Thick pectin	
Amaranthus......	" "	" "	
Barley..........	" "	" "	
Corn............	Thick "	....	
Corn salad.......	Thin "	Thick "	Callose
Cabbage........	" "	Thin "	
Daucus carota....	" "	" "	
Morning-glory....	" "	" "	
Mustard........	" "	" "	Callose
Nasturtium......	Thick "	Thick "	
Pea.............	" "	" "	Callose
Sisymbrium......	Thin "	Thin "	
Tobacco.........	" "	Thick "	
Tradescantia.....	Thick "	Thin "	Callose
Vanilla..........	Thin "	" "	
Ceraptopteris.....	Thick "	" "	Callose

The walls *2*, *4*, *6*, and *1* are alike in that they all have an inner membrane of cellulose and an outer membrane of calcium pectate, but differ in that the calcium pectate membrane on wall *1* is thicker, and in some instances in the presence of callose at the tip of the hair. The calcium pectate membrane on side *1* is continuous with the middle lamella of calcium pectate of walls *2*, *4*, *6*. There is then a secretion of calcium pectate about the entire cell.

The relative thickness of the membranes having cellulose and pectin layers varies with the different plants, the cellulose layer being uniform over the hair, while the pectin is quite often thinner near the tip. Fig. 16 shows the tip of the root hair of *Nasturtium* with the two layers, the outer of pectin, and the inner of cellulose. Fig. 17 shows another *Nasturtium* root hair after it had been in ammonium oxalate; the calcium pectate has been changed to calcium oxalate and pectic acid. The calcium oxalate crystals are shown in the figure. The addition of Ruthenium red now gives no color except a faint tinge of pink about the crystals, due to the presence of pectic acid. Within the cell there are always masses of pectic substances; this is not calcium pectate, as it does not break down on the addition of ammonium oxalate, but still gives the color with Ruthenium red after treatment with ammonium oxalate.

FIG. 16 FIG. 17

FIGS. 16, 17.—Two layers of the root hair membrane; fig. 17, *Nasturtium* root hair after being in ammonium oxalate.

Either of the membranes may be removed by treating the cell with their respective solvents. The calcium pectate membrane is soluble in 2 per cent HCl and 2 per cent KOH, the cellulose is soluble in copper-oxide-ammonia.

The fact that the soil particles are held to the hair by the transformation of the outer layer into mucilage has long been accepted, but what that mucilage is has not been known. In the case of corn growing in the soil or in quartz sand, the mucilage was found to be of cellulose, whereas in a *Coleus* root growing in the soil the soil particles are held to the hair by a pectin mucilage.

It has been known that the epidermal portion of the seedling from which hairs arise stains brown when put in a 2 per cent solution of potassium permanganate, and that the region just above that of the hairs is not colored by the solution. This has often been used to differentiate in a general way the stem and root regions of a seedling. The chemical nature of these walls was determined. The external wall of the epidermal cells above those forming root hairs has without the calcium pectate membrane on side *1* of the epidermal cell a third thin layer which is of cutin. It stains red with Sudan III and is insoluble in 50 per cent chromic acid. This wall *1* of the epidermal cell of the stem is then chemically different and physically of greater rigidity.

c) The osmotic pressure on both sides of walls 2, 4, 6, and on inside of wall 1.—The cortical cell is always plasmolyzed by a solution of sucrose which is 0.02M–0.04M stronger than that which plasmolyzes the epidermal cell; this indicates that on the outside of the wall *6* there is a greater pressure than on the inside; whereas on the outside of *2* and *4* there is a pressure equal to that on the inside. On the outside of wall *1* there is a pressure of one atmosphere. The variation between the outside and inside pressures of wall *6* is small, but there is a great variation in the pressures upon the two sides of wall *1*. Table IV gives the osmotic pressure of the root hairs of different plants grown under similar conditions.

TABLE IV

Plant	Osmotic pressure of root hair	Plant	Osmotic pressure of root hair
Alfalfa	0.24M sucrose	Lettuce	0.30M "
Amaranthus	0.30M "	Mustard	0.22M "
Barley	0.40M "	Nasturtium	0.28M "
Corn	0.28M "	Pea	0.22M "
Corn salad	0.28M "	Radish	0.28M "
Cabbage	0.28M "	Sisymbrium	0.24M "
Celery	0.30M "	Sunflower	0.22M "
Daucus carota	0.24M "	Turnip	0.30M "
Endive	0.24M "		

It will be noted in table IV that all are plasmolyzed by a solution of sucrose between 0.22M–0.30M sucrose, except barley. This is the equivalent of 5.10–7.70 atmospheres. Two facts are indicated here: first, that in moist air there is a minimum of difference of 4 atmospheres between the pressure upon the inside and upon the outside of wall *1*; and second, that under similar conditions there is a remarkably slight variation in the osmotic pressure of root hairs of seedlings of different genera. Some roots respond more readily than others to the percentages of moisture present in the media. Cabbage, alfalfa, squash, and corn show a definite response. The formation of the root hair is inhibited by removing the covers of the Petri dishes in which the seedlings are growing. Regions of no root hairs, corresponding to the growth made during the removal of the cover, followed in regions of hair formation at the period of its replacement.

A series of corn seedlings was grown in air of a known moisture content. This was controlled by percentage solutions of sulphuric acid. The corn was soaked for 24 hours and then hung on cork plates in bottles which were one-third filled with the varying solutions. The bottles, which were corked and paraffined, were all placed in a water bath kept at 24°C. The measurements in table V represent in each case the average of 5 sets.

TABLE V

Solution	Length of root in cm.	Diameter of root in mm.	Length of hair in mm.
Water..............	3.0	70	80
1 per cent H_2SO_4....	1.0	64	70
2 " " "	2.0	65	70
3 " " "	3.0	63	66
4 " " "	2.0	60	50
5 " " "	1.5	70	70
6 " " "	1.5	60	50
7 " " "	1.5	55	50
8 " " "	1.0	65	60
9 " " "	1.5	66	41
10 " " "	1.0	64	23
11 " " "	1.0	61	24
12 " " "	1.0	70	10
13 " " "	1.0	70	10

Table V shows that the cutting down of the moisture content affects the length of the root hair more than the length of the root or the diameter.

2. IN LIQUID MEDIUM

a) *The physical character of the walls.*—There is a variation in the wall of the corn in different media. In moist air the wall is uniform; in water when formed the hair is thickened at the tip.

b) *The chemical nature of the walls.*—The chemical study of the walls is under investigation.

c) *The osmotic pressure on both sides of 1, 2, 4, 6.*—The only wall on which the osmotic pressure may be made to vary at will is on the outside of wall *1*. Radish seedlings were grown in sucrose solutions of increasing concentrations in order to vary the osmotic pressure on the outer wall. The results obtained as the average of several series are given in table VI.

TABLE VI

Grown in	Diameter root in 1/100 mm.	Length hair in 1/100 mm.	Osmotic pressure	Difference between osmotic pressure of root hair and medium
Air......................	60	....	28	28
Water....................	40	50	38	38
0.02 molecular sucrose.....	40	30	42	40
0.04 " "	40	20	42	38
0.06 " "	40	40	42	36
0.08 " "	40	50	49	32
0.10 " "	20	20	40	30
0.12 " "	40	60	42	30
0.14 " "	55	60	44	30
0.16 " "	30	40	48	32
0.18 " "	0	10	0	0
0.20 " "	0	20	0	0
0.22 " "	20	10	50	38
0.24 " "	30	10	65	41
0.26 " "	30	20	60	34
0.28 " "	50	20	65	37
0.30 " "	40	40	65	35
0.32 " "	30	10	0	0
0.34 " "	40	40	70	36
0.36 " "	15	10	75	39
0.38 " "	20	10	65	27
0.40 " "	30	20	0	0
0.42 " "	20	15	0	0
0.44 " "	50	15	70	36
0.48 " "	70	15	75	29
0.48 " "	0	0	0	0
0.52 " "	50	20	80	28
0.60 " "	50	0	90	30
0.65 " "	50	10	100	35

It is seen that as the concentration of the media increases the osmotic pressure of the root hair increases almost in direct proportion. Thus the root hair maintains an osmotic pressure from 4 to 6 atmospheres above that of the medium.

Differential media.—The effect of different media on the two sides of a seedling root was determined. Petri dishes were filled with the solutions, seeds were attached to the edge, and the roots grew upon the solutions. The upper half of the root was in moist air and the lower half in the solutions. The roots grew straight, so that curvature could not account for the difference in the formation of the root hairs.

TABLE VII

Plant	Diameter of root in mm.	Length of hair (in mm.)	
		In air	In solution
Helianthus..........	8	8	1 (0.25M sucrose)
"	8	5	1 (0.20M "
"	5	5	1 (0.10M "
"	5	5	1 (0.05M "
Radish..............	5	8	2 (0.10M "

Discussion

The measurement of the length of cell walls as an indication of differential growth seems of little value unless the number of the cells in any region is taken into account. Since there is not an epidermal cell corresponding to each individual cortical cell, it would seem that the sum total of the epidermal cells should be known in order to determine differential elongation as a factor. So far as known, the measurements taken were of individual adjoining cortical and epidermal cells.

BARDELL's own measurements show that for corresponding epidermal and cortical cells there is no definite relation in length which would cause the epidermal cell to produce a root hair, as neither do the measurements found here on corn.

The factors which determine the initial swelling of the outer wall of the epidermal cell are of primary importance, while other factors come in which determine the later growth into a root hair.

It has been proved in root hairs that there are portions of wall *1* less resistant than others. The bursting of the root hair, the swelling, the branching, and the varying thickness of the membrane, all give evidence of this. The indications are that such weaker places exist in the membrane at the time of root hair formation, although this has not yet been proved definitely. These weaker regions would determine the region of the wall in which the localization of swelling takes place, which immediately follows the initial swelling of wall *1*.

The position of the nucleus can have nothing to do with the initial swelling, as the swelling is over the entire length of the wall *1*. Since the weaker places bear no relation to the position of the nucleus, the nuclear position can in no way affect the formation of the root hair.

In this discussion the second type given by LEAVITT is not considered, the type in which only specialized epidermal cells form root hairs. Preliminary observations indicate that the osmotic pressure of the short cells varies from that of the other cells, but there are doubtless other factors determining the hair formation.

Investigations so far indicate that there are two factors of importance in the initial formation of root hairs. One is the unequal pressure acting upon either side of wall *1;* the other is the variation in the physical character of the wall.

The difference in pressures on the two sides of walls *2, 4, 6* is so much less than the difference on the two sides of wall *1* that it is negligible. The osmotic pressure of the root hairs of the plants investigated when grown in moist air shows slight variation. In the plants examined the osmotic pressure approximates 5 atmospheres. The walls *2, 4, 6* have an opposing pressure equal to or greater than 5 atmospheres, but wall *1* must sustain a pressure of 4 atmospheres, for on this wall the internal pressure is opposed by only one atmosphere, when the root is grown in moist air. This pressure is sufficient to account for the initial swelling of wall *1*.

The result of increasing the osmotic pressure on the outside of wall *1* by growing radish seedlings in sucrose solutions of increasing osmotic value proved that the opposing pressures on either side of wall *1* still maintained a balance of at least 4 atmospheres in favor of an outward pressure.

Root hair formation is retarded when the moisture content of the air is decreased. This is shown by the experiments with seedlings grown in Petri dishes in alternating dry and moist air, and in those grown over sulphuric acid solutions. Reduced moisture affects the membranes by decreasing the extensibility of the walls due to an increase in the elasticity of the colloids. The osmotic pressure of the cell content is also increased by the modification of the moisture content of the air, but this is less effective because of the greater effect of the reduced moisture content upon the character of the wall.

The effect of reduced moisture content is again demonstrated in the experiments with differential media. In the case of *Helianthus* the effect of the sucrose molecule upon the colloids of the membrane and in the radish of glucose is to decrease the moisture content and the extensibility of the wall.

The wall is found to be composed of an outer membrane of calcium pectate and an inner membrane of cellulose. The only exception so far is in corn. A partial third membrane of callose is present in some. The amount of callose is so small and so little is known of its chemical nature that for the present it seems unimportant. The cellulose layer is important in that it gives greater strength to the wall.

The pectin layer and also the pectic substances within the cell are very important in the absorption and the retention of water, since pectin is a hydrophyllous colloid; and for this reason must influence the development of root hairs. The cations and the anions of the mineral substances in solution increase or decrease the absorptive capacity of this membrane according to a definite known manner, as indicated in the lyotropic series, investigations upon which should be made.

Summary

1. The initial formation of the root hair is indicated by a general swelling of the outer wall of the epidermal cell.
2. The swelling is produced if the physical resistance of the wall is overbalanced by the higher osmotic pressure which is maintained on the inside of the wall.
3. Further swelling followed by growth takes place at the less resistant portion of the wall.
4. This region bears no relation to the position of the nucleus.
5. The wall of the root hair is composed of two parts, an inner membrane of cellulose and an outer membrane of calcium pectate.

6. The presence of this membrane, together with the fact that the soil particles are held to it by a pectin mucilage, accounts for the high efficiency of the root hair as an absorbing organ.

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BRIEFER ARTICLES

OCTAVE LIGNIER

(WITH PORTRAIT)

The subject of this sketch was born at Pangy in Champagne in 1855, and died at the comparatively early age of 61. His studies were carried on at Lille, under BERTRAND, GIARD, and GOSSELET. He received the doctorate later at Paris, but the environment of Lille seems to have exercised a greater influence on his subsequent scientific work. Lacking personal fortune, his life was a perpetual struggle to improve the small initial equipment he found at Caën, where he was made professor in 1887. His scientific achievement, in spite of this handicap, was more notable in the 25 years of his professorial activities (celebrated in January 1913) than usually distinguishes capacity favored by fortune and environment. More than 200 articles emanated from LIGNIER'S laboratories, and his activities covered the important fields of general morphology, anatomy, paleobotany, and plant geography. This wide range of interest was due partially to the fact that at first he had to cover the entire field of botany (including even a large herbarium), for only in later years was it possible to relegate certain curatorial and instructional duties to others. More decisive than necessity in the wide scope of his scientific activities were his untiring energy, capacity, and optimism.

Notable among his paleobotanical studies are those on Cycadofilicales, Bennettitales, Coniferales, etc. In the first group he described an interesting new genus *Mittagia* (resembling *Lagenostoma*), characterized by megasporangia not solitary but in sori, and also by the presence of 4 functional megaspores in each megasporangium.

The author's studies on the fossil floras of Normandy are of importance. In dealing with fossil conifers he did not make the mistake of certain German and English anatomists, in regarding rays as of paramount diagnostic significance. A supposed palm leaf *Propalmophyllum liasanum*, described by our author from the Lias, is according to ZEILLER of very problematical value. From the same geological level LIGNIER described remains of Cordaitales and Calamodendreae, a much later occurrence of these ancient types than has hitherto been recorded.

Important anatomical results reached by the author independently of, but in conformity with, workers on this side of the Atlantic were the conclusion that the herbaceous is derived from the woody type of stem, and that leaves are of great importance in the evolutionary history of plants. He divided vascular plants on the basis of the morphology of the leaf into Phylloideae and Phyllineae. The Phylloideae represent the Lycopsida, with the Equisetales and Sphenophyllales left out. The Phyllineae correspond to the Pteropsida, with the Equisetales and Sphenophyllales added (under the caption of Articulatae). SCOTT has further added to the latter the Psilotaceae and erected them into the phylum Sphenopsida.

LIGNIER'S activities were so numerous and important that it is impossible to do justice to them here. Botanical evolution has suffered a great loss in his death.—E. C. JEFFREY, *Harvard University*.

CURRENT LITERATURE

NOTES FOR STUDENTS

Current taxonomic literature.—B. BALFOUR and W. W. SMITH (Notes Roy. Bot. Gard. Edin. **9**:63, 64. *pl. 148.* 1915) describe and illustrate a new genus (*Beesia*) of the Ranunculaceae from northern Burma and Yunnan, China. —R. BENOIST (Not. Syst. **3**:176–180. 1915) has published 6 new species of *Qualea* and *Lecythis* from South America.—E. P. BICKNELL (Bull. Torr. Bot. Club **42**:549–570. 1915) in the 16th article on the "Ferns and flowering plants of Nantucket" enumerates the Compositae and describes a new golden-rod (*Solidago aestivalis*).—S. F. BLAKE (Jour. Bot. **53**:56–58, 103–104, 135–137, 153–158, 193–202, 225–235, 306–307, 322–324. 1915) has published a "Revision of *Salmea* and allied genera," describes new speceis in *Amaranthus*, *Heterosperma*, *Notoptera*, *Otopappus*, *Perymenium*, *Verbesina*, and *Zexmenia* from Mexico, Central and South America, and includes also a new genus (*Steiractinia*) of the Compositae to which 6 species are referred, all being indigenous to northern South America. The same author (Kew Bull. 348. 1915) has published a new genus (*Stenocarpha*) of the Compositae from Mexico.—F. BÖDEKER (Monats. für Kakteenkunde **25**:76–80. 1915) describes and illustrates a new species of *Mamillaria* (*M. multihamata*) from Mexico.—A. BRAND (Rep. Sp. Nov. **14**:146–156. 1915) under the title "Neue Borraginaceen Studien" describes several new species and raises the section *Mattiastrum* Boissier of *Paracaryum* to generic rank and transfers thereto 26 species. The same author (*ibid.* **13**:545–550. 1915) in an article entitled "Neue Gattungen und Arten der Cynoglosseae" describes several species new to science and characterizes two new genera, namely *Adelocaryum* from India and Central Asia, and *Bilegnum*, which is based on *Mattia Bungei* Boiss., from Persia.—T. S. BRANDEGEE (Univ. Calif. Pub. Botany **6**:177–197. 1915) in a 7th article under the caption "Plantae mexicanae Purpusianae" has published 61 new species of flowering plants, mainly from Chiapas and Oaxaca.—N. L. BRITTON (Ann. Mo. Bot. Gard. **2**:33–58. *pls. 1, 2.* 1915) gives a brief account of the vegetation of Mona and Desecheo islands, which lie between Porto Rico and Santo Domingo, and estimates that the total land flora of the former consists of as high as 500 species and of the latter of at least 200 species. A partial list of the plants collected on Mona Island is appended. New species are recorded in *Pedilanthus*, *Tabebuia*, and *Riccia*. One new genus (*Mallotonia*) is proposed, which is based on *Tournefortia gnaphalodes* R. Br. The same author (Bull. Torr. Bot. Club **42**:365–392. 1915) under the general title "Studies of West Indian plants VI" includes a synoptical revison of the genus

Coccolobis in Cuba, recognizing 23 species of which 6 are new to science; a revision of *Anastraphia* with 21 species and 12 new; 14 new species are added to *Tabebuia;* and 12 new species are recorded in various genera of flowering plants. In a 7th article of the same series (*ibid.* 487–517) are included synoptical revisions of the Cuban *Scleria* with 28 species of which 4 are new, of *Tricera* with 18 species of which 9 are new, of *Plumiera* with 9 species of which 5 are new, of *Guettarda* with 26 species of which 7 are new; additional new species are described in *Badiera, Coccolobis, Portulaca, Chamaecrista, Rhamnidium, Nashia,* and *Exostema.*—V. F. BROTHERUS and S. OKAMURA (Bot. Mag. Tokyo **29**:186–188. *pl. 8.* 1915) describe and illustrate a new genus of moss (*Ishibaea*) from Japan. The genus is dedicated to EKICHI ISHIBA, a noted Japanese bryologist.—F. BUBÁK and H. SYDOW (Ann. Mycol. **13**:7–12. 1915) under the title "Einige neue Pilze" have published several new species of fungi including a new genus (*Pachybasidiella*) found on leaves of *Acer dasycarpa.*—L. BUSCALIONI and G. MUSCATELLO (Malpighia **27**:127–190. 1915–1916) in continuation of their studies of the genus *Saurauia* have published two new species from Costa Rica.—A. H. CHIVERS (Mem. Torr. Bot. Club **14**:155–240. *pls. 6–17.* 1915) has published a "Monograph of the genera *Chetomium* and *Ascotricha,*" recognizing 28 species of the former genus and 2 of the latter.—E. CHIOVENDA (Annali di Botanica **13**:371–410. 1915) under the title "Plantae novae vel minus notae e Regione Aethiopica" describes several species and proposes the following new genera: *Afrotrichloris* of the Gramineae and *Peltophoropsis* of the Leguminosae.—R. CHODAT (Bull. Soc. Bot. Genève II. **7**:65, 66. 1915) has published a new species of *Prosopanche* (*P. clavata*) from South America.—O. F. COOK (Jour. Wash. Acad. Sci. **5**:236–241. 1915) has proposed two new genera: *Glaucothea*, based on *Brahea armata* Wats. of northern Lower California, and *Styloma*, based on *Pritchardia pacifica* Seem. and Wendl. a palm of the Fiji Islands. The same author (*ibid.* 287–289) characterizes a new genus (*Tribroma*), based on *Theobroma bicolor* Humb. and Bonpl.—A. DAVIDSON (Bull. S. Cal. Acad. Sci. **14**:11, 12. 1915) has described two new species of *Calochortus* (*C. discolor* and *C. campestris*) from California. K. DOMIN (Bibl. Botanica, Heft 85^{iv}. 401–551. *pls. 14–18.* 1915) in continuation of his studies on the flora and plant geography of Australia has published upward of 50 new species and varieties of monocotyledonous plants and describes a new genus (*Queenslandiella*) of the Gramineae.—H. A. EDSON (Jour. Agric. Research **4**:279–291. *pls. 44–48.* 1915) gives an account of a new fungus disease of sugar beets and radishes for which he proposes the generic and specific name *Rheosporangium aphanidermatus.*—A. D. E. ELMER (Leafl. Philipp. Bot. **7–8**:2543–2919. 1915) has described 248 new species of flowering plants of the Philippine Islands. One new genus (*Quadrasia*) of the Flacourtiaceae is included.—A. ENGLER (Bot. Jahrb. **53**:9–274, 312–605. 1915) in cooperation with several specialists has published the 44th and 45th articles of the series "Beiträge zur Flora von Afrika." Approximately 440 species new to science are recorded, and 3 new genera are proposed, namely, *Synandrodaphne* Gilg of the Thymelaeaceae, *Centrostigma* and *Stolzia* Schltr. of the Orchidaceae.—A. W. EVANS (Bull. Torr. Bot. Club 42:259–308. 1915) presents a discussion of the genus *Plagiochasma* in North America. Six species are recognized, one of which (*P. Landii*) from Mexico is new to science. —O. A. FARWELL (*ibid.* 247–257. *pls. 12–18.* 1915) presents a paper on the genus *Polygonatum* in Michigan; 5 species and several varieties are recognized. —R. G. FRAGOSO (Bot. Soc. Esp. Hist. Nat. 15:120–132. 1915) under the title "Hongos parásitos de la florula hispalense, nuevos ó poco conocidos" describes several species of fungi and includes a new genus (*Septoriopsis* Fragoso and Paul) which is parasitic on citrus fruits.—F. GAGNEPAIN (Not. Syst 3:180–192. 1915) under the title "Papilionacées nouvelles ou critiques" has published several species new to science and describes the following new genera: *Antheroporum*, *Diphyllarium*, and *Endomallus* from China.— H. M. HALL (Univ. Calif. Publ. Bot. 6:165–176. *pl. 20.* 1915) has published 7 new species of flowering plants from California and records important data on other species occurring in the state.—H. HARMS (Rep. Sp. Nov. 13:523–527. 1915) has published 2 new species of *Prosopis* from Paraguay and 7 new species of *Inga* from Central and South America.—E. HASSLER (Rep. Sp. Nov. 14:157–159. 1915) describes a new species of *Zexmenia* from Argentina and transfers two additional species to this genus from *Verbesina*. The same author (*ibid.* 161–180) has published several new species and varieties of flowering plants from Paraguay.—L. HAUMAN (Anal. Mus. Nac. Hist. Nat. Buenos Aires 27:285–306. 1915) under the title "Note sur les Joncacées des petits genres Andins" describes and illustrates a new genus *Andesia*, based on *Oxychloe bisexualis* Kuntze. The same author (*ibid.* 441–516) gives a synoptical revision of the Dioscoreaceae of Argentina, describing and illustrating several new species and varieties.—B. HAYATA (Tokyo Bot. Mag. 29:31–34. 1915) has proposed a new genus (*Pseudixus*), based on *Viscum japonicum* Thunb.—T. HERZOG (Mededeelingen Rijks Herb. no. 27. pp. 1–90. 1914–1915) in cooperation with several specialists records the plants collected by Dr. TH. HERZOG in Bolivia during the years 1910 and 1911. Upward of 60 species new to science are described, and one new genus (*Eleutherostemon*) of the Ericaceae is included.—A. S. HITCHCOCK (Am. Jour. Bot. 2:299–310. 1915) under the title "New or noteworthy grasses" records important data concerning a number of species, makes several new combinations, and describes 7 species new to science.—A. S. HITCHCOCK and A. CHASE (Contrib. U.S. Nat. Herb. 17:459–539. 1915) under the title "Tropical North American species of *Panicum*" have published an important contribution to our knowledge of this genus supplementing their recent revision of the group. In the present paper the authors recognize 116 species and 3 subspecies; and of these, 9 species are new to science. A valuable factor in this paper is the introduction of an outline map on which the geographical distribution of each species is indicated.—H. D. HOUSE (N.Y. State Mus. Bull. 176. p. 38. 1915) describes a new species of *Physostegia* (*P. latidens*) from near Utica, New York.—F. T. HUBBARD (Am. Jour. Bot. **2**:169–198. 1915) presents the results of a "Taxonomic study of *Setaria italica* and its immediate allies." Several new varieties, forms, and combinations are proposed.—A. HUE (Bull. Soc. Bot. France **15**:13–23. 1915) in an article entitled "Tribus Umbilicariacearum genera exposuit" characterizes a new genus (*Charcotia*), based on *Umbilicaria rufidula* Hue.—S. ITO (Bot. Mag. Tokyo **29**:15–22. *pl. 1.* 1915) describes and illustrates a new genus (*Typhulochaeta*) of the Erysiphaceae.—E. KOEHNE (Bot. Jahrb. **52**:277, 278. 1915) has published two new species of *Amelanchier* from western North America. The same author (*ibid.* 279–333) describes upward of 30 new species and varieties of *Prunus*, and (*ibid.* 334–345) records 8 species of *Pygeum* new to science.—B. KOSO-POLIANSKY (Jour. Russe Bot. 1915. pp. 1–21) under the title "A revision of the oriental genus *Grammosciadium* DC." recharacterizes this genus and proposes the following related genera: *Stenodiptera*, *Golenkinianthe*, and *Chrysophaë*.—F. KRÄNZLIN (Arkiv för Botanik **14**:no. 10. pp. 1–8. 1915) has published 7 new species of orchids from Mexico and Brazil.—I. M. LEWIS (Bull. Univ. Texas. no. 22, pp. 1–169. 1915) has recently published a small volume entitled "The trees of Texas: an illustrated manual of the native and introduced trees of the state."—TH. LOESENER (Rep. Sp. Nov. **14**:97–113. 1915) in a 5th article in the series "Mexikanische und zentralamerikanische Novitäten" in cooperation with several specialists records further collections of flowering plants made in Mexico mainly by RUDOLF ENDLICH. A new species and a new variety are described in *Plantago*.—J. LUNELL (Am. Mid. Nat. **4**:152–165. 1915) begins an enumeration of the vascular plants of North Dakota. One new variety of *Alisma* is characterized.—B. LYNGE (Arkiv för Botanik **13**:no. 13. pp. 1–172. *pls. 1–5.* 1914) under the title "Die Flechten der ersten Regnellischen Expedition" characterizes a new genus (*Pseudoparmelia*) and gives a synopsis of *Parmelia*, adding several new species to the latter genus.—K. K. MACKENZIE (Bull. Torr. Bot. Club **42**:405–422. 1915) in continuation of his studies in the genus *Carex* records a new species (*C. egregia*) from northern United States. The same author (Smiths. Misc. Coll. **65**:no. 7. pp. 1–3. 1915) describes two new species of *Carex* from the southwestern United States.—T. MAKINO (Bot. Mag. Tokyo **29**:279–283. 1915) proposes two new genera (*Matsumurella* and *Ajugoides*) of the Labiatae from Japan.—O. MATTIROLO (Mem. Accad. R. Scienze Torino II. **63**:213–218. 1912) has described and illustrated a new genus (*Jaczewskia*) of the Hysterangiaceae.—A. MAUBLANC and E. RANGEL (Bol. Agr. São Paulo, sér XVI. pp. 310–328. *pls. 4–9.* 1915) under the title "Alguns fungos do Brasil, novos ou mal conhecidos" have published several new species of fungi and propose three new genera, namely *Chaetolentomita* of the Pyrenomycetes, *Leandria* and *Didymothozetia* of the Hyphomycetes.—W. R. MAXON (Bull. Torr. Bot. Club **42**: 79–86. 1915) has published a synoptical revision of the North American species of *Psilogramme*, recognizing 8 species of which 3 are new to science. The same author (Smiths. Misc. Coll. **65**:no. 8. pp. 1–12. 1915) presents a "Report upon a collection of ferns from western South America" and includes the descriptions of 6 new species.—E. D. MERRILL (Philipp. Jour. Sci. **10**:1–84. 1915) in the 11th paper in the series entitled "New or noteworthy Philippine plants" has described about 90 species of flowering plants; and (*ibid.* 99–144) in continuation of his studies of the Rubiaceae of the Philippine Islands has published 42 new species in various genera of this family; and (*ibid.* 195–225) 12 new species of *Schefflera* and 22 of *Eugenia* are described from the Philippine Islands; and (*ibid.* 227–264) under the title "Studies on Philippine Anonaceae I" he records 70 species of this family of which 25 are described as new to science. Two new genera are proposed, namely *Griffithianthus* and *Guamia;* and (*ibid.* 265–285) under the caption "Plantae Wenzelianae III" 21 new species of flowering plants are recorded and one new genus (*Wenzelia*) of the Rutaceae is proposed. These are based primarily on plants collected in Leyte by Mr. C. A. WENZEL; and (*ibid.* 287–349) in continuation of his researches on the Philippine flora he has published 63 new species and several varieties of flowering plants. The same author (Jour. Wash. Acad. Sci. **5**:530–542. 1915) has taken up the generic name *Nauclea* L. and transferred thereto certain species hitherto referred to *Sarcocephalus.* A new generic name (*Neonauclea*) is proposed for *Nauclea* of KORTHALS and subsequent authors, and to *Neonauclea* the author transfers about 50 species.—G. MOESZ (Bot. Közlemények **14**:145–158. 1915) under the title "Mykologiai Közlemények" describes several new species of fungi and characterizes a new genus (*Chaetosphaeronema*), based on *Sphaeronema hispidulum* Corda.—A. M. MOSS (Jour. Bot. **53**:1–8. *pl. 535.* 1915) in an article entitled "The Aristolochias of Pará (Brazil)" includes descriptions of 4 new species of this genus by SPENCER MOORE.—J. A. NIEUWLAND (Am. Mid. Nat. **4**:89–95. 1915) has proposed two new genera, namely *Apetlorhamnus* and *Litanum.*—V. NORLIND (Arkiv för Botanik **14**:no. 6. pp. 1–18. *pls. 1–4.* 1915) has published 8 new species of *Oxalis* from South America.—C. H. OSTENFELD (Bull. Torr. Bot. Club **42**: 659–662. *pl. 32.* 1915) describes and illustrates a new species of *Ruppia* (*R. anomala*) from Porto Rico.—N. PATOUILLARD (Philip. Jour. Bot. **10**:85–98. 1915) under the heading "Champignons des Philippines communiques" has published several new species and includes one new genus, namely *Duportiella.*—R. PILGER (Notizblatt Königl. Bot. Gart. u. Mus. Berlin-Dahlem **6**:261–310. 1915) under the general title "Plantae Uleanae" in cooperation with several specialists has published upward of 60 new species and varieties of flowering plants based on the collections of E. ULE in South America. The same author (*ibid.* 311–396) has published approximately 75 species new to science. The following new genera are included: *Abutilothamnus, Codonochlamys,* and *Triplochlamys* Ulbrich of the Malvaceae, and *Microbignonia* Kränzl. of the Bignoniaceae.—H. PITTIER (Jour. Wash. Acad. Sci. **5**:468–474. 1915) has published 4 new species of leguminous trees from Panama.—E. L. REED (Torreya **15**:246, 247. 1915) describes a new species of sundew (*Drosera annua*) from Texas.—A. REHDER (Proc. Amer. Acad. **50**:225–241. 1915) presents a "Synopsis of the Chinese species of *Pyrus*" in which 12 species are recognized, 6 being new to science.—J. F. ROCK (Bull. Torr. Bot. Club **42**:77, 78. *pl. 8.* 1915) describes and illustrates a new species of *Cyanea* (*C. Larrisonii*) from the Hawaiian Islands.—R. A. ROLFE (Curtis' Bot. Mag. *pl. 8635.* 1915) describes and illustrates a new orchid (*Sievekingia Shepheardii*) from Colombia.—E. ROSENSTOCK (Hedwigia **56**:355–371. 1915) under the title "Filices brasilienses novae" has published 29 new species and varieties of ferns. —W. ROTHE (Bot. Jahrb. **52**:355–434. 1915) presents a revision of the genus *Marsdenia* recognizing 47 species of which 9 are new to science.—P. A. SACCARDO (Annal. Mycol. **13**:115–138. 1915) under the title "Notae mycologicae" describes new species of fungi from North America, Philippine Islands, etc., and includes a new genus (*Heteroceras*) found on dead leaves of *Tilia europaea.* —W. E. SAFFORD (Jour. Wash. Acad. Sci. **5**:355–360. 1915) has described 3 new species of bullhorn acacias from Mexico.—R. SCHLECHTER (Rep. Sp. Nov. **13**:537–544, 554–566. 1915) under the caption "Asclepiadaceae Philippinenses" has published 37 new species and proposes the following new genera: *Clemensia*, *Clemensiella*, *Dolichostegia*, and *Petalonema;* and (*ibid.* **14**:114–133. 1915) has described 40 new species of orchids from Ecuador, based on collections made by A. SODIRO and by L. MILLE. The same author (Orchis **9**:4–9, 17–20, 49–54, 90–96. 1915) has published several new species of orchids from Mexico, Central and South America, and includes a new genus (*Jainiopsis*) from India.—H. SCHINZ (Vierteljahrschr. Zürich Naturf. Gesells. **60**:389–422. 1915) under the title "Beiträge zur Kenntnis der afrikanischen Flora xxvi" in cooperation with the specialists F. KRÄNZLIN and A. THELLUNG has published several new species of flowering plants and includes the following new genera: *Manuleopsis* Thell. of the Scrophulariaeceae and *Dinteracanthus* C. B. Clarke of the Acanthaceae. The same author (*ibid.* 423–432) records 10 new species of flowering plants, 7 of which are from Mexico and South America.—W. A. SETCHELL (Univ. Calif. Publ. Bot. **6**:79–152. *pls. 10–16.* 1914) in an article entitled "The *Scinaia* assemblage" presents the results of a detailed study of the genus *Scinaia* and its near allies. Several new species are described and one new genus (*Pseudoscinaia*) is characterized.—C. SPEGAZZINI (Anal. Mus. Nac. Hist. Nat. Buenos Aires **27**:37–74. 1915) under the title "Segunda contribución al conocimiento de las Laboulbeniales Italianas" has published upward of 30 new species and proposes the following new genera: *Parahydraeomyces* and *Thripomyces.*—P. C. STANDLEY (Jour. Wash. Acad. Sci. **5**:57–59. 1915) describes a new genus (*Zuckia*) of the Chenopodiaceae from Arizona; and (*ibid.* 72–76, 391–396) presents the results of studies in the Amaranthaceae, reinstates *Centrostachys* Wallich, records new combinations in this genus as well as in *Iresine*, *Lagrezia*, and *Lithophila*. The same author (*ibid.* 600–602) describes a new species of *Geranium* (*G. Jahnii*) from Venezuela. —O. STAPF (Hook. Ic. *pl. 3033.* 1915) describes and illustrates a new genus (*Homozeugos*) of Gramineae from west tropical Africa.—G. K. SUTHERLAND (Trans. Brit. Myc. Soc. **5**:147–155. *pl. 3.* 1915) under the title "New marine

Pyrenomycetes" describes and illustrates two new genera, namely *Trailia* and *Orcadia*, both parasitic on the thallus of *Ascophyllum* and found along the shores of the Orkney Islands.—W. T. SWINGLE (Jour. Wash. Acad. Sci. **5**:165–176. 1915) has published an interesting article dealing with the kumquat oranges and describes a new genus (*Fortunella*) based on *Citrus margarita* Lour. Four species, all apparently originating from China, are referred to the new genus and they promise to be of considerable economic value in breeding citrus fruits. The same author (*ibid.* 569–578) records another new genus (*Microcitrus*) of citrus fruits, based on *Citrus australasica* F. Muell. Four species are recognized, all being natives of Australia.—H. and P. SYDOW (Ann. Mycol. **13**:35–43. 1915) under the heading of "Novae fungorum species XIII" include descriptions of two new genera, namely *Myelosperma* found on leaves of *Cocos nucifera* at Peradeniya and *Cheiropodium* found in Japan on leaves of *Carex breviculmis*.—R. THAXTER (Proc. Amer. Acad. **51**:1–51. 1915) under the title "New Indo-Malayan Laboulbeniales" has described 46 species new to science and proposes a new genus, namely *Tettigomyces*.—F. THEISSEN and H. SYDOW (Ann. Mycol. **13**:149–746. *pls. 1–6*. 1915) in a monographic study of the *Dothideales* have characterized the following new genera: *Achorella, Agostaea, Amerodothis, Anisochora, Armatella, Bagnisiopsis, Camarotella, Catabotrys, Catacaumella, Coccodothella, Crotone, Dibotryon, Dictyochorella, Dictyodothis, Dothidina, Elmerococcum, Endodothella, Englerodothis, Epibotrys, Haplothecium, Hyalocurreya, Hysterostomina, Inocyclus, Isomunkia, Leveillella, Leveillina, Metameris, Monopus, Monorhiza, Monorhizina, Munkiodothis, Omphalospora, Ophiocarpella, Pachypatella, Phaeocorella, Phaeotrabutiella, Phragmocarpella, Phragmocauma, Phragmodothella, Polycyclina, Rhopographina, Rosenscheldiella, Scirrhiachora, Scirrhophragma, Scirrhodothis, Septomazzantia, Symphaster, Syncarpella, Thyriopsis, Trichochora*, and *Uleodothis*. Many new species are described.—E. N. TRANSEAU (Ohio Jour. Sci. **16**:17–31. 1915) under the title "Notes on the Zygnemales" has published 9 species and 2 varieties new to science.—W. TRELEASE (Trans. Acad. Sci. St. Louis **23**:129–152. *pls. 6–35*. 1915) in a paper entitles "The Agaveae of Guatemala" gives a synoptical revision of the agaves of Guatemala, recognizing 19 species of which 5 are described as new; also a revision of *Furcraea* with 5 species of which 4 are new.—W. B. TURRILL (Jour. Linn. Soc. **43**:15–39. 1915) under the title "A contribution to the flora of Fiji" enumerates 72 species of phanerogams, collected by Sir EVERARD IM THURN in 1905–1907. Of the species recorded 24 are new to science. One new genus (*Pareugenia*) of the Myrtaceae is proposed.—E. ULBRICH (Rep. Sp. Nov. **13**:498–518. 1915) has published several new species and varieties of the Malvaceae from South America.—I. URBAN (Rep. Sp. Nov. **13**:465–484. 1915) has published 24 species of flowering plants new to science from the West Indies. One new genus (*Selleophytum*) related to *Zinnia* is described.—M. VICTORIN (Ottawa Nat. **28**:155–160. 1915) describes and illustrates a new aster (*Aster linariifolius* var. *Victorinii* Fernald) from Quebec.—H. F. WERNHAM (Jour. Bot. **53**:15. 1915) records a new species of the recently established genus *Tournefortiopsis* from South America.—F. v. WETTSTEIN (Oesterr. Bot. Zeitschr. **65**:145–156. *pls. 3, 4.* 1915) gives an account of an alga found in Kremsmünster, upper Austria, identifying it with *Botrydium pyriforme* Ktz., for which he proposes the generic name *Geosiphon.*—R. S. WILLIAMS (Bull. Torr. Bot. Club **42**:393–404. *pls. 21–25.* 1915) in an article entitled "Mosses from the west coast of South America" describes several new species and records a new genus (*Pseudocrossidium*).—J. C. WILLIS (Jour. Linn. Soc. **43**:49–54. 1915) in continuation of his studies on the Podostemaceae, particularly in the light of material from Brazil, has proposed a new family (Tristichaceae) to include *Tristicha*, *Lawia*, and *Weddellina.*—N. WORONICHIN (Bull. App. Bot. **8**:769–807. *pls. 134–136.* 1915) has published several new species of fungi, including also a description of a new genus, namely *Antennulariella.*—A. ZAHLBRUCKNER (Rep. Sp. Nov. **13**:528–537. 1915) has published 13 new species of *Burmeistera* and *Centropogon* from Ecuador and Colombia. The same author (*ibid.* **14**:133–142. 1915) records 21 additional new species of Lobeliaceae from South America.—J. M. GREENMAN.

Botanical researches of the Carnegie Institution.—The annual report[1] of the year 1915 affords a brief review of the varied lines of botanical research in progress at the Desert Laboratory and elsewhere under the general direction of the Carnegie Institution. It also announces the report of investigations that are now being published, or that are to appear in the near future. Only a few of the more prominent lines of investigation can be noted here, and among them none is more important than the group of problems relating to the conditions of plant growth, especially in relation to soil aeration and temperature, as well as to the processes of respiration and photosynthesis, by MACDOUGAL, LIVINGSTON, CANNON, SPOEHR, and their associates. Studies upon succulents are being continued by MACDOUGAL, LANG, and BROWN; while the special water relations of plants have engaged the attention of LIVINGSTON in an improved form of atmometer and in some transpiration studies. This latter field has also been worked by BAKKE and SHREVE, using the method of standardized hygrometric paper.

Among the more important lines of phytogeographic research are investigations of osmotic pressure of cell sap in relation to environmental conditions by HARRIS, of the vegetation of Arizona mountains by SHREVE, and of climatic cycles and successions by CLEMENTS. The investigations of various root systems by CANNON, of the Salton Sea region by a corps of workers, and of the relationships of the Cactaceae by BRITTON and ROSE are being continued. There have been also climatic studies by HUNTINGTON, and genetic analyses by LLOYD, MACCALLUM, and TOWER.—GEO. D. FULLER.

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[1] MACDOUGAL, D. T., Annual report of the director of the department of botanical research. Carnegie Inst. Washington Year Book no. 14 for 1915. pp. 55–106. 1916.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold face** type; synonyms in *italic.*