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TRANSACTIONS

OF THE

WISCONSIN ACADEMY

OF

SCIENCES, ARTS, AND LETTERS

VOL. XV, PART I

1904

WITH EIGHT PLATES

EDITED BY THE SECRETARY

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TABLE OF CONTENTS

LIST OF PLATES

THE REPRODUCTIVE ORGANS OF THE FEMALE MAIA MOTH, *Hemileuca Maia* (Drury).

BY WM. S. MARSHALL.

Hemileuca maia is found late in the summer near Madison occurring very abundantly in the marsh land at the margin of one of the lakes adjacent to the city. Here for a few days both the males and females fly low over the marsh or settle on the grass and small twigs, the latter to lay their eggs. One year a number of the moths were collected and saved for study; some were prepared by having the body cut open before throwing them into alcohol, while from others the reproductive organs were removed and hardened in corrosive sublimate or Flemming's solution.

The reproducitve organs of the female moth are, in general, similar to those which have been described for other Lepidoptera. Each ovary consists of four long ovarian tubules all of which are bent and coiled forming a large irregular mass within the abdomen. When the tubules are separated from each other each one is seen to be a long tube having the same diameter throughout, except for a short distance, at the distal end, where it is narrower (Fig. 4.) Those moths from which the eggs have been expelled show the ovarian tubules shorter and much narrower. The eggs within the tubules, which, before egg-laying generally exceed forty in number, give to each tubule the appearance of a string of beads. After the expulsion of part of the eggs there are here and there in the tubule considerable distances between neighboring eggs, no regularity, however, being apparent in this respect; such spaces in the tubules being found independently in each one, and all showing slight differences. The four ovarian tubules of each ovary meet to form a short oviduct and the two oviducts join near the median line of the body to form a wide oviductus communis. This passes towards the posterior end of the body soon, however, enlarging to form the vagina into which open the bursa copulatrix, dorsally, and the receptaculum seminis on its ventral surface. The vagina has dorsally at its distal end an enlarged saccular evagination or pouch into the apex of which the tube from the receptaculum seminis opens. The bursa copulatrix, a pyriform sac, is situated on the left side of the body. It communicates with the exterior through the ostium bursae, ventrally on the eighth abdominal segment, and with the vagina through a narrow tube, the doctus seminalis, which in *Hemileuca* is without the vesicular swelling present in so many Lepidoptera. The receptaculum seminis, generally on the left side of the body, is double, consisting of a large rounded part and of a second smaller more tubular piece which, near its distal end, bears a narrow, tubular appendix. The cement or sebaceous glands, glandulae sebaceae, two long narrow tubes, lie in the right side of the body where they form a bent and coiled mass. The proximal part of each gland enlarges to form a reservoir, these two parts then uniting and emptying by a common duct into the vagina. As already mentioned, the glands lie in the right side of the body, but the enlarged portions are dorsal in position, just above the vagina. The intestine is entirely functionless, the moths taking no food; it is a long narrow tube which, near its posterior end, becomes much enlarged to form the rectum on the distal end of which is a large saccular outgrowth.

A ventral view of the eighth abdominal segment discloses a large genital plate the shape of which is shown in (Fig. 3). This plate extends from near the middle of this segment to its anterior margin, where, in the median line, is the ostium bursae. The same figure shows the opening of the vagina on the ventral surface of the ninth segment.

Ovary. Each of the two ovaries is made up of four ovarian tubules, these are not bound together in any way, but each one throughout its entire length is separated from the others. Before the expulsion of the eggs each tubule is from 70mm. to 80mm. in length. The number of eggs each contains is not constant, thirty-eight being the average in those counted. The four tubules on each side unite to form an oviduct, 1.5 mm. in length, and the two oviducts join in the median line to form an oviductus communis 2 mm. long.

Throughout the entire length, from the distal end of the tubule to that point where the oviductus communis passes into the vagina, the wall is very similar in structure. On the inner surface there is a folded chitinous layer, somewhat thinner near the distal end. The next layer, the epithelial, has, as such, nearly disappeared, and in its place is an empty space containing a few scattered nuclei each of which shows a few irregular chromatin granules. The cytoplasm, which we can assume was present when the cells were active, has entirely disappeared. Externally each tubule is lined with two muscular layers, an inner circular, and an outer longitudinal layer. Throughout the entire length of the tubule there is only a very slight, if any, difference in the comparative thickness of these two layers (Figs. 5 and 6). Sections cut through the distal end and the middle of the tubule will show this.

The oviductus communis shows a slight change from what we have just described for the ovarian tubule. The remains of the epithelial cells are much more marked, the nuclei appearing at fairly regular intervals, and the cell boundaries, while not complete, are present in such a condition as would allow us to limit the boundaries of the cells which were earlier present in this layer. Both muscular layers show a greater development (Fig. 7), and while the circular longitudinal layers bear to each other the same relative thickness that they did in the tubule, both are here very much thicker. In all specimens of *Hemileuca* examined the eggs were fully developed, and in most of the moths they had been in part or entirely expelled from the body. The egg-laying goes on rapidly, and but a short time is needed for the expulsion of all the eggs from the body. The only use the ovarian tubules and oviducts could at this stage possess would be to aid in expelling the eggs, and we find the parts which are useful in doing this, the muscular layers, still normal, while the other part of the wall, epithelial cells, shows a marked degeneration.

Receptaculum seminis.—The seminal receptacle is double consisting of a larger bladder-like, and a smaller tubular piece, the latter of which is pointed and near its distal end bears a thin coiled appendix (Fig. 8). The two parts have a common duct which opens into the apex of a saccular outgrowth near the distal end of the vagina. The relative size and shape of the two parts can best be understood by the figure from which there are, however, many variations, either in a general increase or diminution in size of the receptacle, or a relative change in one part to the other. From an external view the duct appears to come equally from each part, a section shows at first a similar condition; the cells lining the duct are more like those in the smaller than in the larger part, being in fact the same cylindrical cells, shortened, but not, however, flattened as in the larger part of the receptacle. The appendix, near the distal end of the smaller part, shows a considerable variation in length. In the specimens of *Hemileuca* examined both parts contained spermatozoa.

An examination of sections shows the wall in the two parts to be different, similar, however, in being lined over the entire internal surface with chitin. In the smaller part of the receptacle (glandular part), the greater portion of the wall is a layer of long cylindrical epithelial cells (Fig. 9) which show a decided striation at both ends but more marked in the free than in the basal end. The large ovoid nucleus in each cell contains a number of chromatin granules connected by a linin network. On the outer surface of the wall are a number of circular muscles which, three or four layers thick near the proximal end, gradually diminish until but a single row is left at the apex. Sections through the tubular appendage of this part (Fig. 10) show differences from what we have just described. The cell boundaries were not visible, the nuclei have the same structure as the others, but are much wider and situated in the basal part of each cell. These cells do not show any longitudinal striation, but large vacuoles are present in the cytoplasm. The inner chitinous layer is much reduced in thickness. Near the free ends of these cells there is a row of bodies which are apparently nuclei. They have the same general structure as the large nuclei, but differ from them in shape and size. These nuclei, if they are such, lie free in the cytoplasm, that which surrounds them being darker than in other parts of the cells, without any boundaries separating them from each other or cutting them off from other parts of the cells.

Where the large and small parts pass into each other the cells of the wall change very gradually (Fig. 12), the cylindrical cells of the smaller part gradually getting shorter and shorter until they become flattened as we find them in the larger part of the receptacle. Here (Fig. 11) the wall is much thinner, due almost entirely to a flattening of the epithelial cells, the chitinous lining and layer of circular muscles both being nearly as thick as in the small part. The epithelial cells in the smaller part have an active glandular appearance, but here in the larger part they are very much reduced. Their nuclei have chromatin granules which are gathered in an irregular mass near the center, and have the appearance of being functionless. Even the circular muscles (we take those bodies we have drawn on the outer surface of the wall to be such) do not have the same appearance as those we find in the small part of the receptacle, and we judge that this wall is entirely inactive.

Bursa copulatrix.—From the opening in the gential plate, ostium bursae, a short tube .1 mm. in length leads into the bursa copulatrix. This is an elongated sac pyriform in shape, some 5mm. in length, 1.25mm. wide at narrowest, and 2mm. at the widest part. The wall is thin, having apparently no function other than that of an enclosing sac; in section it appears to be without any definite structure, but composed of a fibrous-like mass in which nuclei lie irregularly scattered. These nuclei show no structure other than that each contains a number of what appear to be small chromatin granules. From the proximal part of the copulatory pouch a narrow tube 1 mm. in length leads into the vagina, opening opposite the duct from the receptaculum seminis.

Cement glands.—The cement glands are two long gradually tapering, tubular parts, very thin distally, but soon beginning to increase in diameter; the proximal part is much larger, this enlarged portion, the reservoir, being about one-quarter of the entire length. Each gland is 30mm. in length, the two uniting, as is common in Lepidoptera, in a common duct 2mm. long, which empties dorsally into the vagina 1mm. anterior to its proximal end and almost opposite, but a little posterior to, the opening of the receptaculum seminis. The entire thinner glandular portions lie in a twisted mass at the right side in the posterior part of the abdomen. The larger reservoir is generally dorsal to the vagina.

Sections through the gland at any place in the distal third show the wall to consist of a layer of epithelial cells, each cell long and narrow, containing an elongated nucleus in the basal half and a number of small vacuoles scattered throughout the cytoplasm. Over the free ends of these cells is a loose chitinous layer which in the sections appears wavy. Scattered just underneath this chitinous layer were a number of small nuclear-like bodies, many of which were elongated and nearly tubular (Fig. 14). Seen in a surface view the chitin appears marked off into small irregular spaces each one of which contains one of these nuclear-like bodies (Fig. 15). The chitinous layer covering this part of the cement gland is a continuation of the same layer which is found to cover the inner surface of the gland throughout its entire length. The nuclear-like bodies appear detached from the epithelial cells, and attached to the chitinous layer, but this might easily be due to poor preservation of the tissue and not normal.

Fig. 16 shows a section cut nearer the proximal end, although still in the narrow part of the gland, and we notice that the very narrow cells just described are replaced by wider ones which are somewhat pointed at their free ends and may be separated from each other either throughout their entire length or, oftener, for only a part thereof. Near the free end of each cell is a peculiar small ovoid body which does not so closely resemble a nucleus as the bodies just described as present under the chitinous layer (Fig. 14). They are quite homogeneous, stain darker than the surrounding cytoplasm, but are not seen distinctly in all cells of this region. Towards the proximal end of the thin glandular part just before it widens to form the reservoir, a distinct change is noted in the structure of the wall. The epithelial cells of which it consists are here of the same length as those just described, but are much wider. The nucleus is also different here, and, instead of lying with its long axis parallel to the long axis of the cell, lies transversely across it; it is also bent with the convex surface towards the basal end of the cell. Along its other surface which is concave, lies a peculiar body, rounded in outline, of a dark homogeneous appearance, with a yet darker small central portion from which radiate a number of dark lines none of which reach the periphery (Fig. 17). Similar cells have been described in many glandular tissues of insects by Dierckx (3), Glison (5), and in the reproductive organs of the Orthoptera by Fenard (4). From the dark central portion of this body a duct passes into the lumen of the gland penetrating the chitinous layer which is here present as in other parts of the gland.

The wide proximal part of the gland has become a reservoir to hold the secretion of the other parts. Internally there is a chitinous layer much wider than in the glandular part, and very much folded. The epithelial layer has undoubtedly become functionless, and is represented by a thin layer of cytoplasm, without cell boundaries, in which are scattered a number of ovoid nuclei here much smaller than in any other portion of the gland. There is an outer layer of longitudinal muscles which is not present in other parts of the gland.

Vagina.—The vagina is a continuation of the oviductus communis from which it differs, externally, only in its slightly greater width. Near its distal margin, along the dorsal wall, it bears a saccular evagination which receives the duct from the receptaculum seminis; nearly opposite to this the ductus seminalis opens.

The wall of the vagina shows in section a greater development of the muscular layers than any other part of the reproductive organs. A similar enlarged muscular layer was found by Verson and Bisson (12) for Bombyx. The thin internal chitinous layer is folded and in the epithelial layer the cells show a partial degeneration; the nuclei appear normal but the cytoplasm has in part disappeared from many of the cells. The layer of circular muscles is wide and followed externally by a narrower longitudinal layer.

Zoological Laboratory,
University of Wisconsin,
February, 1905.

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EXPLANATION OF PLATES.

b. c.	Bursa copulatrix.
ch.	Chitinous layer.
c. m.	Circular muscles.
d. s.	Ductus seminalis.
ep.	Epithelial cells.
gls.	Glandulae sebaceae.
it.	Intestine.
l. m.	Longitudinal muscles.
n. ep.	Nuclei of epithelial cells.
o.	Oviductus.
o. b.	Ostium bursae.
o. c.	Oviductus communis.
o. t.	Ovarian tubules.
r.	Rectum.
r. s.	Receptaculum seminis.
sec.	Secretion of cement gland.
v.	Vagina.

All figures except 1. 2. 3, 4, and 8 drawn with camera lucida.

PLATE I.

EXPLANATION OF PLATE I.

Fig. 1. Reproductive organs, the eight avarian tubules have been removed. x 3.

Fig. 2. More enlarged view of part of same. The different parts are not in their normal positions, the receptaculum seminis is thrown over to the right; the vagina is shown in lateral and the oviducts in dorsal view.

Fig. 3. Ventral view of eighth and ninth abdominal segments showing the opening o. b. into the bursa copulatrix on the anterior margin of the eighth, and the opening of the vagina on the ninth segment. Enlarged.

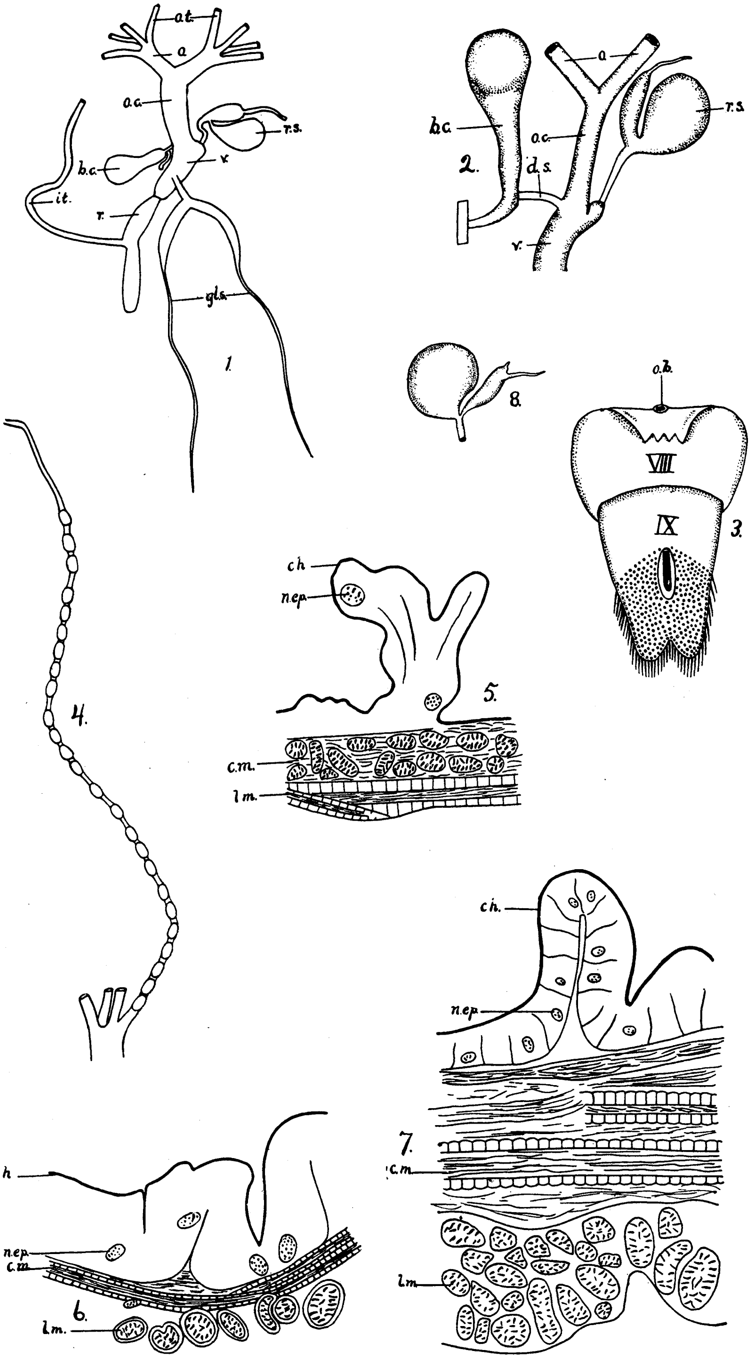
Fig. 4. A single ovarian tubule.

Fig. 5. Longitudinal section through the wall of distal part of an ovarian tubule. x 320.

F.g. 6. Transverse section of wall of same, near the middle. x 250.

Fig. 8. Receptaculum seminis. Enlarged.

Plate I.



W. S. M., del.

PLATE II.

EXPLANATION OF PLATE II.

Fig. 9. Longitudinal section through wall of receptaculum seminis, smaller part. x 800.

Fig. 10. Longitudinal section through the tip of its smaller part. The structure of the tubular appendix is similar to this figure. x 800.

Fig. 11. Longitudinal section of the receptaculum seminis, larger part. x 800.

Fig. 12. Section of wall of seminal receptacle where the two parts pass into each other. Epithelial cells of the smaller part are much longer than those of the larger part. x 250.

Fig. 13. Longitudinal section of bursa copulatrix. a, inner surface. x 800.

Fig. 14. Section of wall of cement gland near distal end. Just under the chitinous layer, ch., are two small nuclear-like bodies which, with the chitinous layer, are represented in the following figure. x 600.

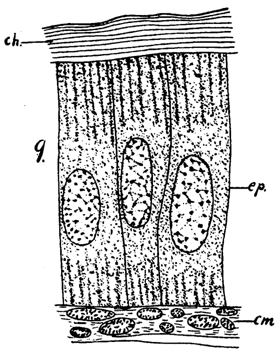
Fig. 15. Surface view of chitinous layer showing markings as if of a division into cells. The nuclear-like bodies and markings may belong to some layer just under the chitin, only the nuclei of which are seen in the section. x 800.

Fig. 16. Wall of cement gland near its proximal end. The epithelial cells show near their free ends peculiar bodies somewhat resembling those seen in the tip of the receptaculum seminis, Fig. 10. x 600.

Fig. 17. Single epithelial cell from proximal end of cement gland just before its enlargement. Between the margin of the nucleus and the free end of the cell is seen the peculiar body from which a duct leads out through teh layer of chitin. x 800.

Fig. 18. Longitudinal section through last part, reservoir, of cement gland. A small portion of the secretion which fills this reservoir is shown. x 800.

Fig. 19. Longitudinal section through wall of the vagina. x 250.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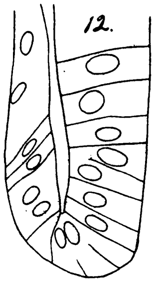
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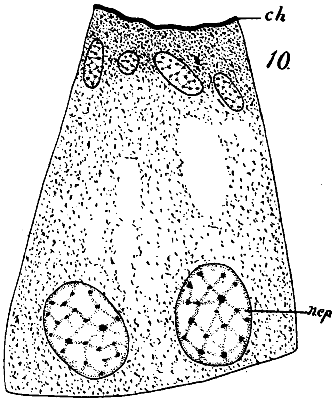
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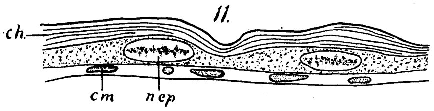
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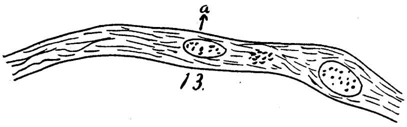
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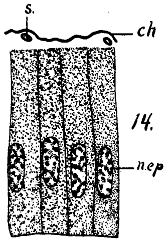
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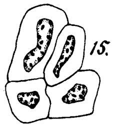
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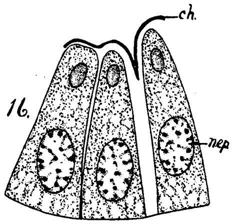
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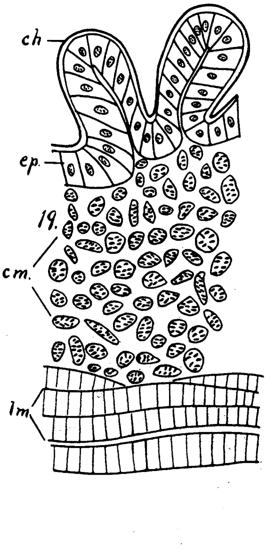
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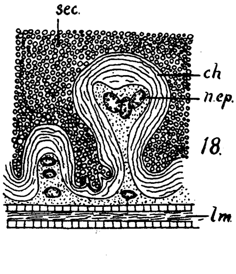
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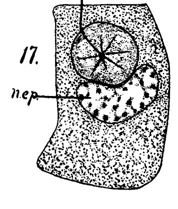
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THE CORRELATION OF FRACTURE SYSTEMS AND THE EVIDENCES OF PLANETARY DISLOCATIONS WITHIN THE EARTH'S CRUST.

BY WILLIAM HERBERT HOBBS.

In a recent paper the author has attempted to correlate within a complex and varied geological province the somewhat scanty observations which deal with the orientation of fracture systems.[1] The results of this correlation possess considerable significance inasmuch as there is a clear indication that over quite an appreciable fraction of the earth's surface the main lines of fracture betray evidences of a common origin. Since the publication of the paper so many verifications of this conclusion have come to the writer from geologists whose work was unpublished, or which apply to the extension of the province treated, that it seems desirable to extend both the area considered and the general topic in order to include the new material.

That the fracture systems observed in a local district betray close relationship to those of neighboring districts, was early shown by Phillips[2] and Haughton,[3] and later by Kinahan[4] for

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[1] Lineaments of the Atlantic Border Region. Bull. Geol. Soc. Am., Vol. 15, Nov. 1904, pp. 483–506. Pls. 45–47. Read by title before the International Geographical Congress at St. Louis, Sept., 1904.

[2] Phillips, John. Illustrations of the Geology of Yorkshire, Part 2. The Mountain Limestone District. London, 1836, pp. 90–98. Also, Manual of Geology, London, 1885 (Etheridge and Seeley Edition), pp. 33–34.

[3] Haughton, Samuel. "On the physical structure of the old red sandstone of the County of Waterford, considered with relation to cleavage, joint surfaces and faults. Trans. Roy. Soc. Lond., Vol. 148, 1858, pp. 133–348. Also, On the joint systems of Ireland and Cornwall and their mechanical origin. Ibid, Vol. 154, 1864, pp. 383–411.

[4] Kinahan, Gerald Henry. Valleys, their relations to fissures, fractures and faults. London, 1875, pp. xiv and 240.

portions of the British Isles, and also by Kjerulf[1] for Norway. Haughton, and more recently Brögger,[2] have conclusively proven that in the districts which they studied the normal faults conform in direction to the general system of joints; but the importance of this result, though reinforced and emphasized by Daubrée,[3] has never been fully appreciated.

For the region of the eastern United States it was developed in the author's paper referred to, that the joints and normal faults of greatest prominence follow in any particular part of the province one or more of three or four general directions. These directions approximate to the meridian and the equator, and to diagonal intermediate bearings. There seems good reason to believe that as regards the first two of the directions mentioned the approximations to meridian and equator are fairly close—generally within 5 degrees. As regards the intermediate directions this is far from being the case, and there are in general not two but several intermediate directions; yet their general tendency to occupy rather distinctly intermediate positions between the meridian and the equator is sufficiently manifest. Wherever close observations have been made it has been found that not four but a considerably larger number of directions may be made out, as will be clearly indicated in the following tables.

These observations receive, moreover, strong support from the studies of Green, Prinz, and others dealing with the orientation of the broader earth features upon the entire planet.

North Carolina.—Since the author's paper[4] was published it was learned that a careful measurement of joint and dike directions had been made by Mr. F. B. Laney within the Newark area of North Carolina, and as a result of his studies Mr. Laney stated that joints and dikes alike were oriented mainly north and south, east and west, northwest and southeast, and northeast and southwest. Mr. Laney has kindly turned over to the author his

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[1] Kjerulf, Theodor. Die Geologie des südlichen und mittleren Norwegen. Authorized German edition by Gurlt. Bonn, 1880, pp. 1–350.

[2] Brögger, W. C. Spaltenverwerfungen in the Gegend Langesundskien, Nyt Magazin for Naturvidenskaberne. Vol. 28, 1884, pp. 253–419, with map.

[3] Daubrée, Géologie experimentale. Vol. I, pp. 289-385.

[4] l. c.

detailed observations, from which it is clearly seen that the intermediate directions which have greatest importance, while properly enough described in general terms as northeast and northwest, fall principally in six and not two series. The comparison of the general results determined for the lineaments of the Atlantic border region with the general directions observed in individual districts examined by the writer expresses a like result. The actual observations made by Mr. Laney have been tabulated by the author with the following result.

N 30° W	**14**	**N–S**	**7**	**N 15° E**	**62**
N 30°–40° W	1			**N 30° E**	**9**
N 30°–35° W	3			N 35° E	2
N 35° W	1			N 40°–50° E	1
N 40° W	1			**N 45° E**	**8**
N 45° W	2			N 50° E	1
N 50° W	2			N 55° E	2
N 55° W	2				
N 55° W	1			**N 60° E**	**12**
N 60° W	**10**			N 65° E	1
N 65° W	1	**E–W**	**8**	N 80° E	1

In the North Carolina Newark both joints and dikes stand approximately vertical, and it appears that the dominant direction is N 15° E. The remaining joint series are so oriented as to fall into three conjugate sets.. Moreover, the joints observed at any locality were found generally to be in pairs corresponding to one or the other of the sets.

The Finger Lakes district of West-Central New York. Mr. Charles G. Brown of Ithaca, N. Y., has measured the directions of more than 1000 joint planes which occur in the vicinity of Ithaca in the basins of the near-lying lakes. In order of relative numerical importance the joint directions of the district are found to be:[1]

N. 20° W. (288), N. 10° W. (139), N. 70°—75° E. (131), N. 15°—16° W. (88), N. 60° W. (86), N. 30° E. (68), N. 80° E. (62), N. 40° W. (51), N. 4°—6° W. (43), N. 30°—34° W. (35), N. 85° E. (35), and N.—S. (15). These twelve directions include 941 of the 1,004 measurements and with the exception of

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[1] Jour. Geol., vol. 13, 1905, pp. 367–374.

the direction N. 12° W., whose 14 observations should perhaps be added to the 139 directed N. 10° W., no other direction is represented by more than six measurements.

The fracture system of which these are the more prominent series has controlled in an important way the topography of the region, as has been described in the paper referred to.

The larger basin of the near lying Lake Ontario has been shown by Wilson[1] to have its lines of drainage determined by fracture planes, though no indication is afforded of the cardinal directions characteristic of the system. This author says:[2] "The direction of the master joint fractures is intimately associated with the trend of all the master valleys, though the modern post-Glacial channels are independent of them. . . . These master joints with other associated valleys and the parallel system of valleys of similar trend on the adjacent Archean areas are probably associated with an extensive system of faults of pre-Ordovician date."

This province like that surrounding the Ausable chasm near Lake Champlain should afford a most promising field for the observation of cardinal directions within fracture systems.

The French River District of Ontario.—Bell has called attention to the marked orientation of the inlets and streams within the French River district of Ontario,[3] and has ascribed this orientation to the control by fissures, joints, and dislocations. The directions are strongly marked in the district and trend E–W or very nearly so, and approximately NW–SE, NE–SW. Bell says:

"The effects of cleavage and bedding, fissures and joints, rock-crushing, dislocations, intrusive dikes, etc., on the production of geographical features are here so well marked as to make it worth calling attention to some points in connection with this subject. In any part of the district we may select, it will be found that the joints, fissures, and dislocations, generally run in two sets intersecting each other at large angles, but those of

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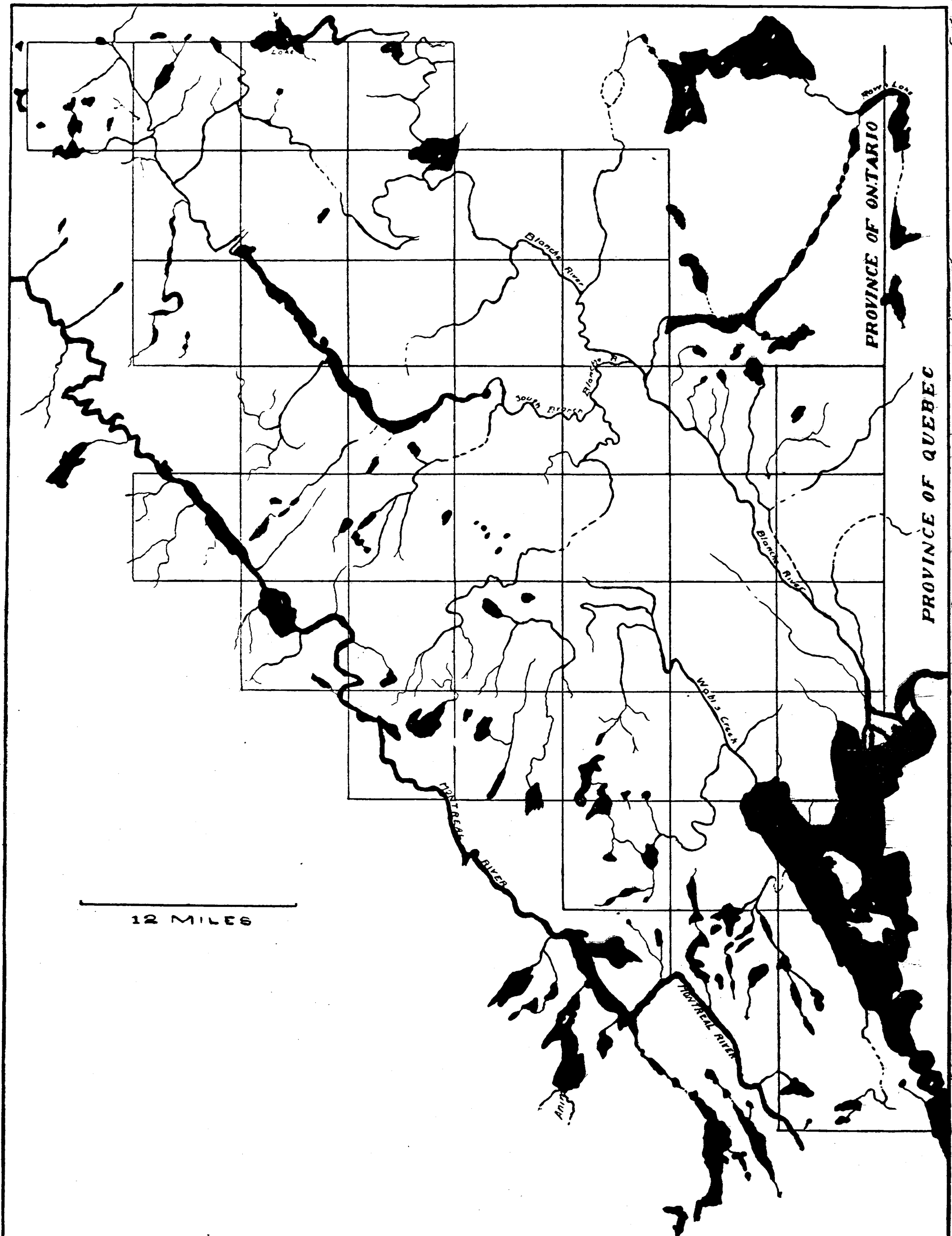
[1] Wilson, A. W. G., Trent River system and St. Lawrence outlet. Bull. Geol. Soc. Am., Vol. 15, pp. 211–242. Pls. 5–10.

[2] Bell, Robert. Report on the geology of the French River district, Ontario. Geol. Surv. Can., Ann. Rept., N. S., Vol ix, 1896, pp. 20–I to 21–I.


PROVINCE OF ONTARIO
PROVINCE OF QUEBEC
Blanche River
South Branch
Blanche R.
Blanche River
Wabis Creek
MONTREAL RIVER
MONTREAL RIVER
12 MILES

either set are parallel to each other. Usually one set is more strongly marked than the other and exercises an important influence in the decay and disintegration of the rocks, and this in its turn affects the contours of hill and valley and determines the positions of streams, inland lakes and of the inlets, etc., of Georgian Bay.

"The dikes which traverse both the Laurentian and Huronian rocks of the district and the fissures and lines of crushing which occur more particularly in the former, have given birth to some of the more striking features of the map. . ."

In an earlier paper Bell[1] had already shown that many of the long and straight valleys within the Archean area of Canada now occupied by rivers, lakes, or by inlets of the larger lakes, have been formed as a result either of the relatively rapid decay on dikes or on lines of close joints. The valleys now occupied by water are further extended by valleys filled in with drift. Examples are Onaping Lake, 30 miles long; Long Lake, 52 miles in length; and Sepiwesk Lake, which with Nelson River forms a trench 96 miles in length. The Mattagami River is thus guided for 160 miles, and Lake Temiscaming, 35 miles in length, with Deep River, forms a rectilinear trench in places more than 2000 feet in depth. Soundings show that the rectilinear inlets of Georgian Bay are extended lakeward by straight channels at their fronts. The prevailing joints of the region correspond perfectly in direction with these trenches.[2]

The map just published[3] by the Department of Crown Lands and covering the area about Lake Temiscaming reveals this orientation in great perfection (see Plate III). Dr. C. K. Leith, who has done much recent geological work in the district confirms the general correctness of this map and the correspondence of drainage lines with the direction of the prevailing joints. As the map indicates, the prevailing joint direction is about N. 40° W., with N. 50° E., and N–S the directions next in importance.

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[1] Bell, Robert. Pre-Paleozoic decay of crystalline rocks north of Lake Huron. Bull. Geol. Soc. Am., Vol. 5, 1894, pp. 357–366, Pls, 15, 16.

[2] Personal communication from Dr. Bell.

[3] Map of part of the District of Nipissing, showing agricultural lands surveyed on Lake Temiscaming, Ontario, 1905.

Southwestern Wisconsin and Northern Illinois.—As early as 1866 Whitney[1] showed that the Galena limestone formation is intersected by two sets of vertical fissures whose directions are approximately east and west, and north and south.

"All through the mining district, indeed, in Wisconsin and Iowa as well as in Illinois, the heaviest diggings will usually be found on crevices varying but little from east to west in their general direction. . . The norths and souths on the other hand or those crevices which have a course approximating to the meridian, are much less important, although these in some instances are the most productive ones of portions of the lead region." Buckley as a result of his measurement of joint directions within the quarries of the State of Wisconsin makes the following general statement.

"As will be seen in the accompanying map the joints of the sedimentary rocks strike in four main directions. The prevailing general direction of the joints is northeast and southwest. The other directions are northwest and southeast, east and west, and north and south."

An examination of Buckley's map will show that the intermediate directions vary in many cases widely from the forty-five degree positions. Under the writer's direction Mr. E. C. Harder[2] has made a careful study of the joint systems which are developed in the rocks of southwestern Wisconsin. For this province as a whole he finds that in order of numerical superiority the dominant joint directions of the district are, N. 35° E., N. 75° E., N. 35° W., N. 55° W., N. 45° E., N. 85–90° E., N. 25° W., N. 45° W., N. 15° W., N. 25° E., N. 65° E., N. 75° W., N. 65° W., and N. 55° E. The tendency of the five degree interval to appear is noticeable and indicates that here as elsewhere the observations of slightly curving planes become adjusted to the larger unit of the compass. Thus the equatorial direction, which is the dominant one, should probably include the measurements ranging between N. 85° W. and N. 85° E., or 25 in all. Only less noticeable aggregations appear elsewhere in the table, where

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[1] Whitney, J. D. Geology of the Lead Region. Geol. Surv. Ills., Vol. 1, 1866, p. 194.

[2] Jour. Geol. Vol. 13, 1905, pp. 363–366.

probably if the joints could be measured with greater accuracy they would fall between two five degree limits on the side of the one having the numerical superiority.

Iowa.—McGee[1] has called attention to the conjugate set of joints which penetrate the quarry rocks of the State of Iowa. Of these joints there are two classes recognized, which are designated by the quarrymen as "clay seams" and "dry seams." The chief set consists of two series of "clay seams" crossing each other approximately at right angles, with two series of "dry seams" also crossing about at right angles and approximately bisecting the angles formed by the principal series. He further adds:

"The phases presented are, however, variable. Either of the two classes or either system of either class may be absent, or additional and generally less conspicuous systems . . . sometimes so blended with the predominant class as to separate the strata into either irregular or tolerably regular polygonal blocks . . . may be introduced; and the two classes pass imperceptibly into each other."

In a personal letter Dr. McGee adds:

"Unhappily my memory is not as clear as I could wish; so far as I am able to recall the less distinct double-sets are orthogonal, departing a few degrees from N–S and E–W, though the direction of the departure from the meridian and parallel escapes me. The more prevalent and conspicuous double-sets are also orthogonal or nearly so and approximately NW–SE, and NE–SW. So nearly as I can recollect the minor and major double-sets do not exactly divide the quadrangle. The difference is somewhat more (or less) than 45°; . . .

"As I dwell on the matter in this writing the impression revives that the dominant single-set trends about N. 55° W., and S. 55° E., with the correlative orthogonal single-set about N. 35° E., and S. 35° W.; and that the stronger joints of the minor double-set trends about N. 5° W. to S. 5° E., and the weaker one about N. 85° E. to S. 85° W. In any event such a scheme will

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[1] McGee, W. J., Note on jointed structure. Am. Jour. Sci. 3rd Ser. Vol. 25, 1883, pp. 152–155.

illustrate approximately the relation of the joints worked out in my building-stone researches in 1880. My best examples were found in the central and southern sections of the state."

Arkansas.—Professor Branner[1] has been good enough to call the author's attention to similar results obtained by Professor Newsom and himself when studying the eastern portion of the Boston Mountains in Arkansas, in which investigation the peculiarities of drainage, the parallelism of streams, the similarity of their elbows, and the relation of these streams to structural features in the adjoining regions were put upon record. The dominant directions of drainage are NE–SW, and NW–SE. With greater definiteness these directions are given as N. 60°–67° E., and N. 51°–65° W. The NE–SW series seems to be controlled by the monoclinal folds. Attention is called, however, to the relationship existing between these stream directions and a series of dislocations in the province, and it is further stated that "there are two other systems of joints in the area here especially considered, one running N. and S., the other E. and W."

The Great Basin of the Western United States.—Since the publication of the early reports upon the geology of the Great Basin of the western United States it has been generally recognized that the dominant faults trend near the meridian. Gilbert has also called attention to the fact that formations rather generally end abruptly on east and west lines. Spurr[1] in his general conclusions upon the origin of the basin ranges of Nevada and California says:

> "The faulting in general seems to be about as frequent as in other regions which show the same amount of folding. The chief faults belong to the north-and-south and east- and-west systems. There are also diagonal ones running northeast and northwest and in each of the systems they may have a very great displacement."

The most important directions described by Spurr are in per-

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[1] Newsom, J. F., and Branner, J. C. The Red River and Clinton Monoclines. Am. Geol., Vol. 20, pp. 1–13.

[2] Spurr, J. E. Origin and Structure of the Basin Ranges. Bull Geol. Soc. Am., Vol. 12, 1901, pp. 217–270. Pls. 20–25.

fect accord with those described by Gilbert as occurring in the clays of Lake Bonneville and controlling the local drainage directions. The data were secured by Mr. Israel C. Russell, then his assistant, of whom Gilbert[2] says:

"He found that the details of drainage were controlled by a compound and extended system of joints. The principal series trend almost precisely north and south and the subordinate series east and west. They are all vertical and straight and (within each series) closely parallel. They are readily traced from top to bottom of the walls of the lateral ravines and not infrequently a wall exposes a broad flat sheet face caused by the removal of the clay from one side of the plane of jointing. Elsewhere the faces of the bluffs are buttressed by square pilasters or ornamented by outstanding rectangular columns, the forms of which have been determined by the two systems of joints . . .

"The point of especial interest is that these joints have been developed in post-glacial time within the series of strata not perceptibly indurated and which repose undisturbed in the place where they were deposited. The strata are nearly horizontal and their inclination of less than ½° northward is presumably the pitch slope of the bottom upon which they were thrown down."

Similar joints were observed by Russell[1] in the clays of Lake Lahontan. He says:

"The marly clays forming the upper and lower members of the Lahontan series usually break into prismatic and cubical blocks on weathering; the vertical faces of the blocks are determined by joint planes and the horizontal by planes of lamination. In many localities a more pronounced jointing occurs, forming two approximately vertical systems that are nearly at right angles to each other. Judging from the number of in-

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[1] Gilbert, G. K. Post-Glacial Joints. Am. Journ. Sci., 3rd Ser., Vol. 23, 1882, pp. 25–27. See also same author, Lake Bonneville. Monograph I, U. S. Geol. Surv., 1890, pp. 211–213.

[2] Russell, I. C. Geological History of Lake Lahontan. Monograph XI, U. S. Geol. Surv., 1885, pp. 162–163.

[1] Iddings, Jos. P. A Fracture Valley System. Journal of Geology, Vol. 12, 1904, pp. 94–105. Pl.

stances observed at widely separated localities, the joints in question may be traced through the entire series of Lacustrian beds."

Yellowstone National Park.—Quite recently Iddings[2] has shown that upon the Livingstone quadrangle, which is included in the area of the Yellowstone National Park, a net-work of faults has determined a fracture valley system in which the dominant directions are NE-SW, NW-SE, N-S, and E-W. More definitely the diagonal directions are given as N. 30° E., and N. 60° W. He says:

"A study of the topographical map reveals the angular character of much of the drainage system, and the prevalence of certain parallel and sub-parallel lines which appear in various streams and occur in quite diverse portions of their channels. Along parallel lines different streams may be flowing in opposite directions: . . . The persistency of these lines becomes more striking when the geological structure of the region is taken into account and it is observed that certain drainage lines traverse rocks of such diverse nature as gneiss, schist, volcanic tuff, breccia, solid lava, limestone, sandstone, and shales.

"The relation of some of these directions of drainage to known fracture planes will be pointed out. The dominant drainage lines in the southern three-fourths of the quadrangle trend about NE-SW, and NW-SE, more nearly N. 30° E. and W. 30° N., the angle between them being approximately 90 degrees. There are other systems of almost rectangular lines somewhat differently oriented, namely, N-S and E-W."

Other Districts.—Only less important in a study of the orientation of fracture systems are other studies which will not be here specifically referred to except as they have been included in the following table. To facilitate the study of directions there are given in separate columns: 1st. Those bearings of fracture systems which approach the direction of the meridian; 2nd. Those which approach the direction of the equator; 3rd. Those which occupy intermediate positions within the quadrants NE and NW; 4th. Those which occupy intermediate positions in the quadrants NW and SE.

TABULATION OF FRACTURE SYSTEMS FOR THE UNITED STATES.

(Partial.)

Bearing of Fracture Series.

DISTRICT AND AUTHORITY.	Meridional or nearly so.	Equatorial or nearly so.	In quadrants NE & SW.	In quadrants NW & SE.	Remarks.
Great Basin of Western United States.					
General direction for Gt. Basin.—Gilbert.	N—S	....	....	....	Formations also end in E—W cliffs.
Basin Ranges in California and Nevada.—Spurr.	N—S	E—W	NE—SW	NW—SE	
Upper Sevier Basin.—Gilbert.	....	....	N 10°—30° E	....	
Utah and Arizona.—Gilbert.	N—S	....	....	....	
General Direction in Colorado plateau —Gilbert.	N—S	....	....	....	
Lake Bonneville (Old River Valley.)—Gilbert.	N—S	E—W	....	....	In clay.
Lake Lahontan.—Russell.	N—S	....	....	....	Also a perpendicular vertical set in clay.
Lake Lahontan (N. of 37° lat.)—Russell.	....	....	N NE—S SW	....	
Telluride quadrangle in Colo.—Purington.	....	....	N 38° E N 53°—63° E	N 21°—51° W N 87° W	Directions N 53°—63° E and N 21°—51° W recognized as each containing several series.
Rico Mts. in Colo.—Ransome.	....	....	N 25°—65° E	N—N 45° W	
Globe Quadrangle in Arizona—Ransome.	....	....	NE—SW	NW—SE	
Bisbee Quadrangle in Arizona.—Ransome.	N—S	E—W	NE—SW	NW—SE to W NW—E NE	Also some others less common.
Albuquerque and Magdalena Mts. in New Mexico.—D. W. Johnson.	N—S	E—W?	....	....	
Same.—Keyes.	N—S	....	....	....	These are the directions of major faults in modern mts.
Death Valley in So. Cal.—Campbell.	N—S	....	NE—SW	....	
Boise Quadrangle, Idaho—Lindgren	....	E—W	E NE—W SW	....	Rarely NE—SW.
Livingston Quadrangle, Montana.—Iddings.	N—S	E—W	N 30° E	N 60° W	
Butte Min. District.—Emmons & Tower.	....	Mainly near E—W.	....	....	In quadrant between NE and SE but mainly near E—W. "Secondary fractures" with dips from 45°—65° at right angles to mineral veins.

TABULATION OF FRACTURE SYSTEMS FOR THE UNITED STATES—Continued.

DISTRICT AND AUTHORITY.	Meridional or nearly so.	Equatorial or nearly so.	In quadrants NE & SW.	In quadrants NW & SE.	Remarks.
High Sierra of California.—Becker.	....	....	ENE—WSW	NNW—SSE	Two diagonal sets sometimes found striking between N—S and NW—SE, and dipping 45° either way. Also rarely horizontal sets.
Same. Upper Kern Valley.—Lawson.	N—S	....	....	....	
Lower Mississippi Valley.					
Boston Mts. in Arkansas.—Branner-Newsom.	N—S	E—W	NE—SW	NW—SE	
Ozark Mts. in Ark.—Powell.	N—S?	....	....	....	
Brunswick Dist. in Indian Territory.—Eldridge.	....	....	....	N 20° W N 50° W	Other directions of minor importance.
Balcones Fault Zone in Texas.–Hill & Vaughan.	....	....	N of E—S of W	....	Also minor faults.
Uvalde Quadrangle in Texas.—Vaughan.	....	....	NE—SW	NW—SE	
Upper Mississippi Valley.					
Entire State of Wisconsin.—Buckley.	N—S	E—W	NE—SW	NW—SE	Main direction NE—SW.
So. Wisconsin—Percival.	....	N 85° E N 85° W	....	NW—SE N 70° W N 80° W	
SW Wisconsin.—Harder.	N 5° E	E—W N 85° W N 85° E	N 35° E N 45° E N 50° E N 55° E N 65° E N 75° E	N 25° W N 35° W N 45° W N 55° W	
Entire State of Iowa.—McGee.	N 5° W circa.	N 85° E circa	N 35° E circa	N 55° W circa	Form two rectangular sets.
Eastern Iowa.—Bain and Calvin.	N—S	Nearly E—W	....	....	Main direction E—W A third series is mentioned at 45° to others.
Northern Illinois. (Also Southwestern Wisconsin.)—Whitney.	N—S	E—W	....	....	
Rosiclare Dist. in So. Ill.—Emmons.	....	....	N 25° E N 32° E N 50°—57° E	....	Not sufficient number of planes observed to clearly indicate these as series.
Minnesota.—Upham.	N—S	E—W	N 60° E	N 45 W	Scattered observations.
Eastern United States.					
So Conn. Valley in Conn.—Davis.	N—S (nearly.)	....	N 30°—45° E	N 20°—30° W	Directions taken from map.
Conn. Valley in Mass.—Emerson.	N—S	....	N 10°—15° E N 20° E N 20°—30° E	N 50° W	Values are approximate only and taken from map.

TABULATION OF FRACTURE SYSTEMS FOR THE UNITED STATES—Continued.

DISTRICT AND AUTHORITY.	Meridional or nearly so.	Equatorial or nearly so.	In quadrants NE & SW.	In quadrants NW & SE.	Remarks.
Pomperaug Valley in Conn.—Hobbs.	N 5° W	N 90° E	N 15° E N 33° E N 55° E	N 34° W N 44° W N 61° W	Unusual directions are, N 20° E, N 25° W. The main directions are the four—N 5° W, N 15° E, N. 34° W, and N 55° E.
River System of State of Conn.—Hobbs.	N. 5° E (eastern upl'nds.) N 5° W (western upl'nds.)	N 90° E	N 15° E N 33° E	N 44° W	Of secondary importance also N 48° E, N 20° E.
Five areas distributed from Stockbridge, Mass. to N. Y.—Hobbs.	N 5° E	N 90° E	N 15° E N 33°—35° E N 50°—55° E N 68°—70° E	N 34°—35° W N 60 W	Both joints and lineaments follow these directions.
Boston Basin—Crosby.	N—S	E—W	... .	....	Dikes take same directions.
Cape Ann.—Shaler.	N—N 5° W.	N 90° E	N 30°—40° E N 45°—50° E (N 75°—80° E)	N 20°—25° W N 30°—35° W	Less important direction between N 10° E and N. 30° E.
White Mt. Notches and River System.—Hobbs.	N 4° E N 5° W	N 90° E	N 12° E N 16° E N 36° E	N 20° W N 25° W N 35° W	In part strong hydrographic lines with few important representatives.
Adirondacks and Lake Champlain. Northern section.—Cushing.	N—S circa	E—W circa	N 45° E circa	N 45° W circa	
North Hudson Dist. in Adirondacks.—Kemp.	N—S	....	NE—SW	NW—SE	Also other directions. See Bull. Geol. Soc. Am., Vol. 15, pl. 47. Also personal communication.
Cayuga Lake Basin.—Brown.	N—S N 4°—6° W	N 85° E	N 15°—16° E N 30° E N 70°—75° E N 80° E	N 10° W N 30°—34 W N 40° W N 60° W	
French Riv. Dist. of Ontario.—Bell.	....	E—W	NE—SW	NW—SE	
Triassic area of N. Carolina.—Laney.	N—S	E—W	N 15° E N 30° E N 45° E N 60° E	N 30° W N 60° W	Directions in part of dikes and in part of joints.
Dominant lineaments of Atlantic border region.—Hobbs.	N 5° E	....	N 50° E	N 33° W N 43° W	Other lineaments directed N 30° E, N 85° W, N 75° W and E—W.

In the above table have been included all reports which have come under the eye of the writer, or have been communicated to him personally, in which the orientation of the fracture systems has been indicated, however crudely; and this entirely without reference to whether the results favor one theory more than another. One condition only has been imposed, namely, that the fracture systems described shall be made up of individual surfaces that in steepness approach the vertical—are in general steeper than 70°—thus insuring that such tilting as may have occurred since their formation has not materially affected the plan of their arrangement.

There are undoubtedly many districts in which no such regularity of arrangement of fracture series can be discovered, but it will generally be found that the planes of jointing or of veins or dikes, are many of them inclined at comparatively low angles to the horizontal. Even where this is not the case, the number of directions of joint planes may be so many that no law of arrangement is discernable; as might well be true when the belt of rocks has been subjected to successive deformation within the zone of fracture either from a single or from several directions. If we are to discover any laws governing the orientation of fracture systems, it will be by proceeding from the simple to the more complex areas, and there is ample ground for assuming that where belts of flat-lying, homogeneous rocks without pre-existing fracture planes are deformed within the zone of fracture, there is normally produced a vertical prismatic system composed of intersecting parallel series. Moreover, observations would appear to show that even where rocks are far from homogeneous and lie in other than horizontal positions, an approximation to this result still obtains. While it has not as yet been demonstrated by experimentation, it is difficult to avoid the conclusion based upon field observation, that a second deformation of rocks which are already possessed of a simple prismatic system of joints through renewal of compression from the original direction, in the main merely increases the number of series within the vertical joint system.

It is certainly of much significance that the systems of fractures which are developed throughout the area of the United

States should so clearly correspond with the orientation of the grander features of the planet as they have been worked out in the studies of Prinz[1] and others. Prinz has found that the major features of the earth are arranged in two nearly rectilinear series running northwest and southeast, and northeast and southwest, with an intermediate series directed nearly along the meridian. It is evident that the present study merely blazes the way for a more thorough and careful correlation to be made when the available data are more nearly adequate for the solution of the problem. It is therefore hoped that geologists will co-operate to the extent of measuring and recording the direction of joint and fault planes within the districts which they individually have opportunity to examine.

University of Wisconsin,
Madison, Wisconsin,
May 20, 1905.

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[1] Prinz, W. Sur les similitudes que présentent les cartes terrestre et planetaires (Torsion apparent des planètes.) Ann. de l'observatoire royale de Bruxelles, 58th year, 1891, pp. 304-337.

THE NATURE AND ORIGIN OF THE BINUCLEATED CELLS IN SOME BASIDIOMYCETES.

SUSIE PERCIVAL NICHOLS.

INTRODUCTION.

Rees (20) was among the first to attempt a careful study of the mycelium of the *Basidiomycetes* with reference to the question of the origin of the carpophore. By making artificial cultures of *Coprinus stercorarius* in dung decoction on slides he was able to observe the formation of erect short hyphae on which he believed sexual cells, spermatia and carpogonia were borne. He also believed that he found a spermatium fused with a carpogonium. After fertilization, branches arise from the base of the carpogonium which developed into the carpophore.

Van Tieghem (25) also germinated the spores of *Coprinus stercorarius* and *radiatus* in dung decoction and studied the development of the carpophore. In his first paper he agreed with Rees. He found the swollen end cells on the lateral branches of the mycelium. These "carpogones" usually terminated in a papilla with which the spermatia fused. The carpogone then divided into three cells, the two lower developing a system of lateral branches which curve around and enclose the terminal cell. Their further development in slide cultures was prevented by lack of nutriment. But by observations made on larger cultures they were seen to be the beginnings of carpophores. Later Van Tieghem reversed this opinion.

Brefeld (3, 4) grew mycelium of *Coprinus stercorarius* from single spores in dung decoction and figures a series of stages in the development of the young carpophore. From the older portion of the mycelium a perpendicular hypha very rich in protoplasm arises. This hypha branches profusely forming a dense snarl from the center of which a bundle of parallel hyphae develop forming the first indication of the stipe. Lateral branches are formed increasing the size of the mass and at the same time the stipe grows rapidly in length. The pileus and gills are differentiated very early in the development of the fruit body. Brefeld found no evidence of the existence of sexual organs at the formation of the carpophore.

With the study of the nuclear phenomena new stand-points arose. The work of Rosen (21), Rosenvinge (22), Wager (26, 27, 28), and Dangeard (5) has established the fact that the cells of the carpophore are frequently multinucleated while the basidia are at first binucleated. In typical basidia the two nuclei fuse and the fusion nucleus divides into the four spore nuclei.

Maire (15) found that the cells of the young carpophore are binucleated and that the cells of the hymenial layer never have more than two nuclei, but that the cells of the stipe and pileus may become multinucleated through the amitotic fragmentation of the two nuclei originally present. The young basidium when it is formed from the hymenial cells receives two and only two nuclei which unite to form the large fusion nucleus of the basidium. He further states that the nuclei in the series of binucleated cells in the young carpophore divide by conjugate divisions so that the two nuclei which fuse in the basidium are of widely different origin. But his evidence is not conclusive on this point.

Maire describes the division of the nucleus in the basidium in detail. The nuclear membrane disappears and the spindle appears at about the same time. The chromatin filaments break up into irregular granules or protochromosomes which are placed on the spindle without any definite order. At the end of the prophase these protochromosomes unite into two definite chromosomes. That the formation of only two chromosomes is not universal among the Basidiomycetes as Maire assumes has been shown by Wager, Ruhland and others. Maire states further that the chromosomes after a longitudinal splitting are pulled apart at the center and move to the poles. The second division is similar to the first. The four centrosomes remain at the summit of the basidium while the nuclei move to the center or base of the basidium. Soon a sterigma is formed above each centrosome, and fibres appear extending from the centrosome to the nuclei which now move to the summit, probably through the influence of these fibres.

Two notes of Maire's (16 & 17) in the Comptes Rendus report that the last two or three cells of the ascogenous hyphae of *Pustularia vesiculosa, Galactinia succosa* and *Acetabula acetabulum* are binucleated. The ascus like the basidium is the last of a series of binucleated cells. In order that such a comparison should have any value we must know how the ascogenous hyphae originate.

In *Hypochnus* Harper (12) was able to trace a series of binucleated cells from the hymenium to the mycelium in the substratum. The mycelium did not form dense wefts or strands but spread through the decaying wood where it could be readily studied. The cells were regularly binucleated. The stages of nuclear fusion and division were similar to those described by Wager. At the equatorial plate stage the chromosomes were distinct and at least six or eight in number.

The origin of the binucleated cells was not determined by these observers. Maire states that the two nuclei in the spore of *Coprinus radiatus* pass into the germ tube and a cross wall may or may not be formed between them. The mycelium is then of two types, the one apocytic, the other cellular. The cells of the latter are uninucleated. He did not observe the transition from these stages to the binucleated condition found in the young carpophore.

In a preliminary notice Blackman (2) has given a brief account of the life history of two of the *Uredineae*. He finds that the spermatia do not have the structure of conidia but of male cells; a thin wall, no reserve material, a very large nucleus with no nucleolus and cytoplasm greatly reduced in amount. He also studied in detail the development of the aecidium of *Phragmidium violaceum*. The aecidium arises as a layer of uninucleated cells just beneath the epidermis of the leaf. Each of these cells divides into a sterile cell above and a fertile cell below. The fertile cell becomes binucleated not by the division of its original single nucleus but by the migration through the wall of the nucleus of a neighboring vegetative cell of the mycelium. He says, "In the presence of the spermatia with their special cytological characters, etc., the only view that seems capable of explaining the facts is that the fertile cell was formerly fertilized by the spermatia, but that now the process has become reduced, fertilization by means of spermatia having been replaced by the more certain method of fertilization by the nucleus of a neighboring vegetative cell." In view of these facts he holds it to be evident "that the Uredineae present an alternation of generations which is as sharply marked as that of the higher plants."

Since the young carpophores invariably have binucleated cells these must originate either at the first formation of the carpophore or sometime during the growth of the mycelium. The latter hypothesis is suggested by Harper's observations on *Hypochnus*. In order to obtain some evidence on this point the study of the nuclei in the mycelium of some of the *Agarics* was begun at the suggestion of Professor R. A. Harper under whose direction the work was carried on.

METHODS.

Spores were collected from a large number of *Agarics* in the following manner. Zinc racks were washed in alcohol and passed through a flame and then placed in plates which had been washed in 95 per cent. alcohol. The racks were covered with bell jars which were also washed in 95 per cent alcohol. Two racks were placed side by side under each jar. Slides were washed in alcohol and passed through a flame and then placed on the lower bars of the racks, each rack holding four. The pileus from a mature fruit body was carefully removed from the stipe and placed on the upper bars of the rack. When the basidia discharge their spores they fall on the slides below thus lessening the danger of infection from the gills. After the spores were discharged the pileus was carefully removed and the bell jar replaced the slides remaining on the racks until needed. Spores preserved in this manner remained pure for a year.

A decoction of string beans, (about 392 grams to a liter of water) proved to be the best nutrient although a decoction of *Coprinus* and of dung was used for some forms. For the early stages in the development of the mycelium small cultures were made in dishes holding 10 c. c. of the nutrient solutions. Large quantities of spores were sown and at the end of 12, 24, and 48 hours, the nutrient solution was removed by a pipette until only 2 c. c. remained, the dish was then filled with fixing solution which would thus be reduced about one-fifth in strength. After fixing 24 hours the spores were stippled on the slide by the method described by Harper (11) in his paper on the nuclear phenomena in the smuts.

Spores were also sown in thin films of agar-agar on sterilized slides. When the mycelium had attained the desired growth the entire slide was immersed in fixing solution. If the film loosened from the slide it was easily fastened again by a film of albumen.

To obtain mycelia, cultures were made similar to those described by Falk (8). Rye bread cut in slices two or three inches thick were moistened in bean decoction and fitted into battery jars five inches deep by four wide. For covers petri dishes four and a half inches in diameter were used. A thin layer of cotton was placed between the cover and the dish to allow free circulation of air. Agar-agar plates were also used.

The spores germinate in from six to eight hours and at the end of two or three days form a growth of mycelium which appears as a white mat about a quarter of inch in diameter on the surface of the bread. The mat increases in size rapidly until it is two or three inches in diameter. At the same time there appear all over the culture small white dot-like masses of mycelium. These are new growths from oidia scattered from the first mycelium. Falk has also described and illustrated such oidial colonies. These small secondary growths were removed whole with about a quarter of an inch of the substratum. Larger mycelia were cut into small pieces for fixation. In order to force the fluid through the thick felt which the mycelium forms the material was placed in a small bottle and well covered with the fixing fluid. The bottle was then fitted into the end of a rubber tube which was connected with the air pump. The air was pumped out from the closely matted hyphae, after which the fixing fluid was renewed.

The material was fixed in Flemming's solutions both the stronger and the weaker. Merkel's and Herman's solutions were also used. The best results were obtained from Flemming's weaker solution. Both Flemming's triple stain and Heidenhain's iron haematoxylin gave satisfactory results.

Hypholoma perplexum, Pk.

The spores of *Hypholoma perplexum* were collected in great abundance. The carpophores appeared in great profusion on decaying stumps and logs of oak throughout September and October. The spores were collected and stored after the manner already described. A large per cent of the spores obtained from mature pilei germinated in a shorter time than those obtained from younger ones. The spores were sown in the hanging drop agar cultures made with bean decoction. The cultures were kept in a dark box at a temperature of 20° c. As all the spores do not germinate at the same time but vary from four to forty-eight hours in the time of the appearance of the germ tube a large number of stages are obtained from the same slide.

The mature spores were studied in the drop cultures and also in the cross sections of the gills where they were still in connection with the sterigmata.

The mature spore of *Hypholoma perplexum* has a dark brown opaque wall which before germinating swells to two or three times its original thickness becoming much lighter in color and transparent. At this time it is easy to distinguish two nuclei lying close together near the center of the spore. They show the usual structure of the resting nucleus and are small spherical bodies with a well defined membrane, a large distinct nucleole and a fine granular chromatin which fills the remaining space (Fig. 1). The chromatin stains a light blue with the triple stain while the nucleolus stains with safranin. Previous to germination there is no indication of a germ pore but the germ tube always appears at the same place on the spore. On germinating a tube in the form of a large round bulb of protoplasm is protruded from the end opposite the point of original attachment to the sterigma. The germ tube increases rapidly in size retaining its spherical form as the cytoplasm passes into it from the spore. One of the nuclei passes from the spore into the tube as soon as there is room (Fig. 2 & 4) the other may pass out at once and it will then be seen near the first (Fig. 3) or it may remain in the spore for some time. Strands of cytoplasm caused by streaming from the spore into the tube are clearly shown in many of the preparations. As the nucleus which has remained in the spore is carried along by the streams of cytoplasm it becomes drawn out into a blunt point on the side towards the germ tube. (Fig. 2). As the nucleus approaches the narrow passage from the spore into the germ tube it is very nearly cone shaped. The vacuoles are very small at this stage with the exception of a single large vacuole which forms in the spore back of the second nucleus.

The tube begins to lengthen immediately in the direction of the long axis of the spore. The bulb has not formed a firm wall at this time and as the tube lengthens it becomes drawn out in the general direction of growth until its diameter is only slightly greater than that of the spore. The two nuclei do not remain together, one moving towards the apex of the tube while the other remains near the base. One of the preparations at this stage showed late division figures (Fig. 5). The chromosomes have already passed to the poles so that it is impossible to determine their number. The spindle fibres still connect the two masses. The two nuclei lie at some distance from each other. One spindle which is near the tip is nearly parallel with the walls of the germ tube. The spindle of the other, near the spore, is placed obliquely. Near one of the nuclei are two small deep staining bodies which may be remnants of the old nucleoles. (Fig. 5a). In an older germ tube the next division was observed. In this hypha three nuclei were in the resting condition. One is somewhat separated from the others towards the tip of the hypha and is a densely staining homogeneous body. Two other nuclei side by side near the center are elongated with the nucleole at one end. The fourth nucleus is dividing and is in the equatorial plate stage. The separate chromosomes could not be distinguished. The spindle fibres show an even distribution at the center and are collected into a definite pole. The presence of a central body could not be distinguished definitely (Fig. 6).

As the germ tube elongates the cytoplasm forms a much thinner layer at the periphery of the cell and a number of large vacuoles appear. The number and size of the vacuoles increase until they are only separated by fine lamellae of granular cytoplasm. The nuclei multiply rapidly and are frequently in pairs for some distance but this arrangement is not at all uniform. The nuclei may be separated from each other by long distances or they may lie in groups of three or four. Cross partitions are not formed in the young germ tube. It frequently branches two or three times before the first cross wall is formed. The cells which are then formed are very variable in length. The number of nuclei which they contain is from one to four (Fig. 7). The mycelium does not consist in these earlier stages of regularly uninucleated or binucleated cells. The mycelium grows rapidly and branches freely and the branches interlace forming a loose net-work. The branches do not show any regularity in their origin. A few are formed near a cross wall but the majority bud out somewhere near the center of the cell. The majority do not show a cross partition at their base. The first cross wall appears at varying distances up the branch. Usually a nucleus is found at the base of each newly forming branch but this rule is not constant for in a few cases a fairly long branch was found without any nucleus near. The lateral branches are narrower than the hyphae from which they arise. The first few cells of the branch contain as a rule fewer nuclei than the cells in the main hypha but the cells nearer the tip may have a large number. The number of nuclei at this stage varies from one to eight or nine; the same branch frequently showing both extremes in number. The nuclei are slightly smaller than those in the young germ tube but show the same general structure, a definite membrane and a nucleole which appears separated from the finely granular chromatin by a small clear space. In some of the preparations the nuclei were drawn out into long rather slender bodies with larger and very irregular chromatin granules loosely scattered throughout their length.

Special lateral branches may also be formed which have regularly uninucleated cells. These branches vary considerably in length and general shape. Some are long and nearly straight, or only slightly coiled, with long slender cells. The nucleus lies near the center of the cell and has the structure described above. Towards the ends of these branches very short cells are formed, only about twice as long as broad. These cells separate readily thus forming oidia. The character of the cytoplasm in these oidial cells is the same as that of the branch from which they originate. It is sometimes dense with very small vacuoles similar to that found in the spore. In other cases it has much larger vacuoles and the cytoplasmic granules as much larger and more irregular. In the majority of cases the nucleus is relatively large occupying nearly the entire diameter of the cell. In some instances the nuclear membrane and nucleole could not be distinguished and the chromatin was collected in two or four deeply staining masses usually oblong in shape. These nuclei may have been in some stage of division.

In one instance a slide culture that was placed in a very warm moist chamber (22° c.) showed a pronounced modification in the usual habit of growth. In many cases the germ tube was divided at once into uninucleated cells that bore numerous short series of oidia. The branches were coiled about the tube making it impossible to determine their number or structure. In other cases the hyphae were much longer and very slender with two or three branches on whose ends were large tangled masses of oidia. The oidia were rather slender and long but showed the usual structure.

Carpophores were not formed in any of the cultures but my studies on *Hypholoma perplexum* were continued on material which had developed spontaneously in nature. During the winter specimens of *Hypholoma perplexum* appeared on an oak log in the green house. The rotten pieces of wood were broken away exposing an expanded sheet-like rhizomorph or perhaps sclerotium, with some young fruit-bodies just forming. A sharp distinction between rhizomorphs and sclerotia probably cannot be made. Between the round tuber-like sclerotium of *Coprinus ephemerus* and the long branched mycelial strands of *Armillaria mellea* are many intermediate forms, among which are the sheet-like mycelial masses of *Hypholoma perplexum.* These have an outer layer of hard brown cells and a central mass of thin walled hyphae bearing, perhaps, a closer resemblance to the structure of the sclerotium than to that of rhizomorphs. But the sheet-like masses do not have any definite shape and, as far as this material showed, may be unlimited in their growth—characteristics not usually associated with a sclerotium. The central portion of this hyphal mass is formed of parallel hyphae which do not show any protoplasmic contents. Their walls have become gelatinous and in the triple stain become light blue. Near the surface on each side there is a layer of thin walled hyphae with irregular cells. The cells are very closely packed together forming a pseudoparenchyma. On the outer surface there is a layer formed by small closely packed cells which are filled with a hard brown substance. When a carpophore is to be formed some of the thin walled hyphae force their way through the hard outer layer where they unite to form the carpophore. Occasionally the carpophores are formed singly but they are usually in clusters.

Closer examination shows that the thin walled hyphae just beneath the surface of hardened cells are formed of short irregular cells that contain very little granular cytoplasm. They are regularly binucleated. The nuclei are large, with a distinct nuclear membrane and a small nucleole. The chromatin does not appear as granular as in the majority of nuclei but takes a uniform light blue stain. The two nuclei are usually pressed close together near the center of the cell. In forming a carpophore a number of the thin walled hyphae force their way through the outer crust, branch profusely at the surface and spread out slightly to form abroad base for the carpophore. The branches are short and branched in turn to form a knot of hyphae which is somewhat similar to the tangled mass formed at the base of the carpophore of *Coprinus.* But the cells are shorter and straighter so that the hyphae are not so matted and difficult to trace. Above the tangled hyphae at the base, the branches gradually assume a more vertical direction. They are still interlaced like the meshes of a net which has been pulled out lengthwise. Near the top of the button the number of branches increase and instead of continuing in a vertical direction they spread out radially. This is the first indication of a pileus. The outer branches of the central system of hyphae are not so closely interlaced but form a loose open network covering the pileus and stipe still further to the exterior. The surface of the button is formed by a layer of nearly straight hyphae. They arise as lateral branches near the base of the button and covering the entire surface, disappear among the hyphae at the apex of the pileus where the loose network of branches is thicker than at the sides. These hyphae are formed of long slender cells with very little protoplasmic contents. The shape of the cells and the direction of growth of the hyphae differentiate this outer layer very sharply from the tissues in the interior of the button. All of the cells are binucleated. The nuclei are large and have a nuclear membrane, a small nucleole and very finely granular chromatin.

At this time the young carpophore is an oval body and as indicated its tissues can be separated roughly into three distinct portions. The central portion formed of closely interlacing hyphae, which may be still further divided into the base of closely packed hyphae, the more vertical intervening hyphae from which the main portion of the stipe is formed, and the rather loosely spreading mass at the top from which the pileus develops. Second and outside of this central portion there is a poorly defined layer of loosely tangled hyphae which will untimately form the veil above and the outer covering of the stipe below. This layer is not sharpely separated from the central portion and is formed by lateral branches from that region. The third and outer portion is a layer of parallel hyphae which form the external covering. This covering is doubtless a simple type of volva.

In a carpophore which is a little further developed the cells above the basal tangle have enlarged and elongated. By the increase in size of the cells the hyphae are pressed close together and with the elongation of the stipe are forced into very nearly vertical series. At the same time the cells in the pileus increase in size but remain more or less intertwined. There is no differentiation of hyphae for the formation of gills as yet. As the stipe elongates the outer covering of parallel hyphae is irregularly broken in several places, and now appears as small particles or scales which soon disappear. The protoplasm in the cells, which are rapidly increasing in length, forms a much thinner peripheral layer, the central vacuole having increased greatly in size. A longitudinal section through a young carpophore just before the breaking of the veil shows the gills already formed. Sections which pass through the center of a gill show the structure very clearly. The hyphae that spread out to form the pileus are very irregularly twisted about each other. Near the lower side of the cap there is a narrower layer of much straighter hyphae which extends from the stipe to the margin of the pileus. From the lower half of the pileus but especially from this layer of straighter hyphae, branches are formed that grow downwards in parallel series forming the trama. The hyphae of the trama branch freely, the branches forming a layer near the surface of the gills, the subhymenium. The hymenium is formed of closely packed basidia which are terminal cells of the hyphae in the subhymenial layer. Fig. 24 is from a section showing the connection of a basidium with the subhymenial hyphae. The large basidium has two nuclei lying near together at the center of the cell which is separated from the stalk cell by a cross wall. A second basidium is just forming from the basal cell but does not as yet contain any nuclei.

The hyphae at the margin of the pileus are continuous with the outer covering of the hyphae on the stipe and form the veil. The original external covering of parallel hyphae has disappeared with the exception of a few small fragments near the base of the stipe. All of the cells are regularly binucleated.

When the basidia are formed there is an uninterrupted series of binucleated cells from the binucleated cells of the sclerotium through the stipe, pileus, trama and subhymenium to the basidia. Later the cells of the stipe and pileus contain from six to eight nuclei.

The stages of nuclear fusion and subsequent division in the basidium have been fully described by Wager, Harper and others. In nearly all the essential points *Hypholoma perplexum* agrees with the forms studied by these authors. The nuclei are in the spirem stage at the time of fusion. The fusion nucleus moves to the summit of the basidium when it divides. The process of the formation of the spindle was not studied. The spindle is always at a right angle to the long axis of the basidia. The chromosomes in the equatorial plate show a tendency to aggregate into a number of larger masses probably as a result of fixation. In every case there are eight or more. Certainly there are many more than two as claimed by Maire.

The second division follows the first very closely. The chromosomes in this case are not scattered on the spindle but definitely arranged in an equatorial plate. The four nuclei move to the center of the basidium. At this time or a little later fibres extending from the sterigmata to the nuclei at the center were observed in a large number of basidia, but their origin was not worked out. As to these fibres Maire says "Après la formation des noyaux-fils dèfinitifs la baside, ceux-si se massent le plus souvent à la base ou au milieu de la cellule, tandis que les centrosomes restent au sommet si les mitoses étaient apicales, s'y rendent dans le cas contraire. En face de chaque centrosome apparaît l'èbouche se produit une diffèrenciation kinoplasmique qui se propage jusque' aux noyaux at mème quelquefois plus loin vers la base de la cellule, orientant tant le cytoplasma de la baside par rapport aux sterigmates."

Each spore receives a single nucleus which immediately divides. In a number of cases the spindle is at right angles to the long axis of the spore. The chromosomes both at the equatorial plate and where they are drawn back to the poles appear to be four or more.

Coprinus.

The dung inhabiting *Coprini* are so easily obtained from dung cultures that the spores were not collected and stored as for the other species studied. The spores were transferred by means of a sterilized needle directly from the gills to the culture medium. For the study of the germinating spore and the early mycelium the agar hanging drop cultures are the most satisfactory. But for the older mycelium and for the formation of the carpophore agar plates in Petri dishes give the best results.

The spores of *Coprinus ephemerus* (?) germinate in from four to forty-eight hours. In the majority of cases of spores obtained from mature carpophores germination occurred in six hours. The spore wall does not swell and become transparent in germination as in *Hypholoma perplexum* and it is impossible to observe the position of the nuclei in the spore. At one end of the spore there is a thinner place in the wall—the germ pore. The germ tube appears as a small globular protrusion. One of the nuclei passes out from the spore at once into the germ tube. The nuclei divide very rapidly so that the young germ tube soon contains from three to six nuclei. At this time a nucleus can frequently be seen in the spore either near its center or just passing through the pore into the germ tube. At the end of the spore opposite the germ tube there is a dark hemispherical body, which perhaps marks the position of a second spore that has been closed by a cellulose thickening (Figs. 8 & 9).

The tube grows very rapidly forming a main hypha which is a direct extension from the spore and also a lateral branch which is nearly at right angles to it. This is well illustrated in Fig. 10 which is drawn from a germinating spore at the end of sixteen hours. The main hypha is slightly constricted at irregular intervals and in nearly every instance it is possible to determine the presence of a cross wall at the constrictions. The number and size of the nuclei vary in the different cells. The first and fourth cells counting from the spore have a very large nucleus which is more than twice the size of the nuclei in the other cells. The nucleole is very large and the chromatin is very much denser than in the nuclei usually found in this species. The other cells of the main hypha have from one to three small nuclei. The lateral branch does not show any of the constrictions found in the main hypha. It is very difficult to locate the cross walls since when newly formed they are extremely delicate and easily confused with a strand of cytoplasm. In one of the cells there are fourteen very small nuclei many of which are only slightly larger than the nucleole of the large nuclei in the main hypha. They have a nuclear membrane, a minute nucleole and a few scattered granules of chromatin. The other cells of the branch have one or two large nuclei.

At the end of twenty-four hours a well developed mycelium is formed. Fig. 11 is drawn from a mycelium that was formed in a hanging drop culture in twenty-four hours. In addition to the main hypha and the lateral branch mentioned in the previous description a second smaller branch is formed close to the spore. These hyphae branch repeatedly, without any regularity. The original hyphae are larger and straighter than any of the lateral branches and for convenience in distinguishing them I shall call them the primary hyphae. In them the cells are very unequal in length but the majority are from seven to eleven times as long as they are broad. The number of nuclei varies also. The cell next to the spore contains a very large nucleus in the center and a small one lying at one side near the junction of one of the branches. The next few (five or six) cells of each of the primary hyphae have three or four nuclei which are slightly smaller than the one found in the first cell. The nuclei are usually near together at the center of the cell but in one or two instances one of them is slightly separated from the others. The next cells for some distance (eight or nine cells) have two nuclei which may be close together or separated. In the remainder of the hyphae the nuclei are separated at some distance from each other but in many cases it is impossible to distinguish a cross wall between them.

The lateral branches are much smaller and more irregular than the primary hyphae and the cells are shorter, three or five times as long as they are wide, and usually contain a single nucleus although cells containing four or five are frequently found. In a few instances where the branches extend to the edge of the agar the terminal cells become smaller and contain a large number of nuclei closely packed together.

Arising from some of the cells near the base of the primary hyphae are short thick conidiophores. Their cells are nearly as broad as they are long. The end cells bear one or two very short branches which produce clusters of very slender hyphae formed of cells two or three times as long as broad. The cells separate readily into rod shaped oidia (Fig. 12). These hyphae do not appear above the surface of the substratum.

Near the center of the oidium there is a small well defined nucleus in which the nucleole and granular chromatin can be distinguished. The remainder of the cell is filled with finely vacuolated cytoplasm. This is in marked contrast with the form found by Maire on *Coprinus radiatus.* He says "Chaque oïdie renferme un seul noyau rèduit d'ordinaire à une petite masse homogène chromatique et un grande vacuole." On account of the number of oidia I was unable to see the nuclei in the cells of the supporting branch.

Occasionally the cells of the main hypha are binucleated. In this case the first few cells near the spore have from one to five nuclei. The cells further from the spore are regularly binucleated. At the end of five or six days branches appear on some of the older cells near the spore that are much larger than any previously formed and are frequently club-shaped. The cells of these branches are binucleated, the nuclei lying near together, sometimes side by side, near the center of the cell. The branches with binucleated cells may be formed from any cell of the mycelium near the spore. Fig. 13 shows one of the branches with binucleated cells formed on a hypha with uninucleated cells. The branch is formed very near the center of the cell instead of at one end as is usual with the mycelial branches. In my opinion the newly formed branch probably received a single nucleus which immediately divided. Fig. 14 is drawn from a hypha with multinucleated cells showing one of the larger branches just forming. Two nuclei of the multinucleated cell are just at the base of the newly forming branch.

Fig. 15 shows a group of these branches closely crowded together—the first indication of a fruit body. The hyphae have many branches spreading outwards. In the center the hyphae are so densely packed that it is impossible to tell whether the branches originate from a single hypha or from several. As a number of hyphae from different directions in the substratum lie near together at this point it is probable that they arise from several.

The cells are regularly binucleated. The cytoplasmic strands are very delicate and form a loose network at the periphery of the cell. Very small convex plates are present at the cross walls marking the regions of protoplasmic connection between the cells. This method of formation of the carpophore was repeatedly observed in the hanging drop cultures. It is probably an adaptation to the culture conditions, especially the small supply of nutrient. In larger cultures of agar-agar in Petri dishes I find quite another method for the formation of the carpophore.

In an agar culture in a Petri dish the main hyphae with their system of branches can frequently be traced for one or two inches. Fig. 16 was made from the end of one of these systems. Squares of the agar a little smaller than a 7/8 inch cover glass can be fixed in Flemming's weaker solution and then fastened to a slide with a film of albumin. Preparations are best stained with iron haematoxylin. The stain washes from the agar in the iron solution leaving the mycelial hyphae sharply defined so that they can easily be studied.

The main hyphae are formed of very long cells which have from five to eleven nuclei that are smaller than in *Hypholoma*, but show the same general structure, they are elliptical with a nucleole at one end, finely granular chromatin, and a small body which is usually opposite the nucleole. This small body is flattened against the nuclear membrane and takes a very deep blue stain. The great regularity of its occurrence and its staining qualities have led me to consider this as the same structure as the central body which Harper described in the ascospores of *Erysiphe*. The primary branches which arise from the main hyphae show great regularity of arrangement. They are formed in pairs at one end of every cell or every other cell. They are never exactly opposite, one always appearing slightly below the other. These branches are approximately of the same size as the main hypha and their base is enlarged in the same fashion as is the base of the germ tube. The young branch receives a single nucleus from the parent cell. A cross wall is then formed separating the branch from the main hypha while it is still very small. The nucleus divides very rapidly so that the young branch soon contains two or three nuclei. These continue to multiply until the mature cells contain as many or nearly as many nuclei as the cells of the main hyphae. The clamp connections between adjacent cells are quite abundant although they are not formed at every cross wall. They are cut off from one of the cells by a cross wall which always shows the deeply staining convex plates originally described by Hoffman (13) and since by Strasburger (24) and other authors. In every instance they show the same cytoplasmic structure as the cells which they connect. I have found one or two cases in *Coprinus* in which a branch has arisen from one of these clamp connections.

The secondary branches which arise from the primary hyphae do not show the same regularity in their formation. Only one is formed from a cell. They are very long and slender and run in every direction through the substratum. They anastomose with other branches frequently thus increasing the complexity of the hyphal system. The nuclei are separated by long distances, they are elliptical with a slightly larger nucleole than is found in the nuclei of the main hyphae. The cross walls are difficult to locate when, as frequently happens, the convex plates at the cross walls are absent.

The mycelium does not appear at the surface of the culture until a fruit body is to be formed. At this stage the character of the branching of the mycelium changes. The cells are shorter and branch three or four times, each successive cell branching in the same way. The cells are also curved backward towards the main hypha, thus a tangled mass is formed (Fig. 17). Other hyphae from various directions pass into this snarl and branch in the same way increasing its size and complexity. Fig. 17a is from one of these tangled masses and shows the Y shaped cells which are frequently found near the center. The cell walls are thick and tinged with yellow. The clamp cells are found at every cross wall connecting the adjacent cells.

All of the cells contain two nuclei which may lie close together but are more frequently separated by the entire length of the cell. In the Y shaped cells I have generally found one nucleus in each of the arms.

In my cultures the hyphal masses developed into carpophores only when they were formed near the surface or some break in the agar. A large number of small tangled masses were formed throughout the cultures but they did not develop into carpophores. Fig. 18 is from a section through a young carpophore which is developing from one of these tangles. The mass of hyphae is much larger than in the earlier stages but it shows the same structure. A large number of hyphae are found converging from different directions in the substratum to form by the interlacing of their branches near the surface of the agar this large tangled mass. The hyphae are so closely packed and so crooked that it is impossible to trace the cells their entire length but as far as I can determine they are in all cases binucleated. The hyphae outside of the tangled mass that are growing towards it and whose branches form the tangle invariably have binucleated cells. In the younger tangles the cells are binucleated and in this large hyphal mass the nuclei are usually found in pairs.

A more complete series of young carpophores of *Coprinus ephemerus* were obtained from dung cultures in the green house. By fixing the young carpophores when they first appear as small white dots on the substratum the main stages in the early development of the fruit body are very easily obtained. They are very similar to the corresponding stages of *Coprinus stercorarius* as described by Brefeld.

The very young carpophore is an oval body formed by a central mass of closely tangled hyphae and an outer covering of very loosely interlaced hyphae. The hyphae near the center are copiously branched. The branches branch in turn and gradually force their way upward and outward, the density of the tangle becoming less as they grow farther from the center until they are only loosely woven together at the surface forming the outer covering. The cells of all the hyphae are very short and are densely filled with a finely vacuolated cytoplasm. They are regularly binucleated, the nuclei showing the same structure as those found in the mycelium.

A section through a carpophore which is more mature shows the hyphae in the central portion arranged in parallel series vertical to the base. At the center there are a number of slender hyphae. Near the top these slender hyphae spread out and slightly downward to form the young pileus. The outer network of loose hyphae still encloses the growing cap and stipe. It is scarcely attached to the cap except near the apex where a few slender hyphae from the center of the pileus pass out into it. Below the cap it is continuous with the hyphae of the stipe. At this time previous to the formation of the gills the cells of the stipe have from four to eight nuclei. The cytoplasm is filled with large vacuoles.

Maire has described the formation and structure of the gills of *Clitocybe aurantiaca, Mycena galericulata, Stropharia semiglobata, Hypholoma appendiculata*, and *Psathyrella disseminata.* He finds that when the basidia are formed the cells of the trama are binucleated but in the stipe and pileus the cells are multinucleated. Harper has found this true also for *Coprinus ephemerus.*

I have also studied the young carpophore of other forms without growing them from spores to determine the arrangement of the nuclei in the young carpophore and in the mycelium in the substratum.

A species of *Crepidotus* was found growing on a rotten log in the green-house during the winter. The specimens were fixed with a portion of the substratum. For the most part the hyphae fill the vascular elements of the wood densely and are difficult to distinguish. But there are also single hyphae which grow in some of the large ducts that are favorable for study. The cells are very poor in protoplasmic contents. Two nuclei lie close together near the center of the cell surrounded by a little granular cytoplasm. At the surface the mycelial hyphae come together and twine around each other forming a short tangled mass which is the base of the carpophore. The hyphae branch profusely and curve upward growing in a vertical direction to form the stipe. At the top of the stipe they spread out in every direction to form the pileus and gills. The cells are regularly binucleated and are filled with very dense cytoplasm.

Certicium lilacino-fuscum was also studied. It appeared in great abundance on a damp log in the green-house. Portions a quarter of an inch square were removed with a thick layer of the substratum and fixed in Flemming's weaker solution.

The surface layer is formed of densely packed short celled hyphae which grow perpendicularly to the substratum. The hyphae branch repeatedly, the branches crowding in between the hyphae. The cells are long and slender and so closely crowded together that I could make out the contents in only a few cases. In favorable preparations in a few instances cells were seen with two nuclei near the center and the remainder of the cell was filled with very granular cytoplasm. The clamp connections between adjacent cells are nearly always present. On the lower surface there are a large number of thick walled hyphae, destitute of cytoplasm, which form a loose felted layer and then penetrate the wood for some distance. They probably serve as a protective layer and also hold the mat close to the substratum. Among these basal thick walled hyphae there are others with thin walls and protoplasmic contents which also penetrate the wood in every direction and are usually single but occasionally are found in masses filling a vascular element of the wood. They are formed of long slender cells with two nuclei.

Rhizomorphs.

In many *Agarics* the hyphae form mycelial strands which often grow to great length. These strands may branch frequently but without regularity and resemble roots. In some species the branches anastromose frequently forming a loose network; a character which is especially pronounced in *Dictyophora duplicata.* The structure of these so-called rhizomorphs is best known through the descriptions of *Armillaria mellea* by De Bary, Goffart and others. The forms which I have studied show a much simpler structure but they apparently have the same origin, the same general character of growth and doubtless serve the same general function. They provide a storage for reserve material, a means of spreading the species in the substratum and are capable of retaining their vitality during a long dormant period.

Poria. (Fig. 19.)

The rhizomorphs of a species of *Poria* were collected in April from the underside of a board sidewalk where they had hibernated. Thus they were in a resting rather than the active growing condition of the other species described later. The main strands were about an eighth of an inch in diameter. These branch dichotomously a few times and from them a number of very slender branches are formed. These branches extend in every direction over the substratum; they subdivide freely, the branches frequently anastomosing and flattening out to form web-like expansions. The younger portions of the strand are a dull yellow while the older portions are coated with a yellowish brown layer.

Their structure is very simple. The entire strand is formed of slender hyphae which are very straight, only twining about each other slightly. At the center a large number of the hyphae have lost their contents and the walls are very thick. But among them are other hyphae with thin walls and rich protoplasmic contents. The cytoplasm is filled with small deeply staining bodies but there are no crystals. Towards the surface these thin walled hyphae become fewer and finally disappear leaving the outer layer formed wholly of thick walled hyphae (Fig. 19a). In a number of the thin walled cells two nuclei were found. They were very small and dense lying near together at the center of the cell. In some cases the deeply staining granules were so abundant and so closely packed that it was impossible to distinguish the nuclei. Among the hyphae there were numerous openings or pockets filled with octahedral crystals which dissolve slowly in hydrochloric acid indicating that they are calcium oxalate (Fig. 19c). Just beneath the surface layer of thick walled hyphae there is a layer of similar crystals very closely packed together (Fig. 19b).

Pholiota praecox. Pers. (Fig. 20.)

The mycelial strands of *Pholiota praecox* were collected from a mass of decaying leaves. A large number of mature caps were removed with all the connecting mycelial strands. The rotten leaves were easily pulled away leaving the strands free from all foreign matter. The strands were cut into pieces one *fourth* inch in length from the tip and from the older parts which show the branching.

The outside of the strand is covered by a thin layer of loosely tangled thick walled hyphae which doubtless serves as a protective layer. Beneath this is the main portion of the strand which has the same structure throughout with no differentiation into medulla and cortex. It is formed principally of large thin walled hyphae which show some slight regularity of arrangement although the longitudinal rows of cells so pronounced in Armillaria were not found. In some parts of the strands there are a few central hyphae that run very nearly straight. On both sides of this central strand the hyphae grow obliquely towards the surface. In other parts bundles of thirty or more hyphae, slightly entwined with one another coil around similar bundles or separate hyphae. Frequently it was impossible to make out any regular arrangement. The great majority of hyphae are formed of large cells but arising as secondary branches from them are a few narrow celled hyphae which run in the same direction as the larger hyphae until they reach the surface. At the surface instead of turning back towards the center as do the larger hyphae their walls become thickened and they form the felted layer. Thus the outer felt consists of the thickened terminal portions of the slender hyphae of the interior.

It is only by very careful staining with the triple stain that I was able to make out the contents of the cells. The walls of the large cells are partly gelatinized and have lost their smooth outline becoming wrinkled and creased. There is a very thin layer of cytoplasm with small light staining granules. The nuclei are flattened against the walls by the central vacuole. They are large and distinct with a very small nucleole on the side towards one end of the cell and a deeply staining body, probably a central body, on the opposite side. The remainder of the nucleus is filled with a finely granular chromatin which takes a stain that is only slightly darker than that of the granules in the surrounding cytoplasm from which it is sharply separated by the nuclear membrane. All of the cells contain two and only two nuclei which may lie near together or separated by the entire length of the cell. Occasionally the cells had one or two small crystals.

The slender hyphae are formed of very long narrow cells which are filled with very dense cytoplasm. The nuclei are large, occupying nearly the entire diameter of the cell and lie close together at the center. When these hyphae reach the surface of the strand their walls become thickened leaving only a very small central strand of cytoplasm.

All of the cells have deeply staining convex plates on opposite sides of their cross walls. These convex plates always appear in pairs with their concave surfaces towards each other.

Lepiota naucina. (Fig. 21.)

The mycelial strands of *Lepiota naucina* are very similar in appearance to those of *Pholiota.* They were found growing in the newly deposited mulch around the base of some young trees. It was impossible to obtain the mature carpophore attached to the strands but by loosening the substratum carefully I could obtain long strands still attached to the young buttons.

The structure of the strands differs in a few respects from those just described. In the interior of the strand the hyphae do not show any regularity of arrangement. The large celled hyphae are separated by a number of fine branches and are much fewer than in *Pholiota.* They are formed of large cells, with slightly rounded ends where the cross walls are formed. The slender hyphae arise as lateral branches of the larger ones and are very abundant. They wind among the larger hyphae forming a dense weft. Near the surface there is a layer of several cells thick entirely composed of slender hyphae. The cells are much shorter than in the center of the strand and each cell branches so that a close net-work is formed. Outside of this there is a felted layer of thick walled hyphae. The thick walled hyphae were in this case so closely packed that it was impossible to trace their origin.

The contents of the cells are very similar to those of *Pholiota.* The large cells have a very thin peripheral layer of cytoplasm which contains a large number of small very deeply staining granules which may be very minute crystals. Larger crystals are very abundant, many of the cells containing twenty-five or thirty. They vary from the smallest size determinable to those which are one fourth of the diameter of the cell. They take a bright red color in the triple stain and are octahedrons in the majority of cases but a few appear to have more faces. Because of the numerous dark staining bodies, it was frequently impossible to differentiate the nuclei but in the cells where there were very few if any crystals or granules two nuclei were found. The large cells have wide pits in their walls through which it is easy to trace a protoplasmic strand. In a few cases the pore is so large that it is possible to trace a connection not only between the peripheral layers of cytoplasm but also between the central vacuoles. The protoplasmic granules frequently accumulate around these pores in large numbers but the convex plates described above are not formed. The slender hyphae are filled with a finely granular cytoplasm without any crystals or large granules. The cells are regularly binucleated. The convex plates are always present at their cross walls.

Dictyophora duplicata. Ed. Fisch. (Fig. 22).

The mycelial strands of *Dictyophora duplicata* were collected from the beds of mulch around the base of young trees on the University drive. By loosening the soil thoroughly large masses of the strands were obtained still in connection with young carpophores. The strands are fine and delicate with numerous irregular swellings. They branch frequently and the branches sometimes anastomose forming a very loose network.

The structure of the strands is decidedly different from the types already described. The very center of the strand is occupied by a few large hyphae which twine about each other slightly. They are formed of very long cells which have lost all of their contents and their walls are slightly gelatinized.

Towards the surface these large hyphae become much fewer and are separated by other hyphae which are only slightly smaller but very rich in protoplasmic contents. This second form of hyphae are the lateral branches of the first and correspond to the slender hyphae of the other species but are very different in appearance. They are loosely twined together towards the interior, forming an open mesh work through which a few of the larger hyphae run and also leaving numerous air-spaces. Towards the outside they become more and more closely woven together until they form a layer of densely packed hyphae just beneath the surface. Their cells are not so long as in the large hyphae but are nearly as wide in many places. They are irregular in width, especially towards the surface and contain large numbers of deeply staining bodies probably of a proteid character. These bodies take a deep blue color with the triple stain which requires a long exposure to Orange G. to remove. On account of the number of these dark bodies it was possible to locate the nuclei in only a few of the cells. In favorably stained cells there were two large nuclei with the usual structure, a small nucleole, and very finely granular chromatin, surrounded by a nuclear membrane with a deeply staining body at one side.

The hyphae do not have their walls thickened when they reach the surface as in all of the preceding species but their cells become shorter and very vesicular. The hyphae are very loosely woven together forming a very thick outer layer. The cells do not contain any of the dark bodies so abundant in the layer just beneath, but have a single large nucleus, which is very irregular in shape, near the center of the cell. This large nucleus is imbedded in a central mass of cytoplasm from which strands radiate in every direction towards the walls, as is the case in *Spirogyra* cells. I did not find any crystals in any of the cells or any convex plates at any of the cross walls.

Lycoperdon pyriforme. Schaeff. (Fig. 23.)

The mycelial strands of *Lycoperdon pyriforme* were found growing in a rotten stump. By breaking off pieces of the wood large quantities of the strands were obtained free from foreign matter. The strands are fine and very tough, in marked contrast to the two preceding species.

In the center of the strand the hyphae run very nearly straight only slightly twining around each other. The large celled hyphae are formed of very long cells so that it was only occasionally that I found a cross wall. The walls are very delicate and do not show either a pore or the lens shaped plates. The cytoplasm forms a peripheral network of deeply staining strands which are very irregular and contain a number of large round granules. Only one or two octahedral crystals were found in any of the cells. The cells contain two large nuclei which take a very light stain as they have apparently only a small amount of chromatin. The nuclear membrane is very distinct and clearly defines the nucleus. The nucleole is very small.

The slender hyphae are very abundant and form the larger portion of the strand. They arise as lateral branches of the larger ones and twining among each other in a slightly oblique direction gradually work their way to the surface. At the surface they form a layer of densely packed hyphae by the interlacing of their branches. On the outside of this layer the hyphae are more loosely woven together and their walls become gradually thickened to form a protective covering. The cells of the fine hyphae are long and slender in the central portion of the strand, becoming much shorter as they approach the surface. At every cross wall very large distinct convex plates are present. The cytoplasm forms a finely granular layer at the periphery. There are always two large distinct nuclei near the center of each cell.

Section which pass through the main strand and the base of a lateral branch show that the hyphae which form the branch do not arise in any very definite fashion. Hyphae from various parts of the central and outer layers curve in the same direction towards one side and unite at the surface to form a branch. In one case I was able to trace a hypha which was growing on the other side of the strand, until it was nearly oposite the branch, where it curved sharply, crossed the main strand and entered the branch on the other side.

CONCLUSIONS.

The results obtained from the above studies show that the binucleated cells do not originate through the formation of any special reproductive apparatus. Neither is there any structure in the formation of the hyphae or in the cells of the hyphae that indicates in any way where the binucleated cells will first appear. That they arise previous to the formation of a carpophore in a large number of forms is very evident. The long series of binucleated cells found in the mycelial strands or rhizomorphs of *Poria, Pholiota, Lepiota, Dictyophora* and *Lycoperdon* show that in these forms the origin of the binucleated cells is only distantly associated with the formation of a carpophore and it is probable that many strands never develop carpophores. In *Hypholoma perplexum* there is an expanded mass of mycelium with binucleated cells formed previous to the appearance of a carpophore. In many forms in which there is not any specialized structure between the filamentous mycelium and the carpophore, as in *Crepidotus, Corticium,* and in *Coprinus ephemerus* it was found that the cells of the mycelium in the substratum in the region of the carpophore were regularly binucleated. Binucleated cells were also observed in the cells of the mycelium of *Hypochnus* by Harper. As was mentioned previously, he was able to trace the series of binucleated cells from the hymenium into the mycelium in the substratum. The above cases show that the formation of binucleated cells is not necessarily followed immediately by the formation of a carpophore.

Further in the fairly complete series of stages from the germination of the spore to the mature carpophore which were obtained for *Hypholoma perplexum* and *Coprinus ephemerus* the same fact appears. A summary of the development of these species is as follows:

The spores of both species contain two sister nuclei which at germination pass from the spore into the germ tube. In *Coprinus* the nuclei divide very rapidly, forming a multinucleated germ tube which may contain twenty or thirty nuclei before the first wall is formed. The nuclei do not divide as rapidly in the germ tube of *Hypholoma* so that it seldom contains more than six or eight when the first wall is formed. In the germ tube of *Hypholoma* the nuclei divide independently as was shown by the appearance of a single nucleus dividing in one of the germ tubes. A septate mycelium is formed in *Hypholoma,* the cells of which contain from one to nine or ten nuclei. The mycelial cells of *Coprinus* frequently contain as many as fifteen nuclei. Lateral branches are formed in both *Hypholoma* and *Coprinus,* the cells of which have a single nucleus. The cells are short and separate from each other readily. In *Hypholoma* these cells germinate forming a normal mycelium, thus proving conclusively that they are oidia. In *Coprinus* the cells did not germinate but their similar origin and structure indicate that they also are oidia.

Apparently after a varying period of growth hyphae are formed which have regularly two and only two nuclei in their cells. In the forms studied this association in pairs does not arise at any otherwise differentiated time or in any special structure.

We must remember as described above in *Coprinus* that binucleated cells were sometimes formed very near the spore, the main hyphae at a distance of two or three cells from the source of its origin having regular binucleated cells. In other cases special branches having binucleated cells were formed. When these branches arise from the uninucleated cells they probably receive a single nucleus which immediately divides. This division is not followed by a cross wall separating the two nuclei and thus the first binucleated cell is formed. If the branch having binucleated cells originates from a multinucleated cell it may receive two nuclei from the parent cell or it may receive a single nucleus that divides to form the second nucleus.

The hyphae with binucleated cells branch frequently and may develop immediately into a carpophore. This method of carpophore formation has been described for *Coprinus ephemerus.* The cells of the young carpophore are always binucleated and a series of binucleated cells extends to the formation of the hymenium. Thus the basidium with its two nuclei is the last of a long series of binucleated cells. That these two nuclei have maintained distinct lines of descent from the first cells with two nuclei occurring in the mycelium is not certain. I have not found evidence of conjugate division in the hyphae but it seems possible that it exists and that the two nuclei of the young basidia have remained distinct throughout the series of binucleated cells.

The evidence seems satisfactory that in the rusts the series of nuclei in the binucleated cells are formed by the association of two nuclei in so called conjugate division. A wall is formed between the two pairs of daughter nuclei. Thus the two nuclei in a cell are not sister nuclei. The nuclei which fuse in the teleutospore have thus remained distinct through generations of binucleated cells. The wide occurrence of a series of binucleated cells in the young carpophore suggests a method of division similar to that in the rusts. Maire believes that the series of nuclei in the binucleated cells of the forms studied by him are formed by conjugate division but his figures do not seem conclusive. In *Hypholoma* and *Coprinus* each of the four spores borne on the basidium receives a single nucleus which immediately divides forming a binucleated spore. Maire states that a similar process occurs in *Clavaria vermicularis, Clitocybe aurantium, Mycena galericulata,* and *Amanita pantherina.* This resemblance between the nuclear history of the *Uredineae* and of the *Basidiomycetes* has been discussed fully by Maire, Harper and others.

In view of the fact that the hyphae with binucleated cells are found originating from uninucleated cells in *Coprinus,* it is very possible that further study may reveal some forms in which the mycelium will show regularly uninucleated cells and that this uninucleated series will extend to the first formation of a carpophore.

At present there is no evidence that the binucleated cells of the *Basidiomycetes* ever originate by a fusion of two adjacent cells such as Blackman (2) finds at the base of the aecidium in *Phragmidium violaceum* and *Gymnosporangium clavariaeforme.*

The occurrence of regular binucleated cells through a large part of the life history of the *Basidiomycetes* leading to the formation of basidia while binucleated cells are unknown in the life history of the *Ascomycetes* makes it difficult to consider the two groups as phylogenetically related. Maire (16) states that binucleated cells occur in the ascogenous hyphae in *Pustularia* *vesiculosa, Galactinia succosa* and *Acetabula acetabulum*. But this hardly seems adequate proof for the conclusion he suggests. The origin of the ascocarp in a sexual process is in marked contrast to the origin of the carpophore as described above. It seems difficult to imagine that they are homologous structures. Certainly neither asci nor basidia can be considered as oogonia as Dangeard proposes.

That true cell fusion does not occur in *Coprinus* is very evident. It is doubtful how far this fact should influence our interpretation of the fusion of nuclei in the basidium. Two nuclei of more or less widely separated origin fuse and this is at least a common characteristic of sexual fertilization. Raciborski (19) believes that the binucleated cells of the rusts represent a prolonged vegetative stage intercalated between the cell fusion and the nuclear fusion just as in the zygospore of *Basidiobolus* a period may intervene between cell and nuclear fusion. He proposes the term zeugite for all cells in which occurs a fusion of nuclei belonging to the same cytoplasmic mass. But the fact that the binucleated cells of the *Basidiomycetes* are not the result of actual cell fusion makes it difficult to compare directly the delayed fusion in the zygospore of *Basidiobolus* and the nuclear fusion in the basidium.

Maire proposes to distinguish two types of fusion: "La sexualité avec fecondation" and "la sexualité avec mixie." The first type is found in the higher plants and animals, where the nucleus resulting from the fusion of two sexual nuclei contains twice the number of chromosomes contained in either of the fusing nuclei. The second type he believes is found in the lower plants and in the basidium. In this type the nucleus resulting from the fusion of two nuclei has the same number of chromosomes as was contained in each of the fusing nuclei.

Maire describes the nuclear fusion and division in the basidia in *Mycena galericulata, Psathyrella disseminata* and a large number of other *Basidiomycetes*. In all of these species he describes the two conjugate nuclei as each having two chromosomes and states that the fusion nucleus of the basidium in the first division has also only two chromosomes. But his figures are somewhat diagrammatic and decidedly inconclusive. On this evidence Maire has formulated a theory of the alternation of generation in the *Basidiomycetes.* He believes that the origin of the binucleated cell is comparable to the fertilization of the higher plants. The carpophore having cells with paired nuclei corresponds to the sporophyte. The fusion of nuclei in the basidium is then not a fertilization but a reduction-process. Blackman's discovery that in the rusts the binucleated cells arise by an actual cell fusion is strong evidence in favor of this view and Blackman develops the conception into very satisfactory form so far as the rusts are concerned. It is possible that the origin of binucleated cells in the ordinary course of mycelial growth as described above may have come to serve as a substitute for cell fusions and that thus the stages of development with binucleated cells in the *Basidiomycetes* may be considered the equivalent of a sporophyte. Since, however, the point or points at which binucleated cells arise is very variable such a sporophyte can by no means be considered as representing a so definitely differentiated stage of development as does the sporophyte of the moss or fern.

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PLATE IV.

EXPLANATION OF PLATE IV.

All figures were drawn with the aid of the Abbé camera lucida.

Fig. 1. The spore of *Hypholoma perplexum* previous to germination. × 2700.
Fig. 2. Spore of *Hypholoma perplexum* with a short germ tube which has received a single nucleus from the spore. × 2700.
Fig. 3. Spore of *Hypholoma perplexum* with a short germ tube which has received both nuclei from the spore. × 2700.
Fig. 4. Same as Fig. 2.
Fig. 5. The nuclei in germ tube of *Hypholoma perplexum* show late division figures. × 2700.
Fig. 6. One nucleus, in a germ tube of *Hypholoma perplexum*, is in the equatorial plate stage of division. × 2700.
Fig. 8. Spore of *Caprinus ephemerus* with a short germ tube containing several nuclei. × 2700.
Fig. 9. Same as Fig. 8.
Fig. 10. A germinating spore of *Caprinus ephemerus* at the end of sixteen hours. × 2700.

Trans. Wis. Acad., Vol. XV.

Plate IV.

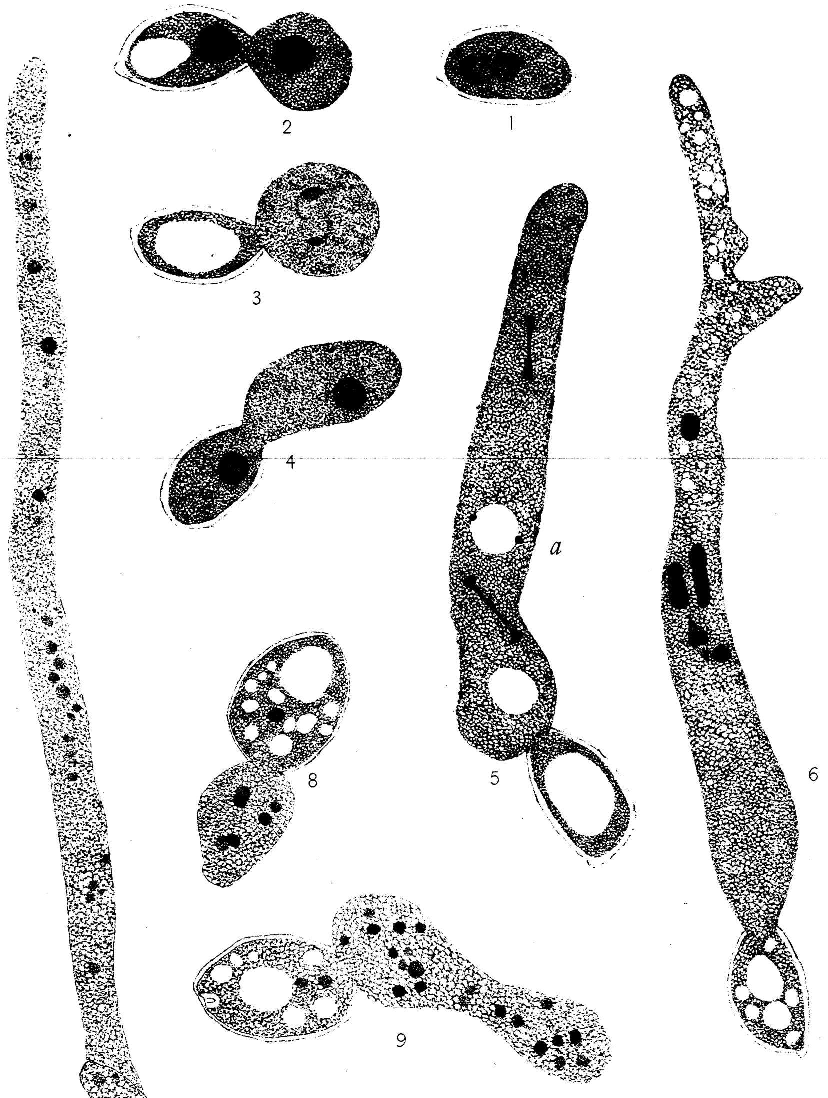

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PLATE V.

EXPLANATION OF PLATE V.

Fig. 7. The mycelium of *Hypholoma perplexum.* × 1200.

Fig. 11. Mycelium of *Caprinus ephemerus.* × 850.

Fig. 12. Oidial branches of *Caprinus ephemerus.* × 1500.

Fig. 13. A branch with binucleated cells formed on a hypha with uninucleated cells. × 1500.

Fig. 14. A large branch forming on a hypha with multinucleated cells × 1500.

Fig. 16. Mycelium of *Caprinus ephemerus* showing the arrangement of nuclei in the cells. × 1200.

Fig. 18. Section through the base of a young carpophore of *Coprinns ephemerus.* × 850.

Fig. 24. A section of *Hypholoma perplexum* showing the connection of a basidium with the subhymeniun. × 1500.

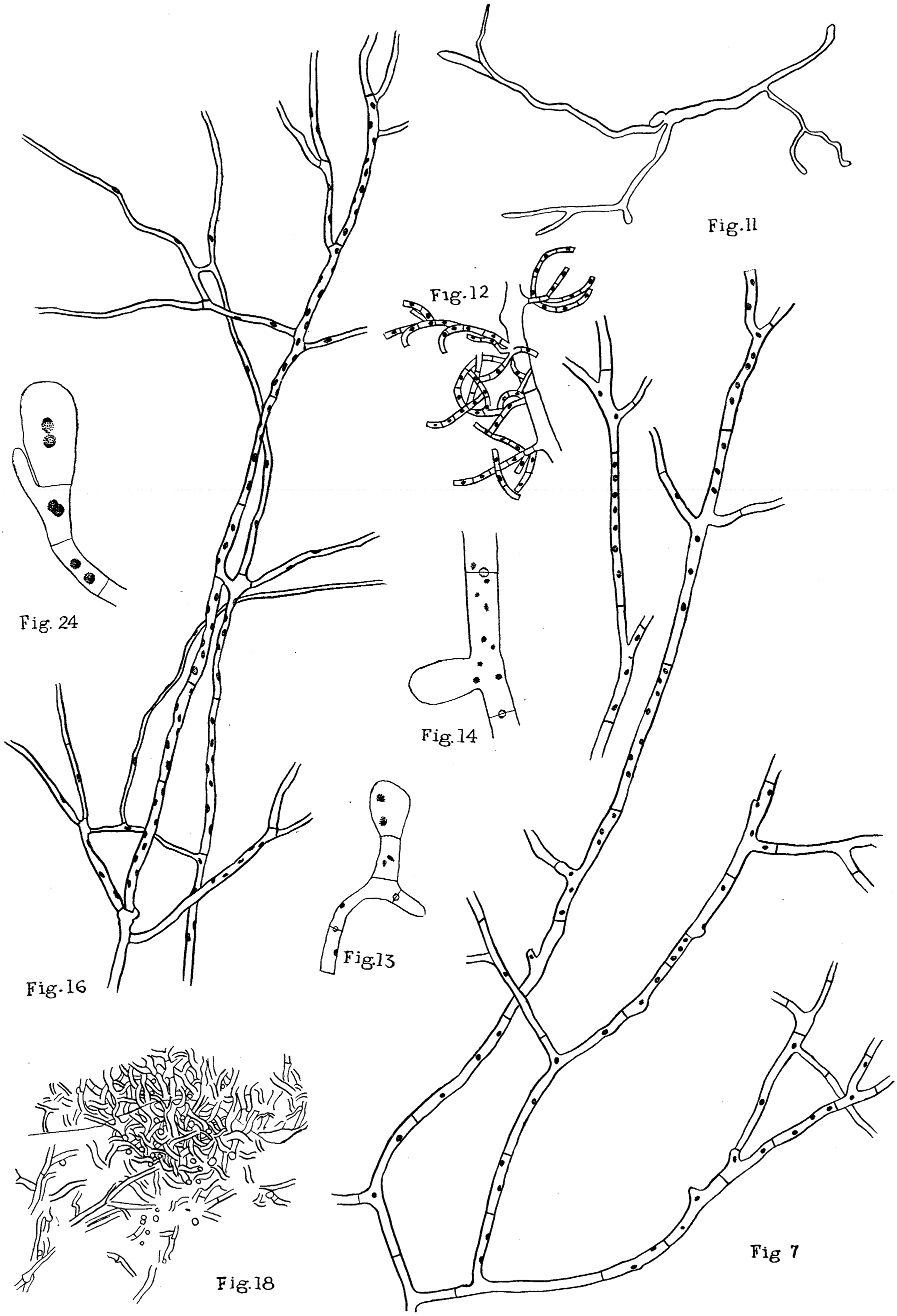


Fig. 11

Fig. 12

Fig. 24

Fig. 14

Fig. 16

Fig. 13

Fig. 18

Fig 7

S. P. Nichols del.

PLATE VI.

EXPLANATION OF PLATE VI.

Fig. 15. The first indication of a fruit body of *Coprinus ephemerus.* × 1500.

Fig. 17. Same as Fig. 15. a, Y-shaped cell. × 1500.

Fig. 19. Longitudinal section of the rhizomorph of *Poria.* a, Outer layer of thick-walled cells; c, Pocket containing crystals; b, Layer of closely packed crystals. × 1200.

Fig. 20. Longitudinal section of the rhizomorph of *Pholiota praecox.* × 1200.

Fig. 21. Longitudinal section of the rhizomorph of *Lepiota naucina.* × 1200.

Fig. 22. Longitudinal section of the rhizomorph of *Dictyophora duplicata.* × 1200.

Fig. 23. Longitudinal section of the rhizomorph of *Lycoperdon pyriforme.* × 1200.

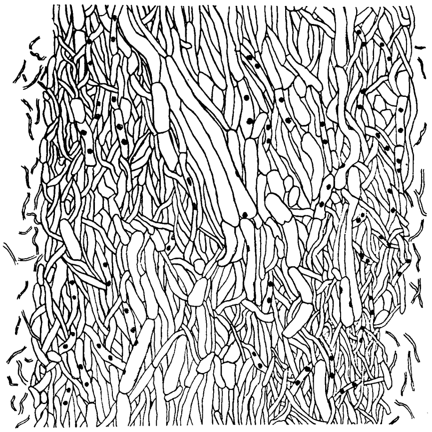


Fig.21

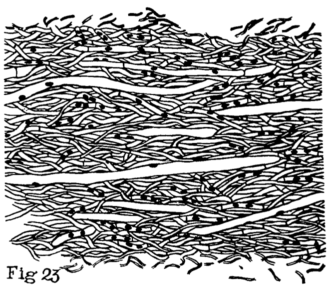


Fig 23

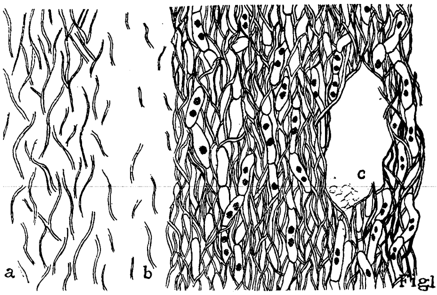


Fig19

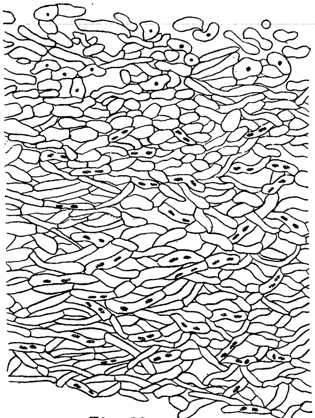


Fig. 22

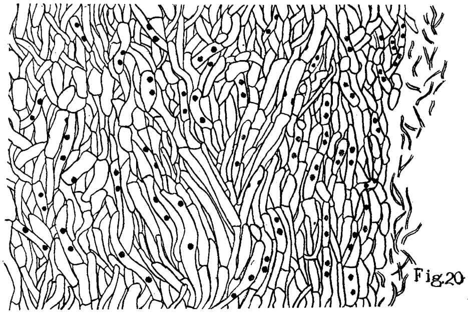


Fig.20

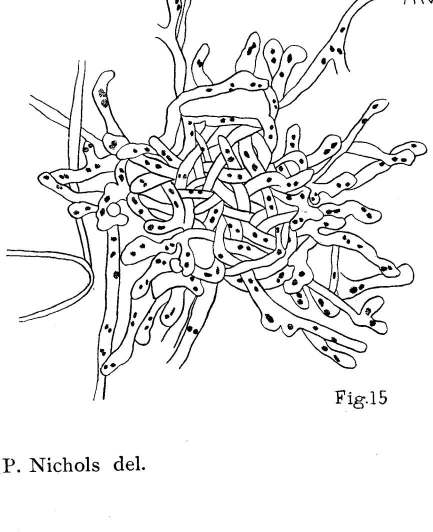


Fig.15

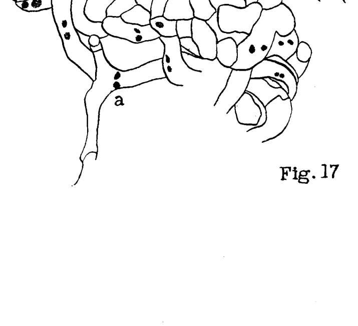


Fig. 17

S. P. Nichols del.

THE RUSSULAS OF MADISON AND VICINITY.

H. R. DENNISTON.

The genus Russula is well represented in Wisconsin, several species being extremely abundant. They grow on the ground and prefer a rich hilly woods where there is considerable moisture, but certain species are also found in low woods or open meadows.

An unusually rich harvest of Russulas was gathered in the summer of 1903, when there was an abundant rainfall throughout the season. It was noted that in such a favorable season the bulk of the Russulas came in the latter part of July and the first part of August; after that, although large numbers of fungi were found, the Russulas were comparatively scarce, and other genera, especially the Cortinarii and Tricholomas, became relatively more abundant.

The Russulas are easily distinguishable by their bright colors, brittle, white or cream colored gills and fleshy stem, but within the genus, a number of species are particularly difficult to separate.

This difficulty is due largely to their extreme variability. If our common *R. integra* is taken as an example, it is found that the color of the pileus may be purple, livid, greenish, brown or tawny. All of these colors may be present in the same individual and are often found in specimens growing in the same neighborhood. This variability of color is a feature of a number of the commoner species of Russula, but not of all of them.

The shape of the pileus usually changes as the plant grows older. On this account it is quite necessary to note the age of the specimen at which it has a certain form. In young plants the pileus is commonly convex, later explanate and finally depressed.

Massee separates the Russulas into two groups according to their acrid or mild taste, and makes this feature the principal basis for classification.

I found that certain specimens of *R. alutacea* were intensely peppery the instant they were tasted, others of the same species, were more slowly peppery. In some cases, the pileus of *R. alutacea* is quite mild, but the gills of the same plant are peppery. For these reasons it does not appear that the taste is a suitable characteristic upon which to divide the genus. The word *acrid* is used by Massee synonomously with *hot* or *peppery*. This use of terms is misleading for, to many people, the words peppery and acrid convey different meanings.

While the taste characteristic is hardly sufficiently constant to be used as the chief basis of classification, it is a point which no collector should fail to test on the fresh specimens.

The flesh is white in most cases and spongy, but may become discolored by being bruised or upon drying. When there is a separable cuticle, a little of it should be removed, for oftentimes the flesh beneath has a characteristic color.

A number of the Russulas have characteristic odors. Our Wisconsin species, *R. foetens,* derives its name from this feature. The odor is pungent and nauseating and is present in the fungus at all stages of its development. Curiously enough, I find the odor of the dried specimen is very pleasant. *R. atropurpurea* develops an exceedingly rank odor while drying.

The gills are usually interveined, a point which should be noted in the fresh specimens, as later it is difficult to observe. The attachment of the gills to the pileus is an important characteristic and on this point it is advisable to examine as many specimens and at as many different ages as possible, for frequently gills which appear in young specimens to be free, upon the expansion of the pileus become apparently decurrent or adnate.

It is of considerable value to compare the color of the fresh gills with a color chart, for there is a color change so delicate from white to straw, from straw to cream and from cream to ocher, that the unaided eye will scarcely distinguish it. In noting the condition of the gills, it is well where possible, to examine a portion under the microscope, for it is frequently found that the spores are tardy in developing, and until they are fully developed, the gills remain perfectly white. In some species the spores cause the gills to have a powdered or dusty appearance.

Probably most of our commoner Russulas are edible, although this is a point which needs further investigation. They are looked upon with suspicion by many people and this is no doubt because of the bright colors and the peppery taste of a number of species. According to McIlvane, all of the Russulas are edible, at least none of them are poisonous. He admits, however, that a number of them are extremely unpleasant in odor and taste.

The species in which the question of edibility is most debated is probably *R. emetica*, a small form with a red cap, white gills and a peppery taste. McIlvane claims to have eaten it in quantities with no unpleasant results.

Miller says it is much eaten in Indiana and Illinois. Stevenson, on the other hand, says that it acts as an emetic and Peck gives it as deleterious.

It is possible that the fungus varies in its effect on the human system but it is also possible that McIlvane and others have mistaken other closely allied species for *R. emetica.*

It is probable that our two commonest species *R. alutacea* and *R. integra* are perfectly edible, since there is nothing unpleasant in their flavor when raw, and no adverse reports against them.

The genus is a favorable one on which to experiment, for the worst that can be said against any of them is that they act as an emetic.

Special emphasis should be placed on the following features to be recorded in field notes from the study of fresh specimens of Russula.

General: All characteristics shall be given from fungi of different ages, and under different conditions of growth.

Pileus.
- Presence of cuticle.
- Color of flesh under cuticle.
- Taste and odor.
- Condition of margin, young and old (striate or even).
- Color of flesh when bruised.

Gills.
- Form.
- Thickness.
- Color, young and old.
- Surface, dusted or smooth.
- Intervenation.
- Equal, heterophyllous or branched. (Relative number.)

Stem.
- Shape.
- Color, young and old.
- Substance.
- Changes in color.

A classification according to color of the Russulas in the vicinity of Madison may be of service to the amateur collector.

Red or blood colored: *R. alutacea, R. emetica, R. roseipes, R. veternosa.*

Purple or livid: *R. atropurpurea, R. integra, R. decolorans, R. amoena, R. ochrophylla, R. ochrophylla* var. *albipes.*

Brown or ferrugineus: *R. foetens, R. pectinata, R. ochracea.*

Yellow: *R. lactea, R. lutea.*

Green: *R. virescens, R. olivascens, R. furcata.*

Black: *R. adusta, R. nigricans.*

White: *R. delica.*

R. adusta (Pers) Fr.

Pileus: convex, explanate or depressed, sometimes unequal; margin inflexed and even; color, fuliginous, smooth, viscid when wet; 6–12 cm.; flesh, white.

Gills: adnate or decurrent, not broad, narrow toward margin; white, changing to lead color, subcrowded, heterophyllous, forked occasionally.

Stem: stout, cylindrical, fleshy, 3–4 cm. long, 1.5–2 cm. thick, smooth, white, soon becoming blackish. July and August.

Spores: globose, slightly echinulate 6–9 u.

Characters on which identification is based.
- Pileus: fuliginous, solid, margin even. 6–9 cm.
- Gills: adnate, white to lead color.
- Stem: stout, short, white becoming blackish.
- Taste: mild.

Characters of dried specimens.
- Pileus: wrinkled, umbrinus to fuliginous, cracking at margin.
- Gills: lead color to blackish.
- Stem: longitudinally wrinkled, umbrinus or blackish.

Habitat. On ground under trees in grass.

Locality. Blue Mounds, Madison.

Edibility. When well cooked it has a good flavor. (McI.)

R. alutacea Fr.

Pileus: convex, explanate or depressed, smooth, pink beneath separable pellicle, rosy, bright red, or purple red, olivaceous, often yellow at center or yellowish spots; margin striate, thin; 4–12 cm.; flesh, white, slightly peppery, gills more so than pileus.

Gills: free, equal, broad (½–1½ cm.), occasionally forked (every 3–10th forked) and heterophyllous, white then ochraceous, rounded anteriorly, not powdered by spores. Sub crowded, interveined.

Stem: cylindrical or tapering downward, smooth, white, tinged rosy, brown where rubbed, fleshy, 6–10 cm. long, 1-2 cm., thick, spongy. August, September, October.

Spores: globose or ellipsoidal, ochraceous, echinulate. 7–7 u., 7–9 u., 9–12 u.

Characters on which identification is based.
- Pileus: rosy red, often yellow at center.
- Stem: stramineus, retaining shape, sometimes reddish.
- Gills: light ochraceous, retaining color when dried.
- Taste: more or less peppery.

Characters of dried specimens.
- Pileus: rosy or wine red, sometimes yellow at center. Thin, margin faintly striate.
- Gills: bright, ochraceous.
- Stem: usually not shrunken, straw colored, rarely brownish.

Habitat. Largest specimen from ground in hilly oak woods.
Locality. Star Lake, Blue Mounds, Madison (Eagle Heights).
Edibility. When fresh it is very good. (McI.)

R. amoena. Quel.

Pileus: convex then depressed; 4–6 cm.; smooth or pulverulent; atroviolaceus; margin not striate; taste mild, odor fruity; flesh white then cream.

Gills: white then cream; reddish at edge when dry; subcrowded, interveined.

Stem: rigid, tapering upward, 4–6 cm. long, 1–1½ cm. thick; powered, purple red. August.

Spores: subglobose, scarcely echinulate. 6x7 u.

Characters on which identification is based.
Pileus: atroviolaceus, powdered, margin even.
Gills: subcrowded, white then yellowish; edge tinged with red.
Stipe: larger toward top; purple red.

Characters of dried specimens.
Pileus: atroviolaceus; depressed at center, wrinkled.
Gills: adnate, ochery with reddish margin.
Stem: larger above, ochery with rosy tint.
Odor, pleasant.

Habitat. On ground in moist woods.
Locality. Blue Mounds.
Edibility. ?

R. atropurpurea Pk.

Pileus: convex, at first globose, finally slightly depressed, smooth; dark purple, livid, or deep purple red, disk often blackish; margin, even at first slightly striate when old. 6–12 cm.; flesh, white, blackish where broken; odor foetid when drying. Taste, mild or slightly peppery.

Gills: free, subdistant, rounded anteriorly, interveined, rarely forked, white to cream color, sometimes with rosy tint.

Stem: smooth, white, tapering upward slightly, 8–10 cm. long, 2 cm. thick, brown where rubbed, fleshy, white, sometimes rosy. July, Aug., Sept., Oct.

Spores: globose or ellipsoidal, stramineus or cream, echinulate, 8x12μ, 7x9μ, 7x7μ.

Characters on which identification is based.
Pileus: dark purple color. Flesh, grayish under pellicle. Odor of drying plant foetid.
Gills and stem brown where bruised.
Stem: sometimes rosy.
Spores with slight rosy tint. 8x12μ.

Characters of dried specimens.
Pileus: dark blackish purple, usually darker in center. Margin even or striate.
Gills: isabellinus, or ochraleucus.
Stem: stramineus, shrinking but little, covered with brown spots.

Habitat. Rich oak woods on ground.

Locality. Madison, Burlington, Blue Mounds.

Edibility. Should be perfectly fresh to have good flavor. (McI.)

R. decolorans Fr.

Pileus: subglobate then convex, smooth, slightly viscid when moist, livid, brownish red, lighter in center. Symmetrical, faintly striate and tuberculate at margin. 6–8 cm.; flesh white. Taste, slowly peppery.

Gills: almost free, subcrowded, equal, rounded in front, interveined, white then yellowish.

Stem: 6 cm. long, 1.5 cm. thick, cylindrical, solid, minutely striate, white then darker. July.

Spores: Globose, ellipsoidal, echinulate. 8x10μ, 9x11μ.

Characters on which identification is made.
Pileus: firm, yellowish red, pale at center.
Gills: subfree, white then yellowish.
Stem: cylindrical, white with darker spots.

Characters of dried specimens.
Pileus: badius at margin, center ferrugineus, retaining its shape well.
Gills: little changed in form, ochraceous.
Stem: stramineus, cylindrical.

Habitat. On ground in oak woods.

Locality. Madison.

Edibility. Esculent and of good quality. (Morgan).

R. delica.

Pileus: infundibuliform, smooth then pruinose, rough, scaly and cracked, white or tan. Margin incurved, entire. 7–12 cm.; flesh, white changing to yellowish, thin, dry, slightly and slowly peppery.

Gills: decurrent, distant, thin, narrow, heterophyllous, interveined, occasionally forked, white or cream.

Stem: stout, cylindrical, smooth and solid, or pruinose. White, 2.5–6 cm. long, 2-2.5 cm. thick. July, August, in woods.

Spores: echinulate, subglobose, 8x9μ, 10x12μ, 7x8μ.

Characters on which identification is made.
Pileus: white, infundibuliform, roughened.
Margin: entire, incurved.
Stem: stout, short.
Gills: decurrent, narrow.
Taste: slightly peppery.

Characters of dried specimens.
Pileus: stramineus or brownish, wrinkled, indifundibuliform. Margin, incurved.
Gills: yellowish or isabellinus.
Stem: shrunken, longitudinally wrinkled.

Habitat. On ground in woods.
Locality. Lake Waubesa, Blue Mounds, Madison.
Edibility. Edible and of fair quality. (McI.)

R. emetica Fr.

Pileus: convex or explanate. Pink or rosy, tawny when old, sometimes white, smooth, margin striate. 4–10 cm. Flesh, reddish under separable pellicle. Taste very peppery.

Gills: almost free, not crowded, heterophyllous, interveined, triangular, white.

Stem: 4–8 cm. long, 1–2 cm. thick, smooth, white or rosy, spongy. Aug., Sept., Oct.

Spores: 8x9μ, ellipsoidal, echinulate white.

Characters on which identification is made.
Pileus: tawny or white color, when old.
Stem: reddish or white.
Gills: always white.
Taste: peppery.

Characters of dried specimens.	Pileus: red or white or tawny. Margin striate and cracked.
	Gills: cream color, wrinkled.
	Stem: stramineus or rosy, longitudinally striate.

Habitat. On ground in hilly woods.

Locality. Madison, Star Lake, Florence.

Edibility. Claimed by some to be poisonous; by others to be perfectly harmless.

R. foetens Fr.

Pileus: globose at first then flat to concave or depressed at center, sometimes gibbous, viscid when moist. Color, young, whitish to pale brown; older, badius or vinosus. Membranous pellicle, elastic and tough, marked with radiating brown lines. Margin thin, striate or pectinate. Size 5–10 cm. Taste mild, nauseous or acrid; odor foetid. Becoming brown when broken.

Gills: adnexed or sinuate, broad, not crowded, interveined, rounded anteriorly, forked near stem, few heterophyllous; white, spotted brownish when old.

Stem: 4–12 cm. firm, tapering upward, smooth, white to grayish, reddish spots and lines. 1.5–2 cm. thick. June, July, Aug.

Spores: white, ellipsoidal, echinulate. 8x10μ.

Characters on which identification is made.	Pileus: brown, depressed with shining viscid pellicle.
	Margin: thin striate.
	Stem: often brown spotted.
	Gills: often brown spotted.
	Odor: foetid, rank.

Characters of dried specimens.	Pileus: reddish brown, much folded, wrinkled and striate.
	Gills: yellowish or salmon.
	Stem: depressed in spots, yellowish, brown spotted.
	Odor, mealy.

Habitat. Moist ground in oak woods.

Locality. Madison, Blue Mounds, Minneapolis, Minn.

Edibility. Not poisonous, but unpleasant in flavor. (McI.)

R. furcata Fr.

Pileus: explanate to depressed. Olivaceus to sordid, smooth or slightly roughened. Margin, incurved, even. 6–8 cm. Flesh white, pinkish under pellicle. Taste mild.

Gills: many, adnate to decurrent, strongly interveined and forked; white.

Stem: 6–8 cm. 1–1½ cm. thick, cylindrical, slightly longitudinally wrinkled, white.

Spores: ellipsoidal, echinulate, 8x10μ.

Characters on which identification is made.
- Pileus: dark-greenish color.
- Stem: cylindical, white.
- Gills: adnate, forked, white.
- Taste: mild.

Characters of dried specimens.
- Pileus: margin even, shining.
- Gills: thick, fumosus.
- Stem: white, wrinkled longitudinally.

Habitat. Ground in woods.
Locality. Parfrey's Glen.
Edibility. Edible (McI).

R. integra Fr.

Pileus: convex, explanate or slightly depressed; smooth, slightly viscid when moist. Purple, livid, brownish, tawny, greenish, darker or lighter at disk. Margin thin, even at first, then pectinate or cracked when old. 6–8 cm. broad; flesh white, dark where broken. Taste, mild.

Gills: free or adnexed, not crowded, 1½ cm. broad, interveined, few forked, heterophyllous, (few short) rounded anteriorly and posteriorly; straw to yellowish. Pulverulent, when mature.

Stem: 6–13 cm.; stout, cylindrical or large above and below, 2½–3 cm. thick; smooth or slightly ridged; white, brown where rubbed, spongy. July, August, September.

Spores: ellipsoidal, globose, echinulate, ochraceous. 7x9μ, 7x7μ, 8x10μ.

Characters on which identification is made.
Pileus: varicolored, dark purple prevailing.
Stem: never reddish. Becoming brown where rubbed.
Gills: powdered when mature.
Taste: never peppery.

Characters of dried specimens.
Pileus: usually showing different colors at margin and disk, dark purple, livid, greenish, yellowish or wine; cracking at margin.
Gills: isabellinus, wrinkled and folded.
Stem: plump or shrunken; straw colored with brownish spots.

Habitat. Largest specimens grow on ground in hilly oak woods.
Locality. Madison (Eagle Heights), Devils Lake, Burlington, Blue Mounds.
Edibility. Of good flavor when fresh. (McI.)

R. lactea Fr.

Pileus: convex, explanate, gibbous or depressed, rigid, surface pruinose then cracked, no separable pellicle; color, stramineus, yellow or pinkish yellow. Margin, even, rounded. 10–19 cm.; flesh, white, bitter; cheesy odor.
Gills: free, equal or slightly forked, broad, solid, subdistant, interveined, rounded anteriorly, white to straw.
Stem: cylindrical or larger at top; solid, white, 2.5–6 cm. long; 1–2 cm. thick. August.
Spores: globose, minutely echinulate, 9μ.

Characters on which identification is based.
Pileus: rigid, yellowish, incurved.
Stem: solid.
Gills: thick, straw color.
Taste: somewhat bitter.

Characters of dried specimens.
Pileus, yellowish or brownish, retaining shape. Margin, incurved.
Gills: thick, narrow near stem, rounded anteriorly.
Stem: longitudinally wrinkled, smaller at base.

Habitat. On ground, not common, in oak woods.
Locality. Blue Mounds, Madison.
Edibility. Edible and of good flavor. (McI.)

R. lutea Fr.

Pileus: conical, convex or depressed, smooth, viscid when moist, pale or bright yellow, surface sometimes puberulent or floccose. Margin even or later striate. 3–8 cm.; flesh white; taste mild.

Gills: adnate to free, interveined, crowded; rounded anteriorly; equal, white then yellowish.

Stem: tapers up, smooth, white, fleshy; 3–5 cm. long; 5–8 cm. thick. July, August, September.

Spores: cream, ellipsoidal, echinulate. 8x10μ.

Characters on which identification is based.
Pileus: pale or bright yellow, small size.
Stem: short.
Gills: crowded, yellowish.
Taste: mild.

Characters of dried specimens.
Pileus: retaining yellow color.
Gills: yellowish or salmon.
Stem: retaining its shape, stramineus.

Habitat. On ground in woods.

Locality. Lake Waubesa, Blue Mounds, Madison.

Edibility. Edible and of delicate flavor. (McI.)

(a) *R. ochrophylla* Pk. *var. albipes* Pk.

Pileus: convex then flattened and depressed, slightly viscid when moist, smooth at first, then broken up into patches; margin at first incurved not striate, blackish or brownish olive. 7–12 cm.; flesh white, reddish when bruised.

Gills: adnexed, rounded near stem, heterophyllous, thick, subdistant, white, reddish where broken.

Stem: 4–7 cm. long; 2½–3 cm. thick; cylindrical, dirty white, then blackish, solid. July.

Spores: 10x11μ, 11x13μ, ellipsoidal, coarsely echinulate.

Characters on which identification is made.
Pileus: fleshy, blackish, margin even.
Gills: thick, subdistant, white, red where bruised.
Stem: thick, cylindrical, blackish.

Characters of dried specimens.
Pileus: umber or blackish, margin wavy. Incurved.
Gills: dark, reddish where bruised.
Stem: umber or blackish.

Habitat. On ground in woods.
Locality. Madison.
Edibility. Edible, but not equal to most Russulas. (McI.)

R. ochracea Fr.

Pileus: flat to convex, fulvus; darker at center. Margin, irregular, thin, cracked, striate incurved; surface, rough, scaly. Size, 4–9 cm. Taste, mild; odor, none.

Gills: free, subcrowded, broad, straight, equal, interveined, white.

Stem: 2–2.5 cm. long, 1–2 cm. thick, tapering upwards or ventricose, surface, smooth, stramineus, hollow. July.

Spores: 6x6μ, 6x7.5μ, echinulate, elliptical or globose.

Characters on which identification is made.
Pileus: fulvus, dark at center, striate, thin.
Gills: free, subcrowded, white.
Stem: short, thick.
No odor or taste.

Characters of dried specimens.
Pileus: fulvus, roughened and dirty, striate.
Gills: wrinkled and wavy, isabellinus.
Stem: short and hollow.
Odor: mealy.

Habitat. Hilly woods on ground.
Locality. Madison (Edgewood).
Edibility. Probably edible.

R. ochrophylla Pk.

Pileus: convex, explanate or depressed; margin, even or slightly striate, 6–10 cm.; atropurpureus, reddish under the separable pellicle. Flesh, white. Taste, mild.

Gills: equal, broad, adnate, subdistant interveined, yellowish then bright ochraceous; dusted by spores.

Stem: Subcylindrical, smooth, whitish with rosy tint; firm or spongy. 5–7 cm. long, 1–2 cm. thick. July, August.

Spores: globose, ellipsoidal, echinulate, 8x10μ, 9x12μ.

Characters on which identification is based.
- Pileus: dark purple color; reddish beneath pellicle.
- Gills: broad, equal, dusted bright ochraceous.
- Stem: rosy.

Characters of dried specimens.
- Pileus: dark purple or livid, smooth; margin slightly striate.
- Gills: bright ochraceous.
- Stem: longitudinally wrinkled, rosy.

Habitat. Open woods on ground.

Locality. Madison, Blue Mounds.

Edibility. Rather tough, but not disagreeable to the taste. Milk in which it is stewed takes on pink color.

(a) R. ochrophylla Pk. *var. albipes* Pk.

Pileus: deep red; stem white; otherwise like the type. A number of specimens answering to this description were found at Blue Mounds in August, 1903.

R. olivascens Fr.

Pileus: explanate or concave, smooth slightly viscid when moist, olivaceous; margin, even; 5–10 cm.; flesh, white. Taste, mild.

Gills: adnexed or adnate, subcrowded, broad at margin, narrow toward stem; interveined, few forked, white.

Stem: 4–7 cm. long, cylindrical or tapering upward slightly, 2 cm. thick, spongy, white, smooth. July, August.

Spores: scarcely echinulate, 8x10μ, 8x9μ, ellipsoidal.

Characters on which identification is based.
- Pileus: smooth, shining, olivaceous.
- Margin: even, taste mild.
- Gills: broad at margin, white.
- Stem: white, smooth.

Characters of dried specimens.
- Pileus: smooth and shining, darker at center, dark olive green or bronze green. Margin entire.
- Gills: stramineus.
- Stem: longitudinally wrinkled, whitish.

Habitat. Rich oak woods on ground.
Locality. Blue Mounds.
Edibility. Taste mild.

R. pectinata Fr.

Pileus: convex, then flat or concave; smooth, ochraleucus to ferrugineus, darker at center; margin, thin, striate tuberculate, at first inflexed. 6–8 cm.; taste, slightly pungent, smell, soapy. No changes.

Gills: free, broad and rounded anteriorly, subcrowded, equal, white.

Stem: 3–6 cm. long, 1½–2½ cm. thick, rigid, equal or ventricose; smooth or pulverulent, white; spongy when old. July and August.

Spores: ellipsoidal or globose, 7x7μ, 7x9μ.

Characters on which identification is made.
- Pileus: ferrugineus, thin, pectinate.
- Stem: slender, white.
- Gills: rounded anteriorly.
- Odor not unpleasant.

Characters of dried specimens.
- Pileus: latericius or yellowish, darker in center; very thin, striate almost to center.
- Gills: yellowish white.
- Stem: stramineus, slightly roughened.

Habitat. On ground in grass under oaks along road.
Locality. Madison, Blue Mounds.
Edibility. Unpleasant in flavor. (McI.)

R. roseipes (Secr.) Bres.

Pileus: convex, plane or depressed, somewhat farinaceous; rosy red, ocher and whitish. Margin, thin, at first incurved to stem; striate at edge. Cracking at edge and divided into areas when old. 3–6 cm. broad. Flesh, white, pinkish under pellicle. Taste mild.

Gills: free or slightly adnexed, subcrowded, rounded posteriorly, stramineus to light yellow.

Stem: 4–6 cm. long, 1½–2 cm. thick, terete, tapering upward, smooth; white, sometimes rosy, spongy.

Spores: globose or ellipsoidal 6x6μ, 7x9μ, light ocher yellow, echinulate.

Characters on which identification is based.
Pileus: rosy and ocher.
Stem: reddish.
Gills: ochery.
Taste: mild.

Characters of dried specimens.
Pileus: brittle and cracked at margin, retaining red color as when fresh.
Gills: ocher, close and wrinkled.
Stem: retaining its shape well; rosy; color persistent.

Habitat. Open mixed woods on ground.

Locality. Madison (Picnic Point).

Edibility. Agreeable in flavor. (Peck.)

R. virescens Fr.

Pileus: convex, flat or depressed; thick at disk, dry, breaking up into darker areas; green or grayish. Margin, striate or even. 5–15 cm.; flesh, white. Taste, mild.

Gills: free, appearing adnate by expansion of pileus, broad and thick, subcrowded, interveined, few forked near stem, white to cream.

Stem: 4–7 cm. long; stout, fleshy, cylindrical or small at base. 1½–3 cm. thick, smooth, white. July and August.

Spores: sparingly echinulate, subglobose, 6x6μ, 6x8μ.

Characters on which identification is made.
Pileus: dry, without pellicle, broken up greenish.
Stem: stout, smooth, fleshy.
Gills: broad, subcrowded.
Taste, mild.

Characters of dried specimens.
Pileus: yellowish tawny with greenish areas.
Gills: regular, changing form but little in drying, stramineus.
Stem: whitish, wrinkled.

Habitat. Rich oak woods on ground.
Locality. Madison, Blue Mounds.
Edibility. Good flavor. Can be eaten raw. (McI.)

R. veternosa Fr.

Pileus: convex then explanate; smooth rosy, with paler yellowish disk. Margin even, 7–8 cm.; flesh, white.

Gills: adnate, subcrowded, broad, heterophyllous, few forked, cream colored.

Stem: tapering upward, 4 cm. long; white; 2 cm. at base; soft, fleshy, then hollow; smooth. June.

Spores: globose, ellipsoidal, echinulate 8x10μ, white.

Characters on which identification is made.
Pileus: rosy or pink, center yellowish.
Gills: subcrowded; few, forked.
Stem: stout, white, becoming hollow.
Taste: stem and pileus, very peppery.

Characters of dried specimens.
Pileus: rosy, paler in center, changing but little
Gills: ochery.
Stem: stout, fleshy, becoming hollow.

Habitat. Ground in woods.
Locality. Cottage City, Minn.
Edibility. ?

R. ————

Pileus: flat to depressed; umber at margin, darker at disk. Margin coarsely striate, thin; smooth or with few yellowish fibrils. 5-12 cm. Taste, mild; odor none.

Gills: free or slightly adnexed, not crowded, equal, thin, interveined, white.

Stem: 4-5 cm. long, 1-2 cm. thick, mealy, cylindrical, white to ocher; fleshy then hollow. July.

Spores: globose, spinulose, 9x9μ.

Characters of dried specimens.
- Gills: wrinkled, thin, drab and leather colored.
- Stem: rough, ocher to brown.
- Pileus: umber, dark at disk, striate, thin.

Habitat. Ground on lawns.

Locality. Madison, Burlington.

Edibility. ?

This Russula is close in its affinities to *R. consobrina var. sororia* Fr. but has a mild taste. It is close also to *R. ochracea* Fr. but has not the ocher gills.

THE RELATIONS OF THE ANDRENINE BEES TO THE ENTOMOPHILOUS FLORA OF MILWAUKEE COUNTY

S. GRAENICHER.

Owing to their large number of species, as also to the close relations of many of the species to certain flowers, the bees of the family *Andreninae* occupy a prominent position among the flower-visiting insects of our neighborhood. This paper deals with 47 species, representing about one-fifth of the entire bee-fauna of our region. Several species make their appearance quite early in the spring, and from this time on the family is represented throughout the floral season, although not a single one of the species extends its time of flight over two months and a half. Some are of rather common occurrence, while others are extremely rare, and are only occasionally met with. For several years past I have given much attention to the bees of this family, and have gradually come across 17 new species of *Andrena* from Milwaukee county, which have been named and described as follows:

Andrena subcommoda	T. D. A. Cockerell, Canadian Entomologist, 1902, p. 45.
Andrena sigmundi	
Andrena multiplicata	
Andrena radiatula	
Andrena rufosignata	
Andrena clypeonitens	

Andrena graenicheri	T. D. A. Cockerell, Annals and Magazine of Natural History, 1902, p. 101.
Andrena parnassiae	
Andrena peckhami	

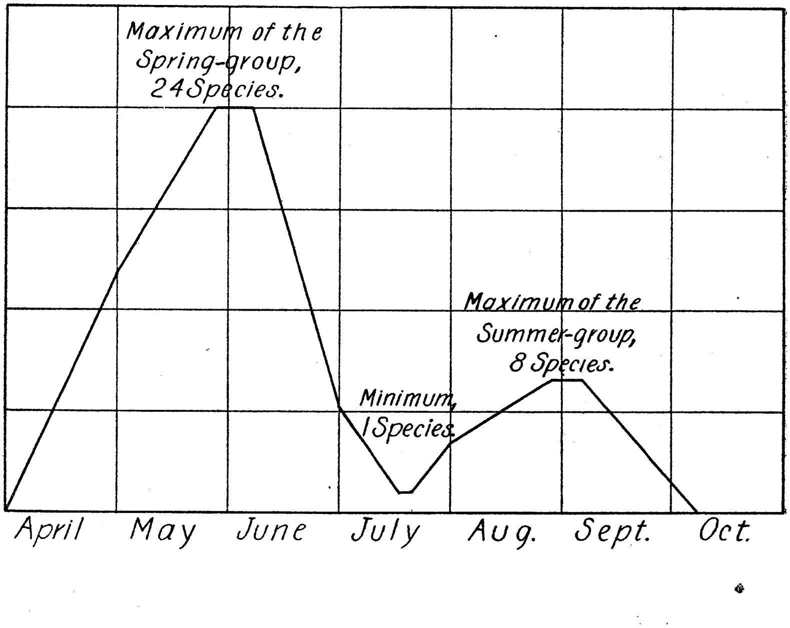
Andrena thaspii	S. Graenicher, Canadian Entomologist, 1903, p. 162.
Andrena cockerelli	
Andrena milwaukeensis	
Andrena viburnella	
Andrena albofoveata	
Andrena fragariana	S. Graenicher, Entomological News, 1904, p. 64.
Andrena wheeleri	
Andrena persimilis	

Polytropic and oligotropic bees. Some bees are in the habit of visiting a great variety of flowers, while others may be observed on the flowers of a single or those of a few species only. *Loew*[1] called the former polytropic and the latter oligotropic. *Robertson*[2] states that "in the economy of the host-bees (those not inquiline) the most important flowers are those from which the female gets the pollen upon which her brood is fed." Accordingly he proposes to use the term oligotropic for a species of bee, of which the female obtains her pollen-supply from a single species, or several closely related species of plants, i. e., plants belonging to the same genus, or the same natural family, and on the other hand to call those bees polytropic that collect pollen from plants of different families. In considering the relations of our *Andrena* species to our flowers, I make use of the terms oligotropic and polytropic in the same sense as *Robertson.* In the following table the time of flight, or in the case of insufficient observation the date of capture of each species is given, as also the names of the plants visited for pollen. When the bee has been recognized as oligotropic this is stated, otherwise the species is considered polytropic. Following *Robertson's* example, I consider in the case of an oligotropic bee the latter adapted to the genus if found collecting pollen from more than one species of that genus, and to the family if it obtains pollen from plants belonging to different genera of that family.

Name of Species.	Time of Flight.	Flowers Visited for Pollen.
1. *Andrena cockerelli*, Graen ..	Mar. 31—May 4	*Oligotropic*, *Salix*.
2. *Andrena illinoensis*, Rob ...	Apr. 6—May 7	*Oligotropic*, *Salix*.
3. *Andrena radiatula*, Ckll....	Apr. 8—June 16	Species of *Salix* and *Thaspium*.
4. *Andrena bipunctata*, Cress..	Apr. 8—June 17	Species of *Salix*, *Prunus*, *Claytonia*, *Cornus*, *Crataegus*, *Viburnum*, *Angelica*, *Symphoricarpos*, etc.
5. *Andrena mariae*, Rob	Apr. 9—June 6	*Oligotropic*, *Salix*.
6. *Andrena erythrogastra*, Ashm.	Apr. 17—June 6	*Oligotropic*, *Salix*.
7. *Andrena carlini*, Ckll	Apr. 18—June 10	Species of *Salix*, *Claytonia* and *Crataegus*.
8. *Andrena vicina*, Sm	Apr. 20—June 15	Species of *Salix*, *Sanguinaria*, *Erythronium*, *Caltha*, *Ribes*, *Rosa*, etc.
9. *Andrena hippotes*, Rob......	Apr. 25—June 26	Species of *Salix*, *Prunus*, *Viburnum*, *Angelica*. *Spiraea*, *Celastrus*, etc.
10. *Andrena cressonii*, Rob	Apr. 28—June 28	Species of *Acer*, *Salix*, *Claytonia*, *Cornus*, *Vagnera*, *Hydrophyllum*, *Geranium*, etc.
11. *Andrena dubia*, Rob	Apr. 28—May 21	This species is known in the male sex only.
Andrena erigeniae, Rob.....	Apr. 28—May 7	*Oligotropic*, *Claytonia Virginica*.
13. *Andrena dunningi*, Ckll	Apr. 30—May 12	Species of *Caltha* and *Lonicera*.
14. *Andrena macgillivrayi*, Ckll.	May 2—May 7	Species of *Salix*.
15. *Andrena milwaukeensis*, Graen.	May 4—June 26	Species of *Claytonia*, *Ribes*, *Streptopus*, *Symphoricarpos*, *Angelica*, *Spiraea*, *Celastrus*, *Diervilla*, etc.
16. *Parandrena andrenoides*, Cress.	May 8—May 28	*Oligotropic*, *Salix*.
17. *Andrena rugosa*, Rob........	May 9—June 12	Species of *Salix*, *Taraxacum* and *Rubus*.
18. *Andrena hartfordensis*, Ckll.	May 9—June 2	Species of *Taraxacum*, *Vagnera*, *Thaspium*, *Angelica*, *Viburnum*, *Symphoricarpos*, etc.
19. *Andrena nivalis*, Sm	May 9—June 27	Species of *Ribes*, *Vagnera*, *Thaspium*, *Heracleum*, *Cornus*, *Rubus*, *Geranium*, etc.
20. *Andrena forbesii*. Rob.......	May 9—June 26	Species of *Salix*, *Taraxacum*, *Viburnum*, *Geranium*, *Crataegus*, *Cornus*, *Thaspium*, *Sanicula*, etc.
21. *Andrena claytoniœ*, Rob.....	May 10—May 14	*Claytonia Virginica*.
22. *Andrena fragariana*, Graen.	May 15—June 15	*Oligotropic*, *Fragaria Virginiana*.
23. *Andrena platyparia*, Rob ..	May 16—July 4	Species of *Salix*, *Thaspium*, *Angelica*, *Cornus*, *Viburnum*, *Rhus*, etc.
24. *Andrena ziziae*, Rob	May 18—June 26	*Oligotropic*, *Umbelliferae*.
25. *Andrena geranii*, Rob......	May 18—July 1	*Oligotropic*, *Hydrophyllum*.
26. *Andrena rufosignata*, Ckll..	May 19	*Uvularia grandiflora*.
27. *Andrena geranii maculati*, Rob.	May 20—June 25	*Oligotropic*, *Geranium maculatum*.
28. *Andrena sigmundi*, Ckll....	May 26—June 6	Species of *Vagnera and Cratœgus*.
29. *Andrena subcommoda*, Ckll	May 27—July 3	Species of *Thaspium*, *Viburnum*, *Cratœgus*, *Symphoricarpos*, *Rosa*, *Spiraea*, *Rhus*, etc.
30. *Andrena corni*, Rob	May 27—July 9	Species of *Viburnum*, *Sanicula*, *Angelica*, *Cornus*, *Rubus*, *Rhus*, *Rosa*, etc.
31. *Andrena multiplicata*, Ckll..	May 29—July 30	Species of *Thaspium*, *Cornus*, *Spiraea*, *Symphoricarpos*, *Rhus*, *Ceanothus*, *Veronica*, etc.
32. *Andrena thaspii*, Graen.....	May 29—June 23	*Oligoitropic*, *Umbelliferœ*.

Name of Species.	Time of Flight.	Flowers Visited for Pollen.
33. *Andrena viburnella*, Graen.	May 29—June 20	Species of *Viburnum and Rubus*.
34. *Andrena wheeleri*, Graen .	June 8—June 18	*Oligotropic*, *Umbelliferæ*.
35. *Andrena albofoveata*, Graen.	June 9—June 16	*Oligotropic*, *Umbelliferæ*.
36. *Andrena robertsonii*, D. T...	June 26—July 16	Species of *Krigia*, *Ceanothus and Rhus*.
37. *Andrena peckhami*, Ckll....	July 21—Aug. 8	*Oligotropic*, *Compositæ*.
38. *Andrena clypeonitens*, Ckll.	July 26—Aug. 20	*Oligotropic*, *Compositæ*.
39. *Andrena nubecula*, Sm......	July 26—Sept. 20	*Oligotropic*, *Compositæ*.
40. *Andrena aliciæ*, Rob	July 27—Sept. 4	*Oligotropic*, *Compositæ*.
41 *Andrena helianthi*, Rob	Aug. 8—Sept. 12	*Oligotropic*, *Compositæ*.
42. *Andrena amerlcana*, D. T...	Aug. 17—Sept. 27	*Oligotropie. Compositæ*.
43. *Andrena solidaginis*, Rob...	Aug. 22	*Oligotropic*, *Compositæ*.
44. *Andrena asteris*, Rob.......	Aug. 24—Oct. 8	*Oligotropic*, *Compositæ*.
45. *Andrena persimtlis*, Graen..	Aug. 24—Sept. 12	*Oligotropic*, *Compositæ*.
46. *Andrena parnassiæ*, Ckll...	Aug. 25—Sept. 26	*Oligotropic*, *Parnassia Caroliniana*.
47. *Andrena graenicheri*, Ckll.	Aug. 28—Sept. 23	*Oligotropic*, *Compositæ*.

The data relating to the time of flight, as contained in this table, enable us to construct a flight-curve for the family *Andrcninae* as follows:



With us the blossoming of the first catkins of our earliest species of willow, *Salix discolor* falls together with the appearance of our earliest species of *Andrena, A. cockerelli,* an oligotropic visitor of the willows. In a certain locality in the Menomonee valley numerous specimens of *Salix discolor* occur, and among these a large specimen, bearing pistillate catkins opens its blossoms regularly in advance of all the other specimens. This particular plant was kept under observation in the early days of spring for 2 successive seasons, with the result, that on the first warm and bright day bringing out its blossoms the presence of the bee *Andrena cockerelli* in both sexes was noticed. This was the case on April 6th, 1902, and again on March 31st in the exceptionally early spring of 1903. I have never succeeded in coming across a species of *Andrena* before our willow-blossoms appear, although two species of entomophilous plants, *Erigenia bulbosa* and *Hepatica acuta* open up their flowers earlier than this willow. As the willow-blossoms become more abundant additional species of *Andrena* arrive on the scene, so that at the end of the third week in April 8 species are on the wing, 4 of which are oligotropic, depending for pollen on the willows exclusively, although they also visit other flowers for nectar. These facts point to the importance of the willows in the economy of our first arrivals among the Andrenine bees. As the season advances several other flowers attractive to insects open up, among them being *Claytonia Virginica* which usually appears towards the end of April, and also has an oligotropic visitor *Andrena erigeniae.* At the beginning of May 13 species of *Andrena* are present, and this number is gradually increased during the month until a maximum with 24 species is reached in the latter part of May, and lasting throughout the first week in June. This is the maximum of our spring-group of *Andreninae,* and it corresponds with the blooming period of a great variety of flowers, representing different families. Seven of these bees are oligotropic, two of which collect pollen from the late species of willows, one from *Fragaria Virginiana,* one from *Hydrophyllum Virginicum,* one from *Geranium maculatum,* and the two remaining ones from umbelliferous plants. Two species of Umbelliferae *Thaspium trifoliatum aureum* and *Taenidia in-*

tegerrima produce flowers in great abundance, and are very attractive to many species of *Andrena* besides the two oligotropic species of the family flying during the maximum. In addition to the plants mentioned in connection with the oligotropic visitors various species of *Viburnum, Crataegus, Cornus, Ribes, Rubus,* etc., supply many of the species forming the spring-maximum with pollen and nectar. From this maximum on there is a gradual decline of the curve until a minimum is reached, extending from about July 17 to July 20, and represented by a single species. At the end of the third week in July a renewed increase sets in, culminating in a maximum of the summer-group of *Andreninae,* with 8 species in evidence at the end of August and the beginning of September. This summer-group comprises altogether 11 species, and these are with but one exception oligotropic bees of the family *Compositae.* The plants of this family with the numerous species of *Solidago, Aster, Helianthus, Rudbeckia, Eupatorium* and many other genera are dominant factors in the make-up of the flora of the late summer months. The earliest species of goldenrod begins its blooming period about the middle of July, around the 20th of the month the first aster appears, and mostly a trifle later the first sunflower. Corresponding with the appearance of these composite flowers the earliest *Andrena* of the summer-group *A. peckhami* begins to fly about July 21, and before the end of the month 3 additional oligotropic visitors are present on these flowers. From the first week in September on there is a falling off in the number of these bees, and around October 8th *Andrena asteris,* the last one of the *Andreninae* disappears. Although any one of these visitors of the *Compositae* may collect pollen from flowers belonging to different genera of the family, they still show a decided preference for certain genera. *A. peckhami, A. clypeonitens, A. aliciae and A. helianthi* favor the sunflowers, *A. nubecula, A. americana, A. solidaginis* and *A. persimilis* the goldenrods, *A. asteris* and *A. graenicheri* the asters.

The exceptional position held by *Andrena parnassiae,* the oligotropic visitor of *Parnassia Caroliniana* has been referred to above. This is so closely related to *A. peckhami,* and the two resemble each other to such an extent as to leave no doubt regarding their origin from a common ancestor. But while *A. peckhami,* a bee adapted to the *Compositae* appears together with the early flowers of *Helianthus, A. parnassiae* is adapted to a plant belonging to quite a different family, and it flies considerably later, from August 25th to September 26th, during the blooming period of *Parnassia.* The first specimens of this bee were taken in a certain locality south of Whitefish Bay on the bluffs bordering Lake Michigan, where the plant *Parnassia Caroliniana* occurs in large patches with an abundance of flowers. The latter are especially attractive to flies, 17 of the 25 recorded visitors belonging to this order, but the bee *Andrena parnassiae* may be observed regularly, season for season, although not a frequent insect. It has up to the present time not been met with at any other point within our County, nor has it been reported from elsewhere.

Oligotropic species of Andrena. In the foregoing several of our oligotropic species have been mentioned in connection with the flowers visited. For the sake of completeness a list of all of our oligotropic *Andreninae* is offered below.

Robertson[2] has published a list of the bees of Carlinville, Southern Illinois regarded by him as oligotropic, and 13 of the 20 species of oligotropic *Andreninae* of that locality occur also in our region. They are as follows:

Name of species.	*Plants visited for pollen.*
Andrena illinoensis, Rob.	*Salix.*
Andrena mariae, Rob.	*Salix.*
Andrena erythrogastra, Ashm.	*Salix.*
Parandrena andrenoides, Cress.	*Salix.*
Andrena erigeniae, Rob.	*Claytonia Virginica.*
Andrena ziziae, Rob.	*Umbelliferae.*
Andrena geranii, Rob.	*Hydrophyllum.*
Andrena geranii maculati, Rob.	*Geranium maculatum.*
Andrena aliciae, Rob.	*Compositae.*
Andrena nubecula, Sm.	*Compositae.*
Andrena helianthi, Rob.	*Compositae.*
Andrena solidaginis, Rob.	*Compositae.*
Andrena asteris, Rob.	*Compositae.*

To these I add 11 species recognized as oligotropic bees of our surroundings:

Name of species.	*Plants visited for pollen.*
Andrena cockerelli, Graen.	*Salix.*
Andrena fragariana, Graen.	*Fragaria Virginiana.*
Andrena thaspii, Graen.	*Umbelliferae.*
Andrena wheeleri, Graen.	*Umbelliferae.*
Andrena albofoveata, Graen.	*Umbelliferae.*
Andrena peckhami, Ckll.	*Compositae.*
Andrena clypeonitens, Ckll.	*Compositae.*
Andrena americana, D. T.	*Compositae.*
Andrena persimilis, Graen.	*Compositae.*
Andrena graenicheri, Ckll.	*Compositae.*
Andrena parnassiae, Ckll.	*Parnassia Caroliniana.*

According to this list 24 of the 47 species of *Andreninae* considered in this paper, or fully one-half are oligotropic. The *Compositae* supply 10 of these with pollen, and the willows come next with 5 oligotropic bees. The importance of the *Umbelliferae* in this respect is also evident, 4 such visitors being adapted to them. As regards *Andrena geranii* this bee figures in Robertson's list as an oligotropic species of *Hydrophyllum appendiculatum,* but in our surroundings it collects pollen from *Hydrophyllum Virginicum,* the only representative of that genus in our flora, and it therefore has to be considered an oligotropic bee of the genus *Hydrophyllum.*

Seasonal forms. There are numerous instances recorded of an insect-species appearing at one period of the season in a form differing more or less from the form assumed at another period. These are called seasonal forms. A few cases are mentioned in the literature, all of them from the Eastern states, in which a species of *Andrena* taken later in the season has been regarded as identical with some species flying in the spring. Observations carried on throughout a number of years warrant the statement, that in our region no seasonal forms of *Andrena* occur. Species after species makes its appearance in the order indicated in the list at the beginning of this paper. As regards their time of flight there is a great diversity among the different species, some of them flying over 2 months, while others are present during a few weeks only. In each species one generation is produced annually, and the bee appears the following season at the time of flight of the respective species.

It has been pointed out that all of our species of the summer-group are oligotropic, and all but one adapted to the *Compositae*, and in this respect they differ essentially from the species of the spring-group. In connection herewith it may be emphasized that while the early *Umbelliferae*, represented especially by the genera *Thaspium, Taenidia, Heracleum, Angelica* and *Sanicula* are very attractive to members of the spring-group of *Andreninae*, the late *Umbelliferae* with the genera *Cicuta, Sium, Oxypolis and Conioselinum* have no relations whatever to the *Andreninae* of the summer-group. *Cornus stolonifera* has its flowering season in the spring, but some specimens produce flowers throughout the summer and as late as the middle of September. It is significant that the flowers of this species are visited very abundantly by many *Andreninae* of the spring-group, but that after the middle of July a single species has been noticed on the flowers, and this is *A. multiplicata,* the latest species of the spring-group, which holds out until the end of July. All of these considerations point to the fact that our *Andreninae* of the summer-group, so far as their relations to flowers are concerned have nothing in common with those of the spring-group, and they furthermore support the statement, that in our region at least, no seasonal forms of *Andreninae* are produced.

January 12, 1905.

References.

1. *E. Loew.* Blumenbesuch von Insekten an Freilandpflanzen, Jahrbuch des botanischen Gartens zu Berlin, III, 1884.

2. *Chas. Robertson.* Flowers and insects, XIX, Botanical Gazette, XXVIII, p. 27.

OBSERVATIONS ON THE WINTERING OF GRAIN RUSTS.

A. H. CHRISTMAN

The manner in which grain rusts pass the winter in northern climates and in regions where the barberry is wanting, is still considered an open question. Eriksson and Henning (1), while they record the germination of uredospores collected during the winter and early spring, conclude that these spores and the mycelia producing them play no important part in perpetuating the fungus. As is well known, they hold that rust may be transmitted as a nonhyphal, so called, mycoplasm in the cells of the host. It is quite possible that their adherence to the mycoplasm hypothesis on other grounds has biased their judgment on the question of the ability of uredospores and mycelium to pass the winter. Ward (2) rejects the mycoplasm hypothesis entirely and probably most mycologists regard it with great skepticism.

On account of the position taken by Eriksson and Henning, it may be well to review some of the earlier observations recorded as bearing on this question. As early as 1875 Kuhn (3) found uredospores of *P. graminis* Pers. in the early spring near Halle, in Germany, and in same year Nielson[4] concluded that in Denmark, *P. rubigo-vera* D. C., passes the winter as a myce-

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[1] Ericksson and Henning,—Die Getreideroste Stockholm, 1896.

[2] Ward, H. M. On the Histol. of *U. dispersa*, Erikss., and the "Mycoplasm" Hypothesis. Phil. Trans. Roy. Soc., Vol. 196, p. 29, '03.

[3] Uber die nothwendigkeit eines Verbotes der Pflanzung und Anlage der Berberitzenstrauches. Kühn J. Landw. Jahrb. Bd. 4, 1875, p. 399.

[4] P. Nielsen,—De for Landbruget farligeste Rustarter og Midlerne mod dem. Ib., 1875, Bd. 1.

lium in the leaves of grain. He gives it as his opinion that the rust retains its vitality so long as the leaves of the host remain green. Blomeyer,[1] in 1876, found uredospores of *P. graminis* Pers. on grain near Leipzig in May and maintains that these had matured too early to be attributed to infections from an aecidium of that season. In England, Plowright[2] found open pustules of uredospores of *P. graminis* on *Triticum repens* on December 31, 1881, and on the same grass again in March of the next year. Von Thümen,[3] in 1886, also observed that in Austria uredospores continue on certain grasses throughout the entire year.

The investigators mentioned above give us no data as to the minimum temperatures in the seasons in which their observations were made. It will be remembered, however, that the minimum temperature for the winter in Denmark, Germany, and Austria is about the same as that of Southern Illinois or Kentucky, while that of England is about the same as that of Tennessee.

Similar investigations were undertaken by H. L. Bolley[4] at Lafayette, Indiana. At various times during the winter of 1888-1889, he found healthy mycelium within the leaves of infected wheat plants. During the first warm days of March there was a general outbreak of uredospores. Bolley concludes that wheat rust passes the winter in Indiana as a mycelium within the host.

Ericksson and Henning,[5] at Stockholm, Sweden, where the winter temperatures are similar to those of Madison, Wisconsin, record the finding of viable uredospores of *Puccinia dispersa* in abundance on November 29th, 1891, and again on the same plants, April 2nd, 1892. The postules then disappeared and fresh ones were not again found until April 30th. Viable

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[1] Blomeyer,—Vom Versuchfelde des Landwirthschaftlichen Institutes zu Leipzig. Landw. Bd., 25, 1876.

[2] Plowright,—The connection of wheat mildew with the barberry aecidium. Gardner's chronicle. Series 2, Vol. 18, 1882.

[3] Von Thümen,—Die Bekampfung der Pilzkrankheiten unserer Kulturgewächse. Wien., 1886.

[4] H. L. Bolley, Wheat rust. Bull. Agr. Exp. Station of Indiana. No. 26, 1889.

[5] Loc. cit.

uredospores of *P. graminis* Pers., were found on *Aira caespitosa* on April 1st, 1892, and of *P. Phlei-pratensis* Erik. and Henn. December 29th, 1891, and again on March 28th, 1892. In experimenting with *P. glumarum* (Schm) Erik. and Henn., certain leaves were marked in the fall of 1892 and the amount of infection noted. After a very severe winter the leaves were again examined April 27th, 1893, and three of ninety which were marked were found to bear open pustules. *P. coronata Corda* was also found in late fall and early spring. Viable uredospores of this rust were found on *Melica nutans*, November 31st, 1891, and in the same place April 5th, 1892. Besides the above records, the abundance of uredospores of *P. glumarum* on February 5th, 1894, is incidentally mentioned. They also noted that a general outbreak of uredospores of the same rust occurred on wheat within a week after the snow disappeared in the spring of 1892.

In spite of these facts, it is Eriksson's conclusion that, in a climate like that of Sweden, the rusts do not winter to any appreciable extent either as uredospores or as a mycelium within the host plant. His statements to this effect are most positive except in the case of *P. glumarum.* Even in the case of that rust, he does not believe that the number of uredospores appearing at the close of winter (three rusted leaves in ninety as shown by his experiments) would be sufficient to insure the continuation of the fungus. He holds for the wintering of rust, as for its propagation through seed, to his well known mycoplasm hypothesis. The evidence for the actual existence of this non-filamentous mycoplasm imbedded in the protoplasm of the host cells as presented by Eriksson and Henning, and later by Eriksson and Tischler[1] seems entirely inconclusive. The structures described are doubtless artefacts or distorted elements of the host cells themselves. The temperatures at Stockholm for the winters of 1890 to 1894 are recorded and may be tabulated as follows for comparison with the temperatures at Madison, as given in a later table.

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[1] **Eriksson and Tischler,—K. Svenska Vetenskaps Akademiens Handlingar. Bd. 37. No. 6, 1904.**

Temperature records at Stockholm.

Years.	Nov.		Dec.		Jan.		Feb.		Mar.	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
1890-1891.	52	2	41	2	41	— 9	52	2	48	0
1891-1892..................	50	16	46	4	41	— 4	46	—18	52	4
1892-1893..................	48	12	45	— 4	34	—16	35	—25	53	—14
1893-1894.........	48	12	45	16	.. ...	......	......	.. ..	......	......

In a recent paper[1] Klebahn describes observations which possibly suggest the existence of a mycoplasm such as Eriksson and Tischler[2] have described in their latest communication on the subject. Klebahn figures and describes minute granules within the host cells which stain, as he says, like the nuclei of the rust hyphae. These granules are very small and do not show the membrane, chromatin, and nucleole, which I have always found in the nuclei of rusts whether collected in winter or summer. Klebahn himself is doubtful whether the structures observed are not artefacts.

In the winter of 1892-1893, Hitchcock and Carleton[3] made a series of observations on the wintering of *P. rubigo-vera* D. C. at Manhattan, Kansas. They record the germination of uredospores collected November 5th, January 9th, January 17th, January 24th, January 25th, February 25th, and March 1st. They also record the maximum and minimum temperatures of each of the winter months.

	Maximum.	Minimum.
December	67°	—9°
January	53°	—1°
February	61°	—6°

Considerable snow covered the ground during the winter. Carleton[4] gives it as his opinion that in the latitude of Man-

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[1] Klebahn,—Einige Bemerkungen uber das mycel des Gelbrostes. Berichte der Bot. Gesell., XXII, 1904.

[2] Loc. cit.

[3] Hitchcock and Carleton,—Preliminary report on the rusts of grains, Kan. Agr. Exp. Station, Bull. 38, 1893.

[4] Hitchcock and Carleton,—Rusts of Grains, Kan. Agr. Exp. Station, Bull. 46, 1894.

hattan the grain rusts winter as a mycelium within the host and produce spores from time to time as the warmer periods occur. In a still more recent paper, Carleton[1] asserts that the uredo on *Poa pratensis* winters alive as far north as Lincoln, Nebr.

In order to further test and determine in a latitude still farther north, the ability of rusts to winter as mycelium and uredospores, the writer undertook to follow the history of several of our common rusts through the winter of 1902-1903.

In the fall of 1902, volunteer grain was very abundant in the vicinity of Madison and well rusted plants could be easily found until late in November. During the winter and early spring material was gathered from plots of Schlansted rye and Red Clausen winter wheat on the University Experimental Farm. The Poa and oat material was gathered from plots in the city. The plots on the University Farm were situated on a piece of ground sloping to the northwest. From these plots the snow drifted leaving the ground bare during the greater part of the winter. The oat and Poa plots were well covered with snow. From time to time plants bearing uredospores were taken into the laboratory and the spores were germinated in water. These water cultures were made within a few hours of the time of gathering the material. The dates of collecting and the prevailing temperatures may be taken from the following table. Initial letters are used to indicate the particular rust collected. There are also shown the maximum and minimum temperatures for each day of the month, as indicated at the head of each vertical column. Spores were germinated on every date noted except in the case of *P. poarum* collected February 18th.

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[1] Carleton,—Investigations of Rusts, Bull. 63, Bur. Plant. Ind., U. S. Dept. Agric., July, 1904.

Prevailing temperatures and dates of collections of rusts, Madison, Wisconsin, 1902–1903.

Day of month		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
																								g. t. c.					r. s. r. t.			
November Temperature	Max	57	64	55	56	54	44	47	58	62	49	62	64	63	57	43	40	41	46	52	51	49	45	44	43	36	31	32	37	34	36	
	Min	48	44	34	44	41	36	28	38	49	34	37	59	42	41	37	34	34	35	30	36	37	35	27	33	27	30	25	20	23	16	
								p.								p.					p. c.											
December Temperature	Max	40	36	31	23	28	27	18	4	22	30	24	27	27	26	31	28	27	33	34	37	36	35	29	31	6	9	16	21	23	17	34
	Min	24	29	19	11	22	15	3	—5	—3	20	17	17	20	17	25	20	13	21	22	33	33	26	24	6	—1	—1	2	10	16	1	12
																		r. s. r. t. c.			r. t.		r t. r. s.				c.					
January Temperature	Max	35	33	33	27	23	24	32	18	14	12	13	2	16	23	34	36	36	13	24	31	19	24	18	27	27	38	36	37	36	16	38
	Min	24	19	21	20	12	14	17	4	—1	—4	—9	—9	—3	9	21	23	12	3	8	14	5	12	3	15	22	25	26	31	15	0	11
		p.					r. t.					p.	r. t. r. s.						p*							r. s.						
February Temperature	Max	37	34	24	21	25	23	28	26	34	42	40	36	25	26	21	6	2	5	19	21	25	34	33	33	37	45	47	37			
	Min	28	22	11	14	6	5	12	15	19	28	32	25	12	10	5	—8	—15	—14	—3	2	13	10	24	15	16	23	33	13			
														p.							r. s. r. t.											
March Temperature	Max	33	40	39	37	40	41	49	42	49	44	42	54	62	41	40	50	47	68	66	56	48	53									
	Min	9	25	31	33	33	32	39	30	30	36	35	36	37	30	30	33	37	41	55	29	25	34									

g. t. = *Puccinia graminis tritici.*
c. = *Puccinia coronata.*
r. s. = *Puccinia rubigo-vera secalis.*
r. t. = *Puccinia rubigo-vera tritici.*
p. = *Puccinia poarum.*
* = *No germination.*

It is plain from the above table that in the latitude of Madison and with a period of three months, during which the temperature scarcely rises above the freezing point viable uredospores may be obtained at practically any time during the winter. As noted also, the spores were taken from very exposed situations. In each case about 10% of spores germinated. The cultures made after January 1st did not vary greatly in this respect excepting in the case of the oat rust spores collected January 26th. Of these spores 60% germinated. In all cases the germ tubes were healthy in appearance and reached a normal development. The time required for germination varied from three to six hours.

In order to make permanent preparations showing the per cent of spores which germinate and the length and appearance of the germ tube, the following method was devised. On one side of a clean slide a thin film of albumen fixative, prepared by mixing equal parts of egg albumen and glycerine, is applied. On a slide so prepared is placed a drop of distilled water containing the spores. When germination has occurred, the water is allowed to evaporate until the slide is nearly dry and the slide is then immersed in a killing solution. I have found Flemming's weak solution very satisfactory. Thirty minutes is usually sufficient time for the exposure. After killing, wash with water and harden by carrying the preparation through the different grades of alcohol, as follows:

30%	3 minutes.
50%	5 minutes.
70%	5 minutes.
80%	5 minutes.
95%	5 minutes.
100%	1 minute.

The slides may then be bleached or stained at once. Short exposures to the stains are most satisfactory. I have used Flemming's triple stain exposing to the Saffranine three-fourths of a minute; Gentian violet, two minutes, and using the shortest possible exposure to the saturated solution of Orange G., or using that solution diluted with three times its volume of water. Mayer's haematoxylin brings out the nuclei perhaps even more clearly. This stain was prepared by mixing a solution of 0.1

gm. of Haematein dissolved in 5cc. of 90% alcohol with a solution prepared by dissolving 5 gm. of alum in 300cc. of water. Thirty minutes' exposure to this stain followed by a very short exposure to the dilute Orange G., gave the most satisfactory stain tried. This method of fixation and staining has been employed with good results in preparing slides of various kinds of germinating spores and also of various kinds of germinating pollen-grains. The albumen fixative seems to have no injurious effect in any case which was tried.

It may be noted early in the winter that in the case of badly rusted grain, many of the leaves become spotted with pale areas. Sections showed that the tissue beneath these spots contained mycelium and undeveloped spores. In order to determine whether such spots developed further and became open pustules during the winter and also to determine whether the oat plants could be wintered over with some protection a clump of badly spotted oat plants was covered in the following way. A bank of earth six inches high, enclosing an area twenty by twenty-two inches, was built around a patch of oat plants and the whole covered with a pane of glass. Certain leaves were placed in such a position that they could be easily seen. From time to time during the winter, the snow was removed from the glass and a little warm water applied when the oat plants could be seen very clearly.

The oat plants were enclosed as above described on November 18th, and a number of the pale spots marked. On December 20th, it was observed that the epidermis was ruptured and open pustules had been formed in certain of the marked spots. Many of the white spots were still apparent. On January 17th the snow was again removed. The pustules and spots were in much the same condition as on December 20th. The oat plants were fresh and healthy except where in contact with the glass. On January 23rd the conditions under the glass remained unchanged. After this date the experiment was interrupted and no observations were made on these plants. It seems probable, however, that uredospores and mycelium of *P. coronata* are at least as resistant as the oat plants on which they grow. As is shown in the table, spores from oats were germinated as late as

January 26th, and it seems probable that the mycelium would have withstood the remainder of the winter if the host could have been kept alive.

Besides the data recorded in the tables, observations were made on the spring development of the rust of wheat and rye. On March 5th, a number of the winter leaves of rye, spotted in the manner described above, were marked. Fifteen days later, or March 20th, it was found that many of the spots had developed to form open pustules. On this last date a general outbreaking of new pustules was noted, which reached its height on about April 3rd, when fresh vigorous spores were abundant. The winter leaves now began to wither and disappear. After April 8th, there was a period of about four weeks when it was impossible to find a single spore. On May 6th, the new leaves began to show a diseased appearance. The plot was visited again May 13th, when open pustules were to be found in abundance.

It will be seen that there were in the spring in question two distinct outbreaks: The first occurred on the old winter leaves in the two weeks following the first warm weather. Uredospores did not appear again until sometime later when a second outbreak occurred on the first of the spring leaves.

Eriksson and Henning also describe two distinct outbreaks and use this fact as evidence for their mycoplasm hypothesis. The first appearance of uredospores they admit is often caused by mycelium that has survived the winter, but the later outbreak comes from the mycoplasm. In their observations on *P. dispersa,* they found uredospores April 2, 1892, but think even in this case that they have no reason for believing that these spores were produced by mycelia living over from the previous year. The explanation of the two separate outbreaks is probably to be sought in another direction.

I have found by experiment that in the cooler weather of spring the incubation period following inoculation with uredospores is usually lengthened to between three and four weeks and this explains the existence of a period with no rust after the first attack. The winter leaves die in early spring and with them the winter mycelium, but not until it has produced uredospores which inoculate the new leaves. Then follows a period of incubation which may be lengthened more or less according to the temperature and other conditions in the spring.

The possibility that uredospores may be carried great distances by the wind and in this way move northward to infect the crops in successive regions is very interesting as bearing on this question. Klebahn has recently attempted a quantitative determination of the number of rust spores occurring in the air. He collected spores on sheets of cotton exposed to the open air during the summer of 1901. His results show that in the course of the summer immense numbers of uredospores are to be found floating in the air. Whether sufficient floating uredospores are present at the first of the growing season to cause the abundant outbreaks of rust frequently observed, requires further proof.

As noted above Carleton thinks that viable spores found during the winter are produced from time to time during the periods of warmer weather. This, indeed, is very likely in a climate like that of Kansas where the temperature rises to about 60° F. as a maximum for each of the winter months. From the table it will be seen that the uredospores of *P. rubigo-vera tritici.* which were gathered on February 6th, were either sixty-seven days old or had matured at a temperature not higher than 6° above the freezing point. It will also be seen that the temperature did not rise above 42° F. for a period of ninety-three days. I am inclined to think that at our winter temperature the uredospores may remain dormant for long periods without losing their vitality. Very likely, however, these spores play little part in producing infections in the spring, since with the first warm days the mycelium produces new pustules with a fresh crop of spores.

As the severity of the weather must affect the amount of healthy host tissue that survives the winter, it must limit the amount of mycelium and so the number of uredospores at hand in the spring, and is, in all probability, one of the chief factors in determining the violence of early outbreaks of rust.

Madison, Wisconsin,
November 1, 1904.

HABITS AND ANATOMY OF THE LARVA OF THE CADDIS-FLY, PLATYPHYLAX DESIGNATUS, WALKER.

C. T. VORHIES.

The larvae of the caddis-fly, *Platyphylax designatus* Walk., are found in great numbers in a certain group of cold springs on the southern shore of Lake Wingra, near Madison, Wisconsin. There are several other large springs about the shores of the same lake, but the larvae are not abundant in any of the others and in some are not found at all. The conditions found in the group inhabited by the larvae are as follows: cold water in abundance throughout the year at a temperature of 8° C. never freezing in the most severe winter weather: plenty of clean rather fine sand, with numerous coarser particles; many larger stones, under which the larvae lie hidden during the daytime; Water-cress, *Nasturtium Officinale,* in great quantities, and some water-milfoil, *Myriophyllum,* on which plants the larvae feed.

A few larvae may be found during the day under the denser clusters of water-cress, where there is little light, but not much evidence of feeding by day may be seen. As the loose stones under which the larvae are hidden are often at a distance of five or six feet or even more from the food plant, and as the intestine is always found distended with food in these specimens, the conclusion is at once forced upon us that they feed almost entirely at night. The fact that during the day the larvae in dishes in the laboratory cluster in the darkest shelter obtainable lends support to this conclusion. When a loose stone is lifted under which dozens of larvae are gathered, what at first appears to be a mass of sand begins to heave and move and soon resolves itself into a number of individual larval cases, each being laboriously dragged away to a new retreat by a visible brown head and six legs. Hundreds of these larvae may be seen in a few minutes time in this one group of springs.

The case is very beautifully constructed of sand grains, and is in the form of a slightly curved tube, (Fig. 21.) open at both ends, though the posterior, narrower end, usually has the margin turned in so as to partially close the orifice. (Fig. 22.) The concavity of the case is ventral and a slight projection or hood extends forward from the dorsal portion of the anterior margin.

The eggs of this caddis-fly are deposited in large numbers in April. They are attached to the lower surfaces of loose stones, mostly at the edge of the water, in very moist situations. The larvae hatch in a short time, probably in less than two weeks, though the exact time has not yet been determined. They are about 1¼ mm. in length when first hatched, and their heads are larger and legs longer relatively than the same parts of older larvae. The interesting fact was noticed that these newly hatched larvae are positively heliotropic to a marked degree when on a dry surface, but at once become negatively heliotropic when placed in a dish of water. The necessity of getting out from beneath the stones where the eggs are placed in order to find water, and of getting beneath stones for protection while building a case, after reaching it, offers an explanation of this peculiarity. The young larvae at once begin building cases when placed in a dish of water with sand in it, and are capable of fashioning a fairly good one in four or five hours. They probably do not feed until safely housed in a case. Small larvae a few millimeters in length are plentiful in the late summer and early fall. From November to January more and more larger larvae are found and small individuals become few in number. About the middle of February the majority of the cases are found to have larger irregular stones attached to the anterior ends, evidence of the approach of pupation, while some are found fastened to the lower surfaces of the large rocks by a mass of silk at the anterior end. Many of the latter are also closed at the posterior end with larger stones of the same kind as those already mentioned. If the closed cases be broken open at such a time very few pupae will be found, but numbers of larvae in an inactive state may be seen. Several days seem to be necessary for pupation after closing the case. The first adult was reared about March 15. On April 18 many adults were taken beneath the stones about the springs and at the same time many eggs were found. Probably some were out as early as the first of April.

Gross dissection was found practicable as a means for much of the work done, the respiratory system being worked out entirely in this way. The full grown larvae,—in the last instar—average about 16 mm. in length by 3½ in breadth of abdomen. The greatest hindrance to dissection was encountered in the numerous leaf-like fat bodies with which the organs are surrounded. In a few specimens dissected after being dead a few hours the fat bodies could be washed out easily by means of a pipette. For microscopic preparations the common fixing agents and stains were used. Delafield's haematoxylin was found serviceable in staining the nuclei of the spinning glands, for whole mounts of the same. These glands, after fixation, may be split with a fine scalpel along one side and spread out. This shows the whole area of the surfaces of the cells and gets rid of the secreted mass of silk within the lumen, which, taking the stain, would otherwise obscure the nuclear structures. A rather long exposure to the haematoxylin is necessary to penetrate the nuclei of such cells. The cytoplasm is thus stained a deep blue which must be washed out with acid alcohol. The addition of a counter stain, such as eosin, rather tends to obscure the nuclei than to aid in differentiation, owing to the thick mass of cytoplasm.

RESPIRATORY SYSTEM.—The internal respiratory system consists of two large longitudinal tracheae extending throughout the body and lying in a series of curves, each the length of a segment. (Fig. 1.) Each trachea has connected with it a series of smaller branches which pass to the external respiratory filaments and to the various organs and muscles of the body. In the prothorax each longitudinal trachea divides into two large branches which pass forward into the head, entering at different levels. Before entering the head each of these again divides, the four subdivisions supplying the head only. The dorsal branch (A) will be described first. One of its two branches lies ventral to the other for a short distance, then curves outward and passes through the muscles directly to the eye, giving off several small branches which at once break up in the muscles. From a point near the eye this branch then curves inward again and, after making a dip posteriorly, where it gives off a small branch, it curves caudad and ventrad and passes beneath the brain, giving off a small branch to that organ, and then another small branch which anastomoses below the brain and dorsal to the oesophagus with its fellow of the opposite side. From here this trachea may be considered as a branch of the ventral trachea (B) with which it is continuous. The dorsal branch of A passes slightly inward so as to lie alongside the corresponding opposite tracheae, giving off immediately after entering the head a branch on the outer side which curves upward and breaks up in the muscles of the top of the head. At a point posterior to the brain the main trachea forks, the inner, larger branch, immediately anastomosing with its opposite, while the outer, smaller one, curves outward and upward and breaks up in the muscles. From the point of anastomosis a median trachea passes forward dorsal to the brain and supplies the muscles of the labrum.

Trachea B divides into two nearly equal branches before entering the head. The outer one curves outward and passes in a direction nearly identical with the outer branch of A but at a lower level, giving off small branches to the muscles, and finally ending in the mandible. The muscles supplied by this branch seem to be mostly mandibular. The inner branch of B passes cephalad and only slightly inward, the first important branch being found posterior to the median anastomosis of A. This branch soon divides, a small part passing forward to the mouth and the main part passing upward to anastomose with the branch described from A. Another important branch from the inner fork of B passes outward and forward and supplies muscles which seem to be manibular. The remaining portion again divides into two parts which pass forward and break up in the muscles of the floor of the mouth. Between the first and second branches of the inner fork of B, but from the inner side, a small branch passes off to the sub-oesophageal ganglion.

The dorsal branches are shown on the right in the figure (Fig. 1.) while those ventral to the intestine or which pass to external respiratory filaments are shown on the left. A regular series of large branches is seen to supply the intestine with the exception of the oesophagus proper. Each of these branches breaks up in a complex fashion on its own side of the intestine. The large intestine is particularly well supplied with numerous small branches which are not arranged on the regular plan of the mid-intestinal supply. A smaller longitudinal trachea lying on either side of the dorsal median line is formed by the union of loops arising from the main trunk. Additional similar loops in the meso- and metathorax are peculiar for the manner in which they dip down toward the leg joints, there giving off in each case a branch which, aided by two others, supplies the two pairs of limbs arising from these segments. There is a suggestion here that these loops may later become the source of supply for the wings also. Of the three branches supplying the meso-and metathoracic legs, the one sweeping in a curve toward the median line before entering breaks up in the first segment of the leg. The second fork of this same branch passes to the second segment, while that from the loop continues to the extremity, supplying the remaining segments. The branches supplying the thoracic ganglia all unite with their opposites by a small anastomosing branch just anterior to the ganglia. The spinning glands, notwithstanding their activity in the secretion of silk, receive no regular supply of important tracheae, the only noticeable branch being a small one in the prothorax. The branches connected with respiratory filaments are marked with a circle at the point of exit through the body wall. Some of the external filaments consist of several branches, in which case the tracheae break up accordingly.

ALIMENTARY TRACT.—The alimentary tract is straight, of the same length as the body, and begins at the mouth as a small thin-walled tube. It extends to about the end of the metathorax with but little variation in size, narrowing slightly at the junction of the head and thorax, thence gradually widening to the beginning of the next division, the mid-intestine. The posterior part of the fore-gut seems to function as a sort of crop, as it is frequently seen somewhat swollen with food when it assumes a rounded outline. (Fig. 1.)

The mid-intestine, as may be expected from the herbivorous habit of the larva, is very large when normally filled with food. It begins with an abrupt enlargement of the alimentary tract at the posterior border of the metathorax. The fore gut apparently is telescoped into this. The width of the mid-intestine at this point is more than one-half that of the average abdominal segment. From here to the posterior end, in the middle of the sixth segment, it tapers gradually to less than one-third the width of the abdomen. The end of the mid-intestine is marked on its external surface by the attachment of the Malpighian tubules, which are six in number. These tubules extend anteriorly to the first abdominal segment; they then turn back on themselves, extending posteriorly to the eighth segment, when they again turn forward and end in the sixth or seventh segment after forming several loops in that region. They are pigmented so as to appear reddish brown, the pigment granules being generally grouped most thickly near the nuclei.

The hind-intestine may be divided macroscopically into a large posterior portion beginning in the seventh segment, and a small intestine only about the length of one segment, and rather narrow. In the circular furrow formed by this narrowing of the alimentary tract the distal folds of the Malpighian tubules are very numerous. The anus is a vertical slit in the posterior end of the ninth segment, between the projecting parts on which are borne the prolegs.

When examined microscopically by means of sections, the oesophagus is found to consist of an epithelium of thin flattened cells, lining which is a chitinous layer bearing groups of chitinous spines, which point backward toward the mid-intestine. From two to eight or nine spines constitute a group. (Fig. 2.) The muscular coats consist of an inner circular and an outer longitudinal layer, both being striated. Sections, both longitudinal and transverse, show clearly that the fore-intestine is telescoped into the mid-intestine for a short distance. The portion thus extending into the mid-intestine as a double fold has the same character of epithelium as the fore-gut, and further proof of its origin is given by the layer of chitin extending to the point where the folded portion meets the anterior end of the mid-intestine. The length of this part is about one-half the width of the anterior end of the intestine,, so that if pushed forward by the food it might nearly, if not quite, close the opening. An oesophageal valve is thus formed. (Fig. 3.) As the posterior portion of the oesophagus as well as the intestine is usually distended with food, the chitinous hooks of the oesophagus probably aid in the function performed by the valve.

At the beginning of the mid-intestine there is a marked change in the epithelial cells, which are here columnar and bear on their inner surfaces a well-marked peritrophic membrane, the thickness of which is about equal to the average width of the cells. (Fig. 5.) Nests of regenerative cells, very similar in appearance and staining reaction to those described by Needham (7) for certain dragon-fly nymphs, are numerous, and placed at regular intervals. A thick basement membrane and longitudinal muscle fibers are present, the latter somewhat scattered. The circular fibers form a nearly continuous coat and are unstriated.

This columnar epithelium extends to a point a little posterior to the openings of the Malpighian tubules, but the peritrophic membrane is supplanted by a chitinous layer just at the posterior border of the lumen of the tubules. That is, the beginning of the hind-intestine is marked only by the change from peritrophic membrane to chitin, and not by an immediate change in the character of the epithelium, except that no nidi are present posterior to the tubules. (Fig. 4.) Within a short distance, however, the columnar epithelium gives way suddenly to a layer of flattened epithelial cells similar to those of the oesophagus. The chitin covering this area of columnar epithelium of the hind-intestine, is beset with numerous spines. At about the middle of the small intestine another band of chitinous spines is developed and a heavy band of circular muscles is present just at this point, indicating that the structure probably acts as a rectal valve. (Fig. 6.)

The larger posterior portion of the hind-intestine can be readily divided microscopically into a large intestine and a rectum. In section the wall of the former is seen to be pouched or folded forward over the posterior end of the small intestine. The epithelium of this part consists of a single layer of large, flat cells of considerable thickness, containing nuclei with finely granular contents. (Fig. 7.) The inner surface of these cells has a peripheral membrane of about the thickness of the peritrophic membrane of the mid-gut and of somewhat similar appearance, being marked with striae perpendicular to the surface. (Fig. 8.) A constant layer of substance,—blue staining in haematoxylin and in triple stain,—lies within the lumen. This makes it appear that the cells are secretory, but Van Gehuchten, (8) who figures similar cells for the larvae of the Dipteron, *Ptychoptera contaminata,* takes the ground that they are exclusively absorptive in function. Circular muscles are found within the peritoneum, but no longitudinal fibers. In the furrows between six irregular outfoldings or loose pouches of the large intestine lie six bands of longitudinal muscles, the latter being outside the peritoneum.

At the beginning of the last segment the character of the epithelium again changes abruptly and the lumen gradually becomes vertical. The epithelium here also consists of a single layer of flat cells, but their size and thickness is much less than that of the cells of the large intestine. This portion is the rectum proper, though no accumulation of waste material is ever found within it, Within the anal aperture and extending out on the external surface a short distance is an area bearing strong, slightly curved chitinous teeth. Betten (1) apparently has overlooked this last division of the intestine.

SPINNING GLANDS.—The spinning glands of the larva of *P. designatus* are very well developed and have been found very interesting as regards the nuclear structures. The glands are about one and one-half times as long as the body and lie ventrad and laterad to the intestine, each forming three principal folds, with the distal end lying in smaller curves of varying shape. (Fig. 12.) Each gland may be readily divided into conducting and secreting portions, a slight enlargement with a constriction in its middle showing the exact location of the dividing lines. In a microscopic preparation the character of the nuclei may be seen to change abruptly at this constriction. The anterior, conducting portion lies in the head and at the base of the labium joins with its fellow in a common duct rather strongly chitinized and leading to an opening at the tip of the labium. In this chitinized portion is the "press" which has been fully described by Gilson. (2, 3.)

The secreting portion is made up throughout its length of cells containing the characteristic branched nuclei of spinning glands of insects but in the small cells near the anterior end the branching is not extensive. (Fig. 14.) Each cell, in surface view is typically the shape of a flattened hexagon, the shorter axes lying in the direction of the length of the gland, two such cells forming the entire circumference. Such a gland when opened along one side and spread out gives the appearance seen in the diagram. (Fig. 13.) The nuclei do not show distinct centers of branching, as figured by Henneguy (6), nor are they broken up into separate fragments, as described and figured by Gilson. (3) They do, however, in some cases of complex branching have this appearance, but a careful examination with a high power shows a connection, in every case, of such apparently detached pieces with the main body of the nucleus. Whether or not the branches ever anastomose is difficult to determine. Such anastomoses are shown by Helm (4) for the nuclei of Lepidopteran glands, but such cases are rare, if they ever exist, in the corresponding structures of *P. designatus*. What at first appear to be such anastomoses are common in the larger and more complex nuclei, but careful focussing and study of the outlines of the nuclear membrane, in a majority of cases, proves beyond a doubt that it is only apparent and not real, the appearance being caused by overlapping branches lying at different levels. (Figs. 15–20.)

NERVOUS SYSTEM.—The nervous system shows a small degree of concentration in the larval stage. It consists of a chain of thirteen double ganglia, (Fig. 9.) which Betten (1.) says is the number given by Klapalek* for Trichoptera, though

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*Klapalek's work was not available.

he (Betten) noted ony six abdominal ganglia, or a total of eleven, in the larva of *Molanna cinerea.* The supra- and subœsophageal ganglia show nothing worthy of particular note. The three thoracic ganglia occupy the pro-, meso-, and metathorax, the third lying slightly anterior to the middle of the metathorax. The first abdominal ganglion, smaller than the third thoracic, lies quite close to it posteriorly, within the metathorax. The second abdominal ganglion, slightly smaller and more elongated than the first, lies near the middle of the first abdominal segment. The third ganglion, about the size of the first, lies at the juncture of the second and third segments, and is the only ganglion found in these two segments. The fourth, fifth, and sixth ganglia lie in their respective segments, but the seventh lies just within the posterior border of the sixth segment and the eighth, closely applied to it posteriorly, lies just within the anterior border of the seventh segment. Although the last two ganglia are closely applied to each other they are entirely separate, as may be readily seen when stained and examined microscopically. This arrangement of ganglia will be seen at once to offer a possible explanation of Betten's failure to find more than six abdominal ganglia, because, in serial sections, only six abdominal segments would probably be noted to contain ganglia. These would be the first, either the second or third, but not both, and the fourth, fifth, sixth and seventh. In the two cases where two ganglia are rather closely applied to each other, very careful observation is probably necessary in order to distinguish that two are present, though the point may be easily seen by means of dissection. The first abdominal ganglion would hardly be looked for in the thorax.*

Reproductive System.—The only traces of reproductive organs found in the larvae are ovaries and testes in the early stages of development. These lie in the fourth and fifth segments, and are found only in the large larvae which are preparing for pupation, which period is determined by the addition ovaries, which are easily distinguishable, are elongate bodies,

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*A dissection of a species of *Molanna* has since been made and the ganglia found substantially the same as in *P. designatus*.

of larger particles of stone to the anterior end of the case. The lying deep down on either side of the mid-intestine. The testes are similarly situated, but are not so readily observable, particularly in their later stages of development, as they are then closely enveloped with a layer of fat of the same color as the surrounding fat bodies. The sperms become quite well developed in the latest larval stages, but accessory organs of reproduction do not appear.

GLANDS OF GILSON.—In the prothorax of the larva careful dissection discloses a small, elongated structure, with irregular, wavy outlines, lying beneath the oesophagus. (Fig. 9.) Its anterior tapering end passes ventrad between the connectives uniting the sub-oesophageal and the first thoracic ganglia, and may be traced to a connection with the base of a curved chitinous spine, which, lying between the first pair of legs, curves forward close to the head. A microscopic examination makes it clear that this is a glandular structure, with its opening at the tip of the spine. (Fig. 10.) The posterior end of the gland is free in the cavity of the body and may lie either to the right or to the left of the median line. This is the only representative in *P. designatus* of the Glands of Gilson, so-called from the investigator who first described them. The original paper was not available, but M. Henseval (5) has given an interesting account of the glands as studied by him in several Trichopterous larvae. In the possession of only one of these structures, *P. designatus* comes in the same group as *Limnophilus flavicornis*, *L. rhombicus*, and *Anabolia nervosa*, as opposed to *Phryganea grandis* and an undetermined Phryganid in which three glands of a somewhat more complex structure are found, one in each thoracic segment. Henseval (5) offers proof of an oily secretion and ascribes an excretory function to these glands.

Under direction of PROF. WM. S. MARSHALL,
Zoological Laboratory, University of Wisconsin,
Madison, May, 1905.

PLATE VII.

EXPLANATION OF PLATE VII.

All figures except dissections are drawn with a camera. Magnification in diameters given after explanation of each figure.

Fig. 1. Dorsal view showing tracheal system and alimentary tract. Secondary longitudinal trachea, Sl., thrown to the outside of the main trunk. A., dorsal branch entering head. B., ventral branch entering head. Pr., prothorax. Mo., mesothorax. Mt., metathorax. Circles on the ends of the tracheal branches indicate points where same enter external respiratory filaments. Oe., oesophagus. C., crop. Md., mid-intestine. Mp., Malpighian tubule. S., small intestine. L., large intestine. R., rectum. × 8.

Fig. 2. Surface view of portion of chitinous lining of oesophagus. p., posterior border. × 300.

Fig. 3. Longitudinal section of oesophageal valve. An., anterior. Ch., chitin. Pm., peritrophic membrane. Cm., circular muscle. Lm., longitudinal muscle. X., point showing end of chitin and beginning of peritrophic membrane. × 110.

Fig. 4. Longitudinal section through junction of mid- and hind-intestine. Mp., Malpighian tubule. Pm., peritrophic membrane. X., beginning of chitinous layer, Ch., of hind-gut. Cm., circular muscle. Lm., longitudinal muscle. Y., beginning of flattened epithelium of small intestine. Pg., pigment granules. × 110.

Fig. 5. Section through nidus of mid-intestine. Cm., circular muscle. Lm., longitudinal muscle. Bm., basement membrane. Pm., peritrophic membrane. × 285.

Fig. 6. Longitudinal section through rectal valve. An., anterior. Ch., chitin. E., epithelium. Cm., circular muscle. Lm., longitudinal muscle. × 90.

Fig. 7. Surface view of epithelial cells of large intestine. × 190.

Fig. 8. Section of same. Cm., circular muscle. Pm., peripheral membrane. × 285.

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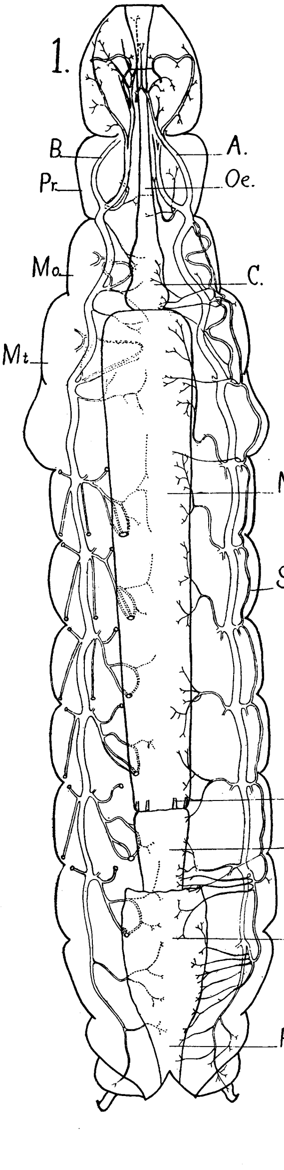

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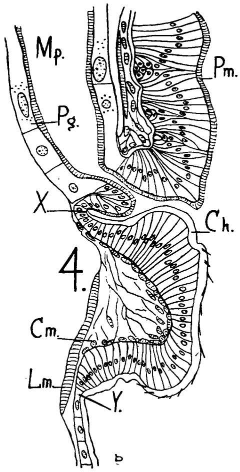

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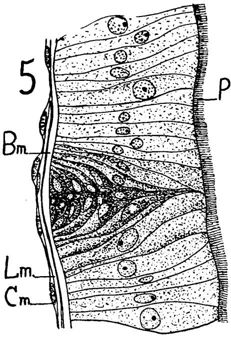

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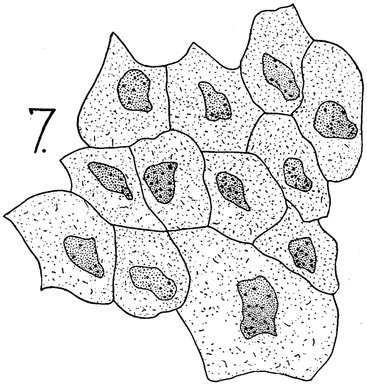

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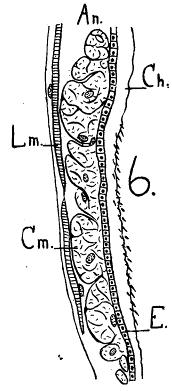

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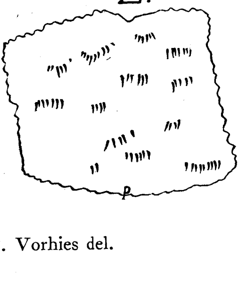

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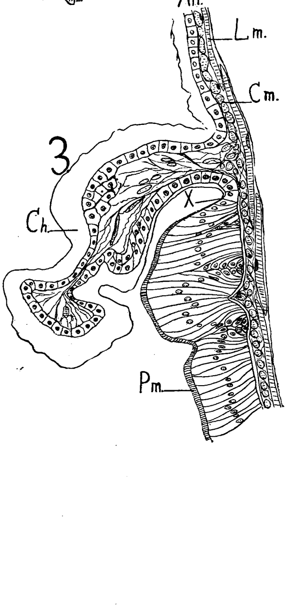

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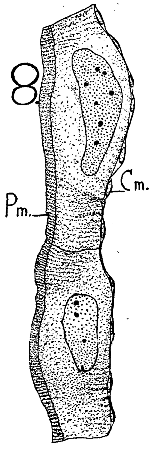

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PLATE VIII.

EXPLANATION OF PLATE VIII.

Fig. 9. Dissection of nervous system and Gland of Gilson, G. 1–9, abdominal segments. I–VIII, abdominal ganglia. × 4.

Fig. 10. Gland of Gilson, showing connection with external hollow spine. × 43.

Fig. 11. Diagram showing number and position of the external respiratory filaments on one side of body. V., ventral half of segment. 1. and 2., first and last segments of abdomen.

Fig. 12. Dissection from dorsal side showing spinning glands. C., conducting portion. S., secreting portion. P., union of conducting portions in press. × 4.

Fig. 13. Diagram showing shape and relation of cells of spinning glands when split along one side and spread out.

Fig. 14. Two small cells, showing simple nuclei, from the small anterior end of the secreting portion. × 160.

Figs. 15 and 16. More complex nuclei in larger cells taken anterior or posterior to the widest part of the secreting portion. × 80.

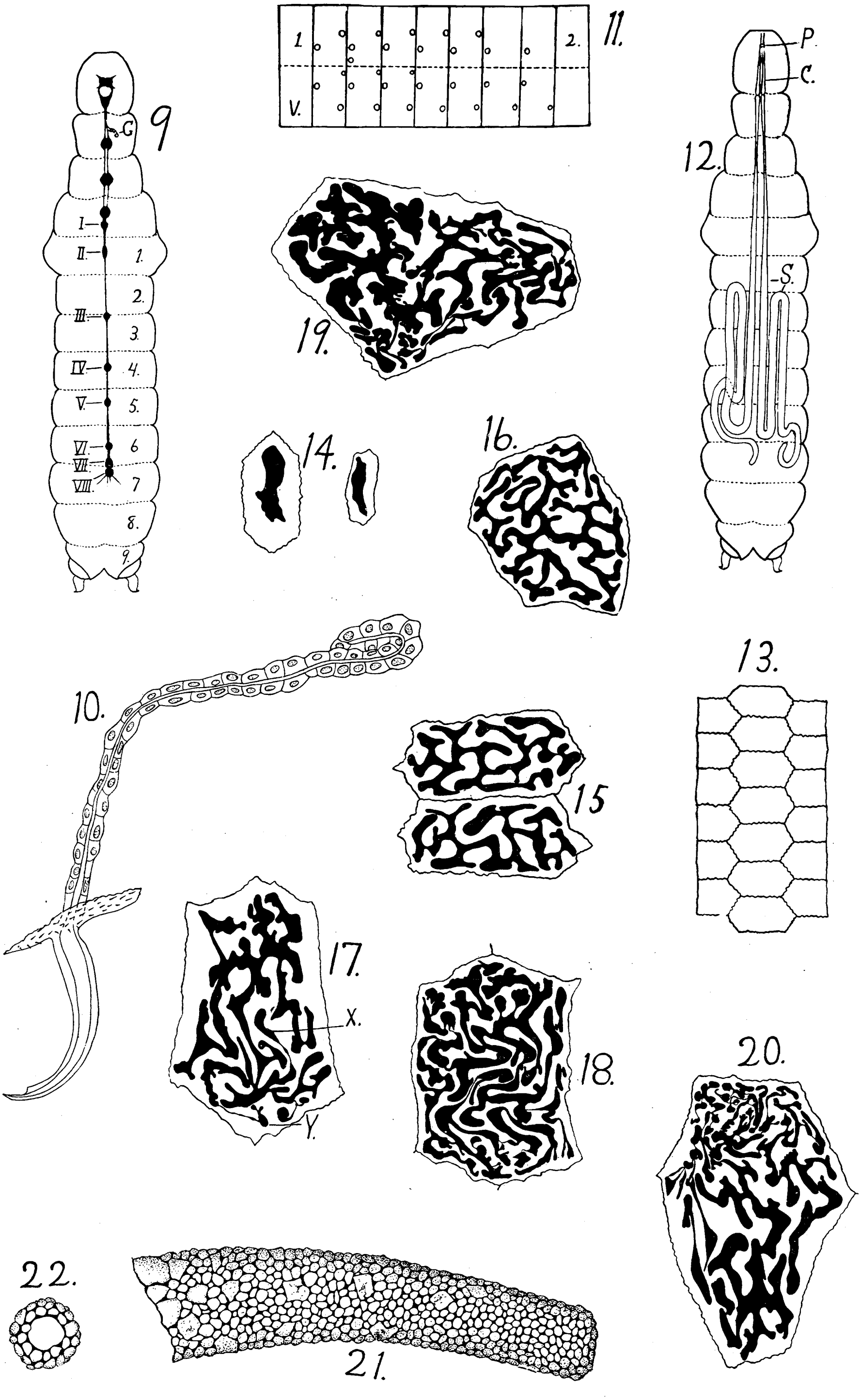
Fig. 17. Still more complex nucleus. Note pieces X, Y, attached by long slender threads to main nucleus. × 80.

Figs. 18 and 19. Complex nuclei filling larger proportion of cell than the preceding. Some apparent anastomoses could not be satisfactorily determined. × 80.

Fig. 20. Complex nucleus showing many pieces attached by slender threads. Many of the apparent anastomoses in this nucleus are undoubtedly not real. × 80.

Fig. 21. Larval case of *P. designatus*, approaching time of pupation, shown by larger stones on anterior end. × 3.

Fig. 22. Posterior end of same, showing how the border of the case is turned in. × 3.



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NOTES ON THE OCCURRENCE OF OSCILLATORIA PROLIFICA (GREVILLE) GOMONT IN THE ICE OF PINE LAKE, WAUKESHA COUNTY, WISCONSIN.*

EDGAR W. OLIVE.

The presence of this minute alga in Pine Lake is of particular interest since, so far as I am able to determine, it has been heretofore reported from but one locality in America, viz., from Jamaica Pond, a small lake in Jamaica Plain, a suburb of Boston. Mr. G. J. Hansen called the attention of Professor Birge to the peculiar growth in Pine Lake, furnishing some notes on its occurrence, and sent to him material which he had collected on March 25, 1905, which was floating on the surface of the lake where the ice had melted. Dr. Birge in turn gave the matter into my hands for further examination.

Isabel F. Hyams and Ellen H. Richards have had *Oscillatoria prolifica* under observation in Jamaica Pond since 1887, and their three papers on the subject so far published embrace the "Life History" (01); "Chemical Composition" (02); and "Coloring Matters" (04). It will be of advantage to review in considerable detail some of the long-continued studies of these writers in order to supplement our meager observations in connection with the occurrence in Pine Lake and thus be able to judge of the conditions which could bring about, in this instance, such an unprecedentedly vigorous growth of the plant.

Oscillatoria prolifica was first noted in Jamaica Pond by these two writers in 1887, and since that time has been so abundant as almost to exclude other forms of plant life. In some years it was much more abundant than in others, dependent upon the

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*This work was done while the writer was serving as a research assistant of the Carnegie Institution of Washington.

temperature and sunlight. During the year in which the plant was most abundant, it did not entirely disappear during the winter, but was found imbedded in the ice which was cut from the water. During the period of most rapid growth—generally in June—the water of Jamaica Pond frequently became turbid and opaque. I have myself noted, some years ago, that from the hills above, the water of this pond appeared reddish or of a brownish chocolate color in bright sunlight. The authors state that after a hot, sunny day, the gas created by the vigorous growth of the plant often caused the alga to rise to the surface and there float as a reddish brown, frothy cream; on June 11 and 12, 1901, for example, the floating masses were of such abundance that fully "one hundred barrels might easily have been obtained" (01, p. 308). When this scum was driven by the breezes on the shore, it decayed on the rocks, giving to them a rich purple coating. The odor of decay was "intensely disagreeable, fetid rather than putrefactive" (01, p. 301).

The authors assert that, whenever the plant was found below the surface, it was blue-green in color; the red pigment, which they have tentatively named "rubescin" (04, p. 274), according to their observations, appeared in the filaments only when a luxuriant growth of the plant had taken place. They suggest the theory that the red substance is probably "an important factor, if not the chief one, in the vigorous growth of plant life." (04, p. 274). The authors, in reaching this conclusion, apparently assume that this red pigment is derived from the chlorophyll of the Oscillatoria. They further bring in for comparison the association of the red coloring matter in the young shoots of oak, maple, etc., with the vigorous growth of these shoots; and, from the apparent close chemical relation of the red color in plants to the haemoglobin of animal blood, they conclude that "good red blood and a portion of red in the chlorophyll of green plants wherever found seem to indicate robust life." (04, p. 274.)

According to their further investigations, the optimum temperature for the growth of the alga lies between 64° and 66° F. After a season of such favorable conditions, a vigorous growth usually culminated in one or two days, never more, when large quantities of the plant would come to the surface, buoyed up by the gas bubbles, a phenomenon to which they refer as the "blooming time" of the Oscillatoria. They say further in this connection that this phenomenon never happened except when an air temperature of 80° F. and over was accompanied by a bright sun and quiet water. (04, p. 270). Only on two days in thirteen years did this "blooming" result in a large amount of scum; while on about five other days of this time a slight scum was formed.

The maximum growth, which took place generally during the long days of June, was followed in July and August by a decreased development, when the plant would be colored usually a more or less brilliant blue-green. In September, the "spore-like" (04, p. 270) bodies were formed, always few in number in comparison with the amount of the plant in the water, which, in their opinion, apparently serve to carry the plant through exceptionally severe conditions. Even in late October, however, these authors have observed that a succession of warm days will frequently permit of a rejuvenescence and a fairly vigorous growth results along the edges where the water is warm. During the winter, according to these investigators, the plant lies dormant, either in the form of broken threads of various lengths and of various stages of arrested development, or in the form of spores. These fragments or spores rest either on the bottom in shallow water or they appear to be held suspended in the denser water near the bottom of the deeper portion. As the spring advances and the surface of the water becomes warmed to a temperature of about 60° F., the authors assert that the plant begins to grow; and growth continues until the water reaches a temperature of 72° F., when rapid breaking up of the filaments occurs.

The alga in Jamaica Pond was at last, in September, 1903, killed by means of an application of copper sulphate, and the authors were able to find during the following spring only the merest trace of its recurrence.

The scum which forms on the surface of many ponds and lakes, following a hot summer season, has been much studied and has been found to consist in most cases of various blue-green algae. The phenomenon has received various names: in England it is called "breaking"; in this locality, it is sometimes called the "working" of the lakes. "Wasserblüthe," "Flos aquae," "waterbloom," are also variously applied to the scum.

In this country, Farlow (77; 83, I; 83, II) has done more work on the subject than any other investigator. According to Trelease (89), Dr. Farlow first observed the purplish color in Jamaica Pond, in Massachusetts, in the spring of 1884, and he gave to the alga the name *Oscillatoria diffusa.* This species was subsequently found, however, to correspond to the earlier described *O. prolifica* (Greville). In his earlier paper (77), Farlow discusses the odors caused by the decay of various organisms sometimes found in water supplies and speaks of Oscillatoria and Lyngbya as causing "indescribably suffocating" odors; of Beggiatoa, sulphurous odors; and of the Nostocs, Plectonema, etc., "pig-pen" odors. In this same paper, he ascribes the death of the algae forming the water-bloom as due to the broiling hot rays of the sun.

Magnus (83) investigated an instance in which the ice cut from a pond near Berlin contained a greenish growth, and, on examination, he found it to contain a species of Aphanizomenon. Trelease (89) studied the "Working" of the lakes at Madison and gives, in connection with his paper, a long list of articles relating to water-bloom. Chodat (96) has recently published observations on *Oscillatoria rubescens* D. C., which, together with *O. prolifica* (see Gomont, 93, p. 225), gives a reddish color to the surface of Lake Morat, in Switzerland. This author appears to agree with Klebahn (95), in that vacuoles of gas are regarded as present in the cortical region of the cells of the Oscillatoria; and he further believes that it is this gas which causes the alga to rise to the surface and float.

Moore (01) also agrees with Klebahn's views concerning the presence of gas-vacuoles in water-bloom, since, after his study of *Oscillatoria prolifica* from Jamaica Pond, he came to the conclusion that the buoyancy of the algal masses was due to the presence of the vacuoles, which he thought contained nitrogen. He was also lead to believe that the red color of the plant was caused by the refraction due to the presence of large numbers of these gas-vacuoles, as had been suggested by Klebahn.

A most recent discussion of Wasserblüthe and of the green, yellow or red colors given to bodies of water by various organisms has been written by Zacharias (O3).

Pine Lake is similar in one respect to Jamaica Pond, viz., in that neither has any outlet to speak of. But, on the other hand, Pine Lake is considerably larger than Jamaica Pond. The former has an area of 1.2 sq. mi. and is about 2 miles long by 1 mile wide; whereas the latter has an area of only 65½ acres. The smaller lake is between 50-60 feet in its deepest part; while the greatest depth of Pine Lake is about 90 feet.

The growth of *Oscillatoria prolifica* in Pine Lake during the summer and fall of 1904 must have surpassed in luxuriance even the richest development of the plant in Jamaica Pond. For, inquiries show that the ice around the shores of the whole lake contained quantities of the alga, as evidenced by the fact that ice harvested on all sides contained the red color imparted by it. Mr. Hansen, as did the great majority of the residents about the shores of the lake, threw away all of his colored ice and replenished his supply from the neighboring Beaver Lake, which did not show any of the reddish growth. Two of the residents, however, retained some of their cut from Pine Lake, and I have examined specimens from the ice-houses on the estate of Messrs. Mayer, situated almost opposite and about one mile from the estate of Mr. Hansen. I was told that this ice was cut in January, 1905. In the most of the ice-cakes examined, the reddish color, resembling the juice of crushed cherries, appeared in small amount only, diffused about air-bubbles and cracks in the ice. One of these colored areas was melted, and, on microscopical examination, the water thus obtained was found to contain the faintly reddish filaments of *Oscillatoria prolifica*. These filaments appear rather rigid and refractive, probably partly owing to the large amount of silica which, in the investigations quoted above, was found to be present. The diameter of a filament from the Pine Lake material measures about 4μ–5μ; while the component cells are likewise about 4μ–5μ long.

I was told that some of the ice at Mr. Mayer's place was colored throughout with the "crushed cherry" color; whereas other cakes showed ten inches or so of clear top, with the reddish substance frozen into the ice only below the ten inches.

The great abundance of the alga is further proved by the large masses which were left floating on the surface of the lake, in March last, where the ice had melted. Mr. Hansen, who collected for the purposes of identification an abundance of the alga on March 25, 1905, says that some of the floating masses were about 12 inches in diameter, while others were small—"the greater part of them being about the size of an oak leaf" (from letter of April 8th to Prof. Birge). Mr. Hansen and many others mentioned the peculiar smell readily noticable at the lake shore, which came from the decaying plant. One described the odor as resembling that from decayed flesh; but Hyams and and Richards describe it rather as fetid, not putrefactive.

An old resident claimed that at intervals during the past twenty years this red color had appeared in the ice taken from Pine Lake. Another said that the ice at North Lake, which is situated only a short distance north of Pine Lake, was colored two or three years ago in this same way. Some ventured the explanation that the fact that Pine Lake has practically no outlet except at high water, might explain the abundance of the alga here, as well as its present confinement to this lake. For, a visit to ice-houses at North and Okauchee Lakes failed to discover any signs of the growth, and careful inquiries at Mouse and Oconomowoc Lakes and Lac la Belle showed that none was present, at least, in the ice harvested from these bodies of water last winter. It is perhaps a significant fact that all of these lakes mentioned have strongly flowing outlets and inlets, excepting Pine Lake and the neighboring Beaver Lake; so that it may well be that this lack may assist in explaining the abundance of the plant in Pine Lake during the past season.

I have had opportunity to examine the plankton of Pine Lake collected on three days only—on Aug. 23, and Oct. 18, 1900, and again on July 26, 1905. The first collections were taken from waters 8—20 meters deep, but only a few blue-green forms were here found, and among them no Oscillatoria.

The more recent material was taken from the surface of both shallow and deep waters, and from various parts of the lake;

the net was also lowered in various localities to a depth of about 25 feet and then hauled straight up. Not a particle of *Oscillatoria prolifica* was found in any instance, but in all of the latter collections there was present in large quantities a species of Gleotrichia, the little colonies of which could readily be seen floating in the lake, with the naked eye, together with a small amount of other common plankton forms.

We are thus struck at once with an important difference in the midsummer conditions of Jamaica Pond and Pine Lake. In the case of Jamaica Pond, other forms of plant life appear to have been practically excluded for years by *Oscillatoria prolifica;* during the months of July and August, moreover, the growth of the alga in this Pond was but somewhat decreased from the earlier more luxuriant development. In Pine Lake, on the other hand, we have in midsummer an abundance of another species of blue-green alga, and the seeming total disappearance of *Oscillatoria prolifica.* This last fact is to me inexplicable, since one can hardly conceive of the killing off entirely of the luxuriant growth of the past year by the severity of the winter cold. I think that it is more than probable that repeated observations will surely reveal this species of Oscillatoria again during another season, if not later during this one. There is thus presented by Pine Lake a most interesting problem involving the seasonal variation and the varying predominance of different plankton forms.

When we try to obtain insight into the conditions of the past season which allowed of the production of *Oscillatoria prolifica* in such phenomenal abundance, we at once note the unusually favorable weather conditions of the latter part of last year. According to data kindly furnished by Mr. J. L. Bartlett, Weather Observer, while last October at Madison was about the average in temperature, November, on the other hand, was 6° warmer than usual. During the month of October 1904, which had a mean temperature of 51° F., several warm spells were recorded at Madison; on Oct. 1, 72°; Oct. 9, 77°; Oct. 17, 75°. On Nov. 3, the temperature reached 68°; on Nov. 19, 67°; while the mean for this month was about 40° F. But the most striking weather conditions of this time were furnished by the long drought which then prevailed; so that possibly this also assisted the favorable conditions for algal growth. The large lakes at Madison, Monona and Mendota, froze on Dec. 13 and 14, 1904, respectively. I have no record concerning Pine Lake, but it is quite probable that it was frozen over at about the same time, notwithstanding the fact that it is considerably smaller than the Madison lakes. The likelihood of the earlier freezing of the smaller lake is somewhat counterbalanced, in this instance, by the fact that the climate of the locality of the latter is to a certain extent influenced by the proximity of Lake Michigan, since it is only 20 miles away from the large lake, and over 50 miles nearer than Madison.

Now, granting that the conditions for the growth of Oscillatoria were unusually favorable in Pine Lake particularly during last October, how can we account for the occurrence of such vast quantities of the alga, frozen up in the ice? Hyams and Richards speak of an occasional fall growth, a sort of rejuvenescence due to a new warm season, which resulted in one instance in some of the alga being found in the ice. Birge (98, p. 420) says that in the autumn there is normally "a period, beginning a little before the first of October and extending to the freezing of the lake, when the algae are present in immense quantities, and are distributed with approximate equality through the whole mass of the water." If those observers were correct, who assert that the first ten inches of the ice from Pine Lake was clear, and the alga appeared only in the lower strata, then we must assume that, after considerable freezing had been accomplished, the severe cold must somehow have killed the alga and thus caused it to rise to the surface. I have not, however, myself seen an instance in which one side only of the ice-cake was colored, but those which I examined were instead reddish in small areas, about cracks and air-bubbles. It has been suggested that possibly those who made the observation recorded above were mistaken and that it was the upper part of the ice which was thus colored and not the lower; I have not had an opportunity, however, of verifying this suggestion. But in the event of this being the case, then we must suppose that the unusually mild and long-prolonged growing season of last fall culminated in a "blooming time," or "working," of the alga. Should this prove true, then the floating scum would have been frozen directly into the top ice. I have no means at present of determinating certainly which of these two ideas is correct, but the weight of the long-continued observations of the above quoted authors on the seasonal habits of the plant inclines me to believe that the alga did not form a scum, but was probably present, late in the season, in vast quantities in the deeper waters of the lake and that somehow the extreme cold of the month of January caused it to rise after the surface had become frozen.

Concerning the reddish color which appears in these plants, particularly on their rising to the surface, or on their undergoing decomposition, I wish to record here a suggestion at considerable variance from the theory held by Hyams and Richards, who come to the conclusion that the reddish substance is an important factor in the vigorous growth of plants.

These authors have themselves stated the fact that ''whenever the plant is found below the surface it is blue-green in color'' (01, p. 310), and that the reddish pigment appeared ''when luxuriant, or whenever the growth is rapid'' (04, p. 271). They have further said that it is the mass of filaments near the surface of the water, and the floating scum, and the decayed alga on the rocks of the shore which display the reddish or violet tints.

While it may be correct, as do the two authors above cited, to assume the probability of the great importance of these reddish pigments and perhaps even their chemical combination with the chlorophyll of these plants, it is hardly allowable, in my opinion, to bring in, to assist in establishing their point of the great importance of these substances, comparisons with the other reddish coloring matters sometimes present in the cell-sap of the young shoots and leaves of higher plants. For, I think that it is not at all established that these reddish pigments of the higher and lower plants are similar to each other, either chemically or physiologically.

The appearance, in the case of *Oscillatoria prolifica* as well as in other common species of Oscillatoria, of the reddish coloring matters in the filaments *after* they have risen to the surface and particularly on their undergoing evident decomposition, suggests that such colors arise as decomposition products, rather than that this reddish pigment is associated with a vigorous growth. The intensification of the color, as decomposition proceeds, argues also strongly for this conclusion.

The daily determinations, from March to October, of the amount of carbon-dioxide dissolved in the water of Jamaica Pond, as made by Hyams and Richards, may be regarded as furnishing a very important clew to the revealing of one cause, at least, of the formation of a surface scum and the appearance of the reddish color. These authors found that whenever the Oscillatoria grew vigorously, the normal content of "*carbon dioxide disappeared and the water became not only neutral but alkaline. With the decay of the plant, the alkalinity disappeared and carbon-dioxide again became normal and in one or two instances appeared in excess.*" (02, p. 310).

It seems to me readily conceivable, at any rate, that we may have in the lack of this important food-substance, carbon-dioxide, a condition perhaps brought about by its being used up by the plant during the vigorous growth, the prime cause of the beginnings of decomposition and the consequent rising of the alga to the surface of the water and the appearance of the red pigment.

Madison, Wisconsin, Aug. 3, 1905.

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INFECTION EXPERIMENTS WITH ERYSIPHE GRAMINIS DC.

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Neger is to be credited with the discovery that among the mildews, as in the rusts, our ordinary morphological species may consist of a number of physiologically specialized forms which are limited in their occurrence to a single host plant or to a group of closely related host plants. Neger's (8) first experiments were made during the summer and fall of 1901. His general conclusion was that, in the mildews he studied, specialization has gone to such an extent that conidia from one species will not infect a species of any other genus. In some cases the specialization has gone still further so that conidia from one species is incapable of infecting another species of the same genus. His results may be summarized as follows:

1. Conidia of *Erysiphe cichoracearum* DC. from *Artemisia vulgaris* will infect *A. vulgaris* but not *A. Absinthium, Alchemilla vulgaris, Galium silvaticum, G. rotundifolia, Hieracium murorum, Lactuca muralis, Leontondon taraxacum, Lithospermum arvense, Plantago lanceolata, Ranunculus repens, Senecio vulgaris,* nor *Sonchus oleraceus.*

Conidia from *Lactuca muralis* will infect *L. muralis* but not *Galium silvaticum, Hieraceum murorum,* nor *Pulmonaria officinalis.*

Conidia from *Hieracium murorum* will infect *H. murorum* but not *Artemisia vulgaris, Galium silvaticum, Hypericum montanum, Lactuca muralis, Leontodon taraxacum* nor *Sonchus oleraceus.*

Conidia from *Senecio vulgaris* will infect *S. vulgaris* but not

Hieracium murorum, Lactuca muralis, Pulmonaria officinalis nor *Symphytum tuberosum.*

Conidia from *Plantago major* will not infect the same host *P. major, Artemisia vulgaris,* nor *Hieracium murorum.*

Conidia from *Lappa major* will not infect *Artemisia vulgaris* nor *Senecio vulgaris.*

Conidia from *Verbascum thapsiforme* will not infect *Artemisia vulgaris.*

Conidia from *Pulmonaria officinalis* will not infect *Hieracium murorum.*

Conidia from *Lithospermum arvense* will infect *L. arvense* but not *Hieracium murorum, Pulmonaria officinalis,* nor *Symphytum tuberosum.*

2. Conidia of *E. polygoni* DC. from *Heracleum spondylium* will infect *H. spondylium* but not *Aegopodium podagraria, Anthriscus silvestris,* nor *Hypericum montanum.*

Conidia from *Galium silvaticum* will infect *G. silvaticum* but not *Aegopodium podagraria, Ranunculus repens, Senecio vulgaris,* nor *Vicia sepium.*

Conidia from *Ranunculus repens* will infect *R. repens* but not *Galium silvaticum.*

Conidia from *Trifolium incarnatum* will infect *T. incarnatum* but not *Galium silvaticum, Hypericum montanum, Trifolium repens,* nor *Vicia sepium.*

Conidia from *Hypericum perforatum* will infect *H. perforatum* but not *H. montanum* nor *Galium silvaticum.*

3. Conidia of *E. galeopsidis* DC. from *Galeopsis tetrahit* will infect *G. tetrahit* but not *Calamintha acinos, Glechoma hederacea,* nor *Stachys rectea.*

4. Conidia of *Microsphaera astragali* DC. (Nev.) from *Astragalus glycyphyllos* will infect *A. glycyphyllos* and *A. cicer* but not *Robinia pseudoacacia* nor *Vicia sepium.*

5. Condia of *Uncinula salicis* DC. (Winter) from *Salix purpurea* will infect *S. purpurea* and *S. caprea.*

6. Conida of *Uncinula aceris* DC. (Sacc.) from *Acer pseudoplatanus* will infect *A. pseudoplatanus* and *A. campestre.*

7. Conidia of *Phyllactinia corylea* (Pers.) Karst. from *Corylus avellana* will infect *C. avellana.*

In his experiments, Neger found that the incubation period was two to three days.

Neger raised the question whether this specialization which he discovered in the conidia of the mildews also extends to the ascospores. He says he has never observed perithecia with the ascospores formed on *Senecio vulgaris, Galium silvaticum, Calamintha acinos, Symphytum tuberosum, Hieraceum murorum* and others. The question arises how these plants become infected each spring. The conidia cannot live over the winter. Neger found that conidia from *Plantago,* if kept dry, lost their germinating power in seven days and conidia from *Artemisia* in twelve days. In order to explain the facts as observed by him, he advances the theory that the ascospores have the capacity to infect a wider range of host-plants than the conidia. To illustrate this we might take the three host-plants, a, b, and c, the conidia of each constituting a well-defined physiological species. We may further assume that perithecia with ascospores may be formed only on a. In the spring, however, these ascospores may be capable of infecting all three host-plants, a, b, and c, and thus spread the fungus.

If the ascospores have a wider range of host-plants than the conidia of the same species, the way in which the conidia become specialized each season has to be explained. Neger attempts to explain this by citing the experiment of Brefeld who found that the spores of certain smuts, if grown in a nutrient solution, lost their ability to infect a living plant. Ward also found that conidia of *Botrytis cinera,* if grown in an artificial nutrient substance, could not infect turnips. It must be remembered, however, that it is often a difficult thing to get spores to germinate in a nutrient solution and the plants produced are frequently not vigorous and so in turn do not produce a very high per cent of viable spores.

Neger compares this assumed capacity of the ascospores to infect a wider range of host-plants than the conidia to a similar capacity which he claims exists in the aecidiospores of the rusts. On this point he cites the work of Eriksson (2) who found that *Avena sativa* and *Alopecurus pratensis* were both infected with aecidospores taken from the same aecidum cup, the uredospores of each constituting distinct forms. It must be stated, however.

that Eriksson himself does not believe that the experiment was carried out with sufficient accuracy to give absolutely certain results.

Neger offers no evidence that the ascospores actually do have a wider range of host-plants than the conidia of the same species. Salmon (10) distinctly states that he has frequently observed perithecia on *Senecio vulgaris* and *Symphytum,* while others have recorded them on *Calamintha* and *Hieraceum,* all of which are plants upon which Neger did not find perithecia. It is possible that with more careful observation, perithecia will be found on all plants on which conidia are produced.

Marchal (7), during the spring of 1902, attempted the infection of several grasses with conidia of *Erysiphe graminis.* He gives no detailed account of his work but states that conidia from wheat would not infect rye, oats and barley; conidia from rye would not infect wheat, oats and barley; conidia from oats would not infect wheat, rye and barley; and conidia from barley would not infect the other cereals. As a result of his work he makes seven physiological species which he names as follows:

1. *E. graminis* f. spec. *Tritici* upon *T. vulgare, T. Spelta, T. polonicum, T. turgidum,* not on *T. durum, T. monococcum, T. dicoccum.*
2. f. spec. *Hordei* upon *H. hexastichon, H. vulgare, H. trifurcatum, H. nudum, H. jubatum,* and *H. murinum,* not on *H. maritimum, H, secalinum,* nor *H. bulbosum.*
3. f. spec. *Secalis* upon *S. cereale* and *S. anatolicum.*
4. f. spec. *Avenae* upon *A. sativa, A. fatua, A. orientalis,* and *Arrhenatherum elatius.*
5. f. spec. *Poae* upon *P. annua, P. trivialis, P. pratensis, P. caesia, P. mutalensis, P. memoralis,* and *P. serotina.*
6. f. spec. *Agropyri* upon *Agropyron.*
7. f. spec. *Bromi* upon various *Bromes.*

It is impossible to tell how far Marchal's results are reliable since he gives no data as to the number of his experiments or the conditions under which they were performed.

There is, in fact, considerable evidence that there are more than seven special forms of mildew which infect these grasses mentioned as hosts.

Salmon (10) made several infection experiments with the mildew of the grasses, especially of the Brome grasses. He finds that, instead of there being one special form for all the Brome grasses, as stated by Marchal, there are at least four and probably five distinct biologic forms on the Bromes.

Salmon also obtained the following results with the mildew from other plants. These results in general confirm the work of Marchal.

1. Conidia of *Erysiphe graminis* DC. from wheat will infect wheat and *Triticum Spelta* but not oats, barley, rye, nor *Agropyron repens.*

Conidia from oats will infect oats, *Avena brevis, A. nuda, A. sterilis, A. strigosa,* and *A. orientalis,* but not wheat, barley, rye, *Festuca elatior, F. heterophylla, Poa annua, Dactylis glomerata, Arrhenatherium elatius, Trisetum pratense, Phleum pratense, Alopecurus pratensis,* nor *Lolium Italicum.*

Conidia from *Avena nuda* will infect oats, *A. brevis,* and *A. nuda.*

2. Conidia of *Erysiphe polygoni* DC. from *Trifolium pratense* will infect *T. pratense,* but not *T. agrarium, T. repens, T. medium, T. montanum, T. incarnatum, T. hybridum, T. filiforme, Lotus corniculatus, Melilotus arvensis, Medicago sativa, Lupinus luteus,* nor *Pisum sativum.*

Conidia from *Pisum sativum* will infect *P. sativum* but not *Lupinus luteus, Colutea arborescens, Onobrychis sativa,* nor *Trifolium pratense.*

It is interesting to note that the mildew on the oats was able to infect a number of other species of grasses belonging to the same genus whereas the mildew on *Trifolium pratense* was unable to pass over to any other species of the same genus.

Salmon (12) studied still further the infection power of the conidia of several species of mildews. His results are as follows:

1. Conidia of *Erysiphe graminis* from *Avena sterilis* will infect *A. sterilis, A. sativa, A. pratensis,* but not *Arrhenatherum avenaceum, Lolium temulentum, Festuca elatior, Bromus unioloides,* nor *B. sterilis.*

Conidia from *Agropyron repens* will infect *A. repens, A. tenerum, A. caninum,* but not *A. glaucum* nor *A. acutum.*

Conidia from *Poa pratensis* will infect P. *pratensis, P. annua,** and *P. nemoralis,** but not *Festuca elatior* var *pratensis, F. arundinacea, F. heterophylla, Lolium perenne, L. temulentum, Dactylis glomerata, Phleum pratense, Alopecurus pratensis, Avena sativa, Hordeum vulgare, Triticum vulgare, Secale cereale,* nor *Agropyron repens.*

Conidia from *Dactylis glomerata* will infect *D. glomerata* but not *Avena sativa, Secale cereale, Triticum vulgare, Lolium temulentum, Hordeum vulgare,* nor *Agropyron repens.*

2. Conidia of *Sphaerotheca humuli* from *Potentilla reptans* will infect *P. reptans* but not *Alchemilla vulgaris, A. arvensis, Fragaria* sp. (cult), *Spiraea Ulmaria, Agrimonia Eupatoria,* nor *Potesium officinale.*

3. Conidia of *Sphaerotheca humuli* var. *fuliginea* from *Taraxacum officinale* will infect *T. officinale* but not *Plantago media, P. lanceolata,* nor *Fragaria* sp. (cult.)

Conidia from *Plantago lanceolata* will infect *P. lanceolata* but not *P. major* nor *Taraxacum officinale.*

4. Conidia of *Erysiphe cichoracearum* DC. from *Plantago major* will infect *P. major, P. media,* but not *P. lanceolata, Galium Aparine* nor *Eupatorium cannabinum.*

5. Conidia of *Erysiphe galeopsidis* DC. from *Ballota nigra* will infect *B. nigra* but not *Salvia verticillata* nor *Leonurus Cardiaca.*

Salmon (11) also made some infection experiments with ascospores from *Hordeum vulgare* during the spring of 1903. The ascospores infected *Hordeum vulgare, H. zeocriton,* and *H. trifurcatum,* but not *Triticum vulgare, Avena sativa, Secale cereale, H. maritimum, H. secalinum, H. jubatum,* nor *H. bulbosum.*

The ascospores corresponded in their infecting powers to the conidia from the same host except in the case of *H. jubatum.* Marchal says that the conidia from *H. vulgare* will infect *H. jubatum.* Salmon states, on the contrary, that he has not been able to infect *H. jubatum* with conidia from *H. vulgare.* However, so far as the infecting power of the ascospores alone is concerned, one experiment is not sufficient to determine.

As a result of the investigations summarized above, it may be

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* "Sub-infection."

assumed as proven that there are physiological differences between the mildews of one morphological species by which it is split up into a number of forms, each limited to one or few host plants. In what these differences consist is by no means yet determined. Salmon (13), during the summer of 1903, carried on a series of experiments to determine the behavior of these specialized forms under specially controlled conditions. He cut out of the leaf to be inoculated some of the epidermal cells on one surface and most of the mesophyll tissue. The epidermal cells on the other surface were left intact. The spores were sown upon these uninjured epidermal cells and the leaf was then placed with the cut surface downwards in a petri dish. Salmon found that when the leaves were injured in this way that they were able to bridge the fungus over to other host plants although the healthy leaves of these same plants are immune to the attacks of the fungus. For example, under these conditions spores from wheat will infect barley, oats and *Hordeum sylvaticum;* spores from barley will infect wheat, oats, *Hordeum murinum* and *H. sylvaticum;* spores from *Bromus commutatus* will infect *B. racemosus, B. asper* and *Hordeum sylvaticum;* spores from *Bromus secalinus* will infect *B. asper* and barley; spores from *Avena strigosa* will infect barley and wheat. He further found that spores from wheat will infect *T. monococcum* and *T. dicoccum.* All of these are cases in which no infection would occur on uninjured leaves.

Salmon also sowed the spores produced on these injured leaves on other injured leaves of the same plants and found that they were able to produce infection. In this way the viability of the spores formed on the injured leaves was proven. It was further discovered that spores produced on injured leaves of *Hordeum sylvaticum* by inoculating with conidia from wheat were in turn able to infect healthy leaves of *H. sylvaticum.*

These investigations indicate that although there are definite physiological species, normally restricted to one or a few host plants, yet injured host plants of one physiological species may be infected by spores of another physiological species and, in this way, the mildew may be bridged over from one host plant to another.

Various terms have been applied to these physiologically different varieties, as ''special forms,'' ''biologic forms,'' ''adapted races,'' ''biologic species,'' ''physiological species.'' Some regard such specialized forms as incipient morphological species. It is possible that the specialized form of mildew adapted to growth upon wheat, but not on other grasses, is in course of becoming a species of *Erysiphe* which may eventually show morphological differences in addition to the physiological ones it already manifests.

Salmon believes that the physiological species are restricted to certain host plants because of the presence of certain enzymes or toxins, or perhaps both, in the cells of the parasite and antitoxins, or similar substances, in the cells of the host plant. Under normal conditions certain substances peculiar to each species of host plant are able to prevent the attacks of any mildew except the specialized form adapted to withstand these substances. When the host plant is injured these substances are not formed by the cells in sufficient quantities to prevent the attack of other specialized forms of the fungus and accordingly infection results.

It is difficult to see how the spores from an injured leaf, which has become infected by the special form from another host plant, are able to infect uninjured leaves of the same plant. The cells of the healthy plant, of course, form these substances which normally inhibit the development of this special form of fungus. In one generation, however, the fungus becomes able to resist these substances and produce infection. The fungus must be extremely variable and must readily adapt itself to the substances formed by the cells of the plant infected. It is evident also that the differences between the various physiological species are very slight. They also probably differ among themselves as to the degree of their specialization.

Just to what extent injured plants may serve to bridge these specialized forms over from their own host plants to others must be determined much more fully. We shall probably find that the various specialized forms differ remarkably in this respect. The farther apart the host plants are in their characters the more difficult will it be for the specialized form on the one to pass over to the other.

Fifty-five species of grasses have been reported as infected by this one mildew, *Erysiphe graminis* DC (14). In fact all grass mildews are included in this one abundant and cosmopolitan species. Of this number only sixteen species have been reported in this country as being infected with the mildew: *Agropyron glaucum* (3) (6), *A. tenerum* (4), *Agrostis exarata* (3), *Avena* sp. *indet.* (3), *Beckmannia erucaeformis* (3), *Bromus unioloides* (4), *B. breviaristatus* (4), *Elymus condensatus* (3), *Glyceria nervata* (3), *Hordeum jubatum* (3), *Poa pratensis* (3) (4) (9) (16), *P. tenuifolia* (3) (6), *P. nemoralis* (4), *P. serotina* (4), *Secale cereale, Triticum vulgare* (3).

This mildew occasionally causes serious damage to forage and cereal crops, especially to wheat. Serious outbreaks of the mildew have occurred in Europe on winter sown cereal crops. Anderson (1), speaking of the attacks of the disease in Montana, says: "It affects chiefly the *Poas* and is especially damaging to *P. tenuifolia,* one of the most valued forage grasses."

During the last year, I have carried on infection experiments using the conidia of *Erysiphe graminis* from the host plants *Poa pratensis* and *Secale cereale.* In the latter part of September, 1903, I brought into the greenhouse several sods of *P. pratensis* infected by the mildew. The grass grew for most of the winter but gradually died out. In order to keep a good supply of conidia on hand, seed of *P. pratensis* was sown and the young seedlings were placed beside the sods. They became infected and served as a source for obtaining conidia for further infections. In this way, the fungus has been kept growing in the greenhouse from the last of September, 1903 to February 1st, 1905.

About the last of October, some rye which was growing in the greenhouse was discovered to be infected with mildew. This mildew was also kept growing in the greenhouse, by the methods noted above, from the last of October to the first of July. During the winter, the rye seedlings lived about six weeks and, in order to have a supply of conidia, it was necessary to plant seed every four or five weeks and inoculate the young seedlings.

With the mildew on these two host plants, a series of infection experiments was carried on to test their power of passing over to and growing upon other grains and grasses. Most of the experiments were performed by placing one and one-half inch flower pots, containing the seedlings used, under a bell jar. The bell jar was left over them from twenty-four to forty-eight hours and, in many cases, during the course of the experiment. All the seedlings in one half of the pot were inoculated, those in the other half serving as controls. An ordinary wooden label bearing the name of the plant, the date when the seed was planted, and the date of the inoculation, was placed in the pot in such a way as to separate the inoculated seedlings from the controls. The cereals were grown in the pots used in the experiments. The other grasses were grown in larger pots and a few seedlings transferred to the small ones for the experiments.

Four or five pots, containing usually as many different grasses, were placed under the same bell jar. In every experiment, one pot contained uninfected seedlings of the host plant used as a source of conidia for inoculation. In this way it was always possible to tell whether good, viable spores had been used in inoculating the seedlings for, if the conidia infected seedlings of their own host plant, their viability was demonstrated.

For most of the experiments in which rye seedlings were inoculated, the Schlamstead variety of rye was used. In a few experiments another variety, the Petkus, was taken. The mildew appeared to infect the two varieties with equal readiness.

The young seedlings of the cereals appeared in from four to six days after sowing the seed. The oat seed was longer in germinating than the others. The grasses came up in from ten days to two weeks after the seed was sown. The age of the seedlings as stated in the tables is reckoned from the time of sowing the seed. In most cases, the first leaf of the seedlings of the grains was inoculated, although in older seedlings other leaves were also inoculated.

In some cases, another method of experimentation, suggested by Salmon (10) was employed. Green, vigorous leaves of grasses or grains were placed on a moist filter paper in a Petri dish. Some of the leaves were inoculated, the rest being kept as controls. Under these conditions the leaves retained their vitality long enough to determine whether infection would take place.

The inoculations were made by removing with a scalpel a few spores from an infected leaf and applying them to the leaf to be inoculated. As the spores, under ordinary conditions, are light and dry, it was difficult to get them to stick to the leaf to be inoculated. This difficulty was overcome by placing infected leaves on a moist filter paper in a Petri dish and leaving them a few hours. In this way a very abundant supply of moist conidia was secured which readily adhered to a leaf when applied. Care had to be taken not to leave the spores too long before using them as they readily germinate under such conditions. The time elapsing between inoculation and the first appearance of infection was fairly constant. Patches of mycelium appeared in from three to four days if infection occurred at all. By the fifth day conidia were very abundant. The first observation recorded was made when the conidia had become abundant, not at the first appearance of the mycelium.

Before taking up the detailed account of my experiments it will be interesting to note some general differences in the behaviour of the mildew on the rye and blue grass. On the young rye seedlings the mildew spread very rapidly. When the leaves of seedlings placed beside well infected plants showed signs of infection, it was only two or three days until all of the seedlings were nearly covered with patches of mycelium bearing abundant conidia. In the case of *Poa pratensis* the conidia were not produced nearly so abundantly, nor did the fungus spread so rapidly. In one case a tuft of *P. pratensis* which was growing about two feet from another that was infected remained free from the mildew for almost three weeks. Then at first only a very few patches of mycelium appeared. It was fully two weeks longer before the mildew had spread over the entire tuft of the grass although the sod only covered an area about three inches in diameter.

The mycelium and conidia also differed markedly in color on the two host plants. The growths on the blue grass were pure white while those on the rye were quite pinkish in color.

When infected leaves were left in a Petri dish they gradually died, turning yellow. The cells in the infected areas of the rye leaf retained their green color for a longer time than the others. The fungus seemed to act upon the infected cells in such a way as to prolong their life. On the other hand the cells in the infected region of the leaves of the blue grass died first, the leaves turning yellow there, while the uninfected parts often remained green for several days afterward.

My first experiments were aimed to show the possibility of readily infecting leaves and seedlings of blue grass with conidia from the same host. The first five or six experiments failed. This was doubtless due to the fact that the spores used were either weak or dead. They were taken from the plants which had just been brought into the greenhouse and it was evident that conidia were not produced at all abundantly until after the plants had been in the greenhouse about three weeks. The mildew seemed to be brought to a standstill by the change to the greenhouse, not growing vigorously for some time. Later an abundance of conidia was produced on the infected grass and I then had no more trouble in transferring the mildew. After these first failures, forty-one additional experiments were performed in which the blue grass was inoculated with conidia from the same host and only three were unsuccessful.

A series of observations was next made to determine to what extent the mildew on the rye and blue grass had become specialized. Spores from both of these host plants were sown on leaves and seedlings of various grasses which are reported as hosts of *Erysiphe graminis.*

I have used for the most part grasses that are commonly cultivated and must hence have been repeatedly exposed in nature to infection from both rye and blue grass. Considerable difficulty was experienced in obtaining seed of many species of grasses. Seed of *Glyceria fluitans* was obtained from Currie Bros., Milwaukee, Wis.; seed of *Bromus mollis* from Vaughn's Seed Store, Chicago, Ill.; seed of *Lolium perenne, Festuca elatior, Dactylis glomerata* and *Poa nemoralis* from J. M. Thorburn & Co., New York; seed of *Poa compressa, Phleum pratense* and *Festuca heterophylla* from Peter Henderson & Co., New York; seed of *Poa trivialis* from the Bureau of Plant Industry,

U. S. Department of Agriculture. The seed of the cereals was obtained from Prof. R. A. Moore, Agronomist of the Wisconsin Agricultural experiment station.

The seed obtained from these various sources, in some cases, was not pure. Great care had to be taken to have only seedlings of the desired species of grass for the experiments. The seedlings of most of the grasses used have certain distinctive characters by which they can readily be distinguished. The greatest difficulty was with the various species of *Poas* as the young seedlings are quite similar in general appearance.

To test the results reported by Marchal spores from rye were sown upon wheat, oats, barley, *H. jubatum, Bromus mollis, Poa pratensis, P. trivialis, P. nemoralis* and *P. compressa.* The results of these experiments are given in the folowing table:

TABLE I.—*Conidia from Secale cereale.*

Plants on which spores were sown.	Total No. of experiments.	Age of seedlings. (Days.)	Total No. inoculated.	Results.		Rye.	
				On inoc. seedlings.	On controls.	No. of trials.	No. successful.
Triticum vulgare	24	7-12	66	—	—	20	18
Avena sativa...........	16	8-19	49	—	—	16	14
Hordeum vulgare	15	11-16	45	—	—	11	11
Hordeum jubatum......	8	16-56	32	—	—	8	8
Bromus mollis	5	28-56	12	—	—	5	5
Poa pratensis	12	16-101	75+	—?	—	10	10
Poa trivialis.............	6	26-44	35+	—	—	6	6
Poa nemoralis	9	52-110	40+	—	—	8	8
Poa compressa...	5	26-44	16	—	—	5	5

The sign — indicates no infection; —? indicates that in one experiment there was, apparently, infection, an account of which is given below. The bell jar was left over the seedlings either for 24 hours or 48 hours, or, in many cases, during the whole course of the experiment. Three to five different kinds of grasses were placed under the same bell jar, one of them always being rye; consequently in the table the record of the observations on the rye is repeated in several cases. The actual number of experiments with rye recorded in the table is fifty-two. Only two were unsuccessful.

These were all made by sowing spores on young seedlings and placing them in a moist atmosphere under a bell jar, except four of the experiments with wheat and two of those with blue grass which were made by Salmon's Petri dish method described above. Seedlings were left uninoculated in each experiment to serve as controls.

With one exception none of the seedlings or leaves inoculated became infected, although the experiments were continued from eight to fifteen days. The controls also remained free from the mildew. In one experiment, leaves of blue grass in a Petri dish were inoculated, and when examined after five days were found badly infected with the mildew. The pot containing the blue grass from which the leaves were taken for the experiment was then examined and it was found that the grass had, in some way, become infected with the mildew. Consequently there was no evidence in this experiment that the rye mildew could infect the blue grass. In the other eleven experiments in which blue grass was inoculated with spores from rye no infection occurred.

On the other hand the rye leaves and seedlings inoculated with conidia from rye in connection with the above experiments in every case except two had infected areas after about five days. During the same interval of time, none of the controls were infected. The seedlings were examined from day to day, the controls especially being watched closely. Patches of mycelium bearing conidia appeared on several of them eight or nine days after the experiment was started. Evidently some of the conidia that were first produced on the inoculated seedlings had fallen upon the controls and then germinated, producing infection. This infection occurred only after a period of time equal to that which had elapsed between inoculation and the first appearance of conidia on the inoculated leaves. The mildew was also much more widely spread over the inoculated seedlings when the controls became infected.

A further interesting experiment was made to show that the spores from rye would not infect the other cereals. On March 25th, seeds of the four cereals, wheat, rye, oats and barley, were sown in a large pot, about fifteen inches in diameter, one kind of seed in each quadrant of the surface. Shortly after the young seedlings came up they were all inoculated with conidia from rye. The seedlings were left growing until about the middle of July when they had matured, producing normal kernels. The rye seedlings became infected about four days after inoculation. The mildew was present on the rye during the whole time, conidia being produced abundantly. On the other hand, not one of the seedlings of wheat, oats or barley became infected, although conidia from the rye must have fallen repeatedly upon them.

Spores from blue grass were also sown upon several grasses that had been experimented with by Marchal and with similar results. Seedlings of rye, wheat, oats, barley, *H. jubatum*, and *B. mollis* were used. The following table presents the results:

TABLE II.—*Conidia from Poa pratensis.*

Plants on which spores were sown.	Total No. of experiments.	Age of seedlings.	No. inoculated.	Results.		Blue grass.	
				On inoc. seedlings.	On controls.	No. of trials.	No. successful.
Secale cereale	12	7-12	28+	—?	—	5	5
Triticum vulgare	9	10-16	28	—	—	9	7
Avena sativa............	9	8-19	29	—	—	9	8
Hordeum vulgare	10	8-16	24	—	—	10	8
Hordeum jubatum	6	16-53	17	—	—	6	6
Bromus mollis....	5	30-40	12	—	—	5	5

The sign — indicates no infection; —? indicates that in one experiment there was, apparently, infection, an account of which is given below. The belljar was left over the seedlings for 24 hours or 48 hours, or in many cases, during the whole course of the experiment. Three to five different kinds of grasses were placed under the same belljar, one of them always being blue grass; consequently in the table the record of the observations on the blue grass is in several cases repeated. The actual number of experiments with blue grass recorded in the table is twenty-three. Only two were unsuccessful.

All of these experiments were made by the bell jar method except four of those with rye which were made by placing rye leaves in a Petri dish. Control seedlings were kept in each experiment. No infection occurred except in one instance. In this case four rye leaves were placed in a Petri dish and two of them inoculated with spores from the blue grass. In the same dish two other rye leaves were inoculated with conidia from rye and two were kept as controls. When examined a week after inoculation all of the eight leaves had patches of mycelium with conidia. This infection was doubtless due to allowing some of the rye spores used for inoculating two of the leaves to fall on the other six. In none of the other eleven experiments in which rye was inoculated with conidia from blue grass did infection occur.

In connection with these same experiments seedlings of blue grass were inoculated with spores from the blue grass. Altogether twenty-three experiments were made, several of the seedlings in each case not being inoculated. Infection occurred on the inoculated seedlings in all of the experiments except two. None of the controls became infected.

My experiments with conidia from rye and blue grass thus confirm Marchal's results for the forms considered.

Marchal also states that the same special form of mildew occurs on all of a number of species of *Poa*. I have made a number of inoculations with the mildew from *P. pratensis* upon *P. nemoralis, P .trivialis* and *P. compressa* and secured some interesting results which do not entirely confirm Marchal's statement. My results are given in detail in the following table and additional notes are given below.

TABLE III.— *Conidia from Poa pratensis.*

No. of Experiment.	Date.	Plants on which spores were sown.	Age of seedlings.	No. inoculated.	No. of controls.	Observations.				
						Date.	Inoc. seedlings.	Controls.	Blue grass.	
									Inoc. seedlings.	Controls.
45 a.	Dec. 31	*Poa nemoralis....*	24	‖	‖	Jan. 7 12	+ "	— "	++	—
46b.	"	" "	57	"	"	7 12	" "	" "	" "	" "
49a.	Jan. 2	" "	26	"	"	7 12	" "	" "	" "	" "
50a.	6	" "	30	"	"	12 19	" "	" "	" "	" "
53a.	9	" "	33	"	"	19 26	— "	" "	" "	" "
54a.	9	" "	33	2	"	19 26	" "	" "	— +	" "
64c.	16	" "	40	‖	"	26 29	" "	" "	++ "	" "
65b.	25	" "	49	"	"	Feb. 1 8	++ "	" "	" "	" "
66b.	25	" "	49	2	"	1 8	" "	" "	" "	" "
67a.	25	" "	49	‖	"	1 8	" "	" "	" "	" "
68b.	27	" "	51	"	"	6 11	— "	" "	" "	" "
69a.	27	" "	51	"	"	6 11	" "	" "	" "	" "
74a.	30	" "	54	"	"	6 15	++ "	" "	" "	" "

Table III.—*Conidia from Poa pratensis*—Continued.

No. of Experiment.	Date.	Plants on which spores were sown.	Age of seedlings.	No. inoculated.	No. of controls.	Observations.				
						Date.	Inoc. seedlings.	Controls.	Blue grass.	
									Inoc. seedlings.	Controls.
76b .	Feb. 2	" "	33	||	||	8 17	+ "	— "	++ "	— "
77a .	2	" "	33	"	"	11 17	" "	" "	" "	" "
78c .	2	" "	33	"	"	11 17	— "	" "	" "	" "
80a .	11	" "	66	"	"	21 26	" "	" "	" "	" "
81d .	13	" "	66	"	"	20 26	++ "	" "	" "	" "
82a .	13	" "	66	"	"	20 26	— "	" "	" "	" "
95d .	Mar. 5	*Poa trivialis*......	30	4	3	Mar. 15 19	" "	" "	— "	" "
100a .	8	" "	33	3	2	19 23	" "	" "	+ "	" "
101b .	14	" "	39	||	"	23 30	" "	" "	++ "	" "
102c .	14	" "	39	"	"	23 30	" "	" "	+ "	" "
103a .	15	" "	40	"	"	23 30	+ —	" "	" "	" "
104b .	15	" "	40	"	"	23 30	" "	" "	++ "	" "
107a .	23	" "	23	8	10	30 Apr. 6	+ "	" "	+ "	" "
108a .	23	" "	23	14	6	Mar. 30 Apr. 6	— "	" "	++ "	" "
95c .	5	*Poa compressa* ...	30	7	3	Mar. 15 19	" "	" "	— "	" "
100b .	8	" "	33	4	3	19 23	" "	" "	+ "	" "
101a .	14	" "	39	||	||	23 30	" "	" "	++ "	" "
102b .	14	" "	39	"	"	23 30	" "	" "	+ "	" "
103c .	15	" "	40	"	"	23 30	" "	" "	" "	" "
104a .	15	" "	40	"	"	23 30	" "	" "	++ "	" "
107b .	23	" "	23	12	14	30 Apr. 6	+ "	" "	+ "	" "
108b .	23	" "	23	5	7	Mar. 30 Apr. 6	— +	" "	++ "	" "

The sign ++ indicates good infection; + fair infection; — no infection. || indicates that several seedlings, usually eight to ten, were either inoculated or kept as controls. In the table the record of the observations on the blue grass is repeated. The actual number of experiments with the blue grass recorded in the table is twenty-seven. Two were unsuccessful. The blue grass seedlings were from 43 to 96 days old.,

Poa nemoralis.

The Wood meadow grass is now classed among our good grasses for shaded pastures. It is particularly valuable for shaded lawns.

The seedlings are very similar to those of blue grass. They can generally be distinguished by their wider leaves and more vivid green color.

Spores from rye produced no infection on this grass. The experiments with conidia from blue grass were doubtful in their results and quite interesting. As seen from the table, nineteen experiments were made in which spores from blue grass were sown upon the seedlings of *P. nemoralis.* The inoculated seedlings in eleven of these experiments became infected with mildew; in the other cases there were no signs of infection. None of the uninoculated seedlings in any experiment became infected. In six of the experiments in which infection occurred there was a vigorous growth of the mildew, fully as good a growth as was present upon the seedlings of blue grass which were under the same bell jar. In the remaining five experiments in which infection occurred, there were only a few small infected areas, while the seedlings of blue grass in the same experiments had numerous small infected areas producing conidia very abundantly.

The seed was not pure as some of the grass was allowed to grow until it flowered and it was found that *P. pratensis* was also present. It is possible that some of the seedlings infected were those of *P. pratensis;* still I am sure that most of the seedlings that were infected were seedlings of wood meadow grass.

Some of the infected seedlings of the wood meadow grass were kept with a view of determining whether the mildew would pass from them to other grasses, especially blue grass. The mildew, however, never developed on the wood meadow grass with sufficient vigor to make it possible to get sufficient spores for such experiments.

Poa trivialis.

The Rough-stalked meadow grass, like Wood meadow grass, is well adapted for shaded places. It is a spreading, thickly matting, stoloniferous species. The young seedlings are narrower leaved than those of the other *Poas* considered and lighter green in color. They resemble the Kentucky blue grass, however, quite closely.

Eight experiments were made in which spores from blue grass were sown upon the seedlings of *P. trivialis.* A slight infection occurred in only two experiments. In each of the experiments 103 a and 107 a one patch of mycelium with conidia was present on an inoculated seedling. These patches had disappeared when the seedlings were examined a second time a few days later. In these two experiments the seedlings of blue grass inoculated with spores from blue grass and placed under the same bell jars with *Poa trivialis,* became only slightly infected.

Poa compressa.

Canada blue grass is not reported so far as I can find as a host of *E. graminis.* As seen from the table eight experiments were made in which seedlings of this grass were inoculated with spores from Kentucky blue grass. It is a very hardy grass, growing on poor and dry soils. It has creeping rootstalks, forming a close and durable turf. It is distinguished from the Kentucky Blue Grass by its flattened wiry stems and by its decidedly bluer color.

None of the seedlings inoculated with spores from the blue grass became infected except in two experiments. In one of these, 107 b, a small patch of mycelium bearing conidia was present on one inoculated leaf. In experiment 108 b there was no visible infection when first examined seven days after inoculation. When examined a week later two small infected areas were present on one inoculated seedling. In the first of these two experiments, the seedlings of blue grass inoculated with spores from the same host and placed under the bell jar with *P.*

compressa bore only a few patches of mycelium; conidia were not produced abundantly. In the other experiment, however, there was a very good infection of blue grass.

As seen from these experiments the mildew on *P. pratensis* will not readily infect the other species of *Poa* experimented with. Still under certain conditions it seems able to pass over to some extent to *P. nemoralis,* and, in a lesser degree, to *P. trivialis* and *P. compressa.* It is possible that there is a "sub-infection", as Salmon calls it, in these cases. The question of the purity of the seed and the identification of the seedlings is very important in connection with experiments on the various species of *Poa.*

My observations indicate that the group is an interesting one and further experiments may be expected to throw light on the real nature of physiological species and their method of origin.

The facts thus far brought out lead us to expect that among the mildews on the remaining grasses which have been reported as hosts many will be found which have become more or less physiologically specialized and limited in their power of producing infection. I have further carried out a series of experiments with spores of rye and blue grass mildews to determine, if possible, whether these forms can pass over to any other grasses the seed of which I could obtain. All of these grasses are reported as hosts of *Erysiphe graminis* DC. The results of these experiments are summarized in the following tables and further notes on each grass are given below.

TABLE IV.—*Conidia from Secale cereale.*

No. of Experiment.	Date.	Plants on which spores were sown.	Age of seedlings.	No. inoculated.	No. of controls.	Observations.				
						Date.	Inoc. seedlings.	Controls.	Rye.	
									Inoc. seedlings.	Controls.
55b.	Jan. 11	*Lolium perenne* ..	......	3	11	Jan. 19 26	— “	— “	++ “	— “
56a.	11	“ “	......	3	“	19 26	“ “	“ “	“ “	“ “
57c.	11	“ “	......	3	“	19 26	“ “	“ “	“ “	“ “
61b.	16	“ “	......	6	4	26 29	“ “	“ “	“ “	“ +
62a.	16	“ “	......	5	4	26 29	“ “	“ “	“ “	— +
72b.	28	“ “	......	11	11	Feb. 5 13	“ “	“ “	“ “	— +
87b.	Feb. 22	“ “	......	10	6	29 Mar. 5	“ “	“ “	“ “	+ ++
88a.	22	“ “	......	3	6	Feb. 29 Mar. 5	“ “	“ “	“ “	— +
93d.	27	“ “	......	6	7	5 10	“ “	“ “	“ “	— “
55a.	Jan. 11	*Festuca elatior* ...	26	4	11	Jan. 19 26	“ “	“ “	“ “	— “
56c.	11	“ “	26	3	“	19 26	“ “	“ “	“ “	“ “
57a.	11	“ “	26	3	“	19 26	“ “	“ “	“ “	“ “
62b.	16	“ “	16	3	3	26 29	“ “	“ “	“ “	“ +
72c.	28	“ “	28	11	11	Feb. 5 13	“ “	“ “	“ “	— +
73c.	30	“ “	30	2	4	6 13	“ “	“ “	“ “	— +
87d.	Feb. 22	“ “	23	3	4	29 Mar. 5	“ “	“ “	“ “	+ ++
88c.	22	“ “	23	3	5	Feb. 29 Mar. 5	“ “	“ “	“ “	— +
90d.	27	*Festuca heterophylla*	23	9	7	5 10	“ “	“ “	“ “	— +
97b.	Mar. 5	“ “	30	5	10	12 19	“ “	“ “	“ “	— +
98b.	5	“ “	30	10	7	12 19	“ “	“ “	“ “	— +
99b.	8	“ “	33	3	3	15 23	“ “	“ “	“ “	— +
105c.	19	“ “	44	3	2	24 30	“ “	“ “	“ “	— +
106d.	19	“ “	44	4	2	24 30	“ “	“ “	“ “	— +
110c.	26	“ “	51	3	6	Mar. 30 Apr. 6	“ “	“ “	“ “	— +
112c.	26	“ “	51	5	5	Mar. 30 Apr. 6	“ “	“ “	“ “	— +

TABLE IV.—*Conidia from Secale cereale*—Continued.

No. of Experiment.	Date.	Plants on which spores were sown.	Age of seedlings.	No. inoculated.	No. of controls.	Observations.				
						Date.	Inoc. seedlings.	Controls.	Rye.	
									Inoc. seedlings.	Controls.
55c .	Jan. 11	*Dactylis glomerata*	35	3	||	Jan. 19	—	—	++	—
						26	"	"	"	"
56b .	11	" "	35	3	"	19	"	"	"	"
						26	"	"	"	"
57b .	11	" "	35	2	"	19	"	"	"	"
						26	"	"	"	"
61a .	16	" "	40	2	3	26	"	"	"	"
						29	"	"	"	+
71c .	28	" "	52	2	3	Feb. 5	"	"	"	—
						13	"	"	"	"
73a .	30	" "	54	2	3	6	"	"	"	"
						13	"	"	"	+
79d .	Feb. 11	" "	57	||	||	17	"	"	"	—
						26	"	"	"	+
89c .	22	" "	68	2	6	29	"	"	"	+
						Mar. 5	"	"	"	++
87a .	22	*Phleum pratense.*	18	8	4	Feb. 29	"	"	"	+
						Mar. 5	"	"	"	++
88d .	22	" "	18	6	11	Feb. 29	"	"	"	—
						Mar. 5	"	"	"	+
89a .	22	" "	18	6	4	Feb. 29	"	"	"	+
						Mar. 5	"	"	"	++
90b .	27	" "	23	9	7	5	"	"	"	—
						10	"	"	"	+
91c .	27	" "	23	4	5	5	"	"	"	—
						10	"	"	"	"
92a .	27	" "	23	12	4	5	"	"	"	—
						10	"	"	"	+
93c .	27	" "	23	||	||	5	"	"	"	—
						10	"	"	"	"
97a .	Mar. 5	" "	30	3	8	12	"	"	"	—
						19	"	"	"	+
111c .	26	" "	51	4	4	30	"	"	"	—
						Apr. 6	"	"	"	+
105d .	19	*Glyceria fluitans*	19	2	3	Mar. 24	"	"	"	—
						30	"	"	"	+
106c .	19	" "	19	6	8	24	"	"	"	—
						30	"	"	"	+
109d .	26	" "	26	5	6	30	"	"	"	—
						Apr. 6	"	"	"	+
110b .	26	" "	26	3	3	Mar. 30	"	"	"	—
						Apr. 6	"	"	"	+
112d .	26	" "	26	3	5	Mar. 30	"	"	"	—
						Apr. 6	"	"	"	+

In the table the record of the observations on the rye is repeated. The actual number of experiments with rye recorded in the table is twenty-six. All were successful. The rye seedlings were from 6 to 19 days old.

The sign ++ indicates good infection, + fair infection, — no infection.

|| indicates that several seedlings, usually eight to ten, were either inoculated or kept as controls.

TABLE V.—*Conidia from Poa pratensis.*

No. of Experiment.	Date.	Plants on which spores were sown.	Age of seedlings.	No. inoculated.	No. of controls.	Observations.				
						Date.	Inoc. seedlings.	Controls.	Blue grass.	
									Inoc. seedlings.	Controls.
45b .	Dec. 31	*Lolium perenne..*	24	11	11	Jan. 7 12	— "	— "	++ "	— "
46a .	31	" "	24	"	"	7 12	" "	" "	" "	" "
49b .	Jan. 2	" "	26	"	"	7 12	" "	" "	" "	" "
50b .	6	" "	30	"	"	12 19	" "	" "	" "	" "
53b .	9	" "	33	"	"	19 26	" "	" "	" "	" "
54c .	9	" "	33	2	"	19 26	" "	" "	— +	" "
63c .	16	" "	40	5	"	26 29	" "	" "	++ "	" "
75a .	30	" "	54	11	"	Feb. 6 15	" "	" "	" "	" "
76d .	Feb. 2	" "	57	"	"	8 17	" "	" "	" "	" "
85a .	15	" "	70	5	7	22 29	" "	" "	" "	" "
51b .	Jan. 6	*Festuca elatior...*	21	11	11	Jan. 12 19	" "	" "	" "	" "
69b .	27	" "	42	"	"	Feb. 6 11	" "	" "	" "	" "
70a .	27	" "	42	"	"	6 11	" "	" "	" "	" "
74c .	30	" "	45	"	"	6 15	" "	" "	" "	" "
76 a.	Feb. 2	" "	48	"	"	8 17	" "	" "	" "	" "
77b .	2	" "	48	"	"	11 17	" "	" "	" "	" "
86c .	17	" "	17	6	2	29 Mar. 2	" "	" "	" "	" "
51a .	Jan. 6	*Dactylis glomerata*	30	11	11	Jan. 12 19	" "	" "	" "	" "
53c .	9	" "	33	"	"	19 26	" "	" "	" "	" "
54b .	9	" "	33	"	"	19 26	" "	" "	— +	" "
68a .	27	" "	51	"	"	Feb. 6 11	" "	" "	++ "	" "
70b .	27	" "	51	"	"	6 11	" "	" "	" "	" "
74b .	30	" "	54	"	"	6 15	" "	" "	" "	" "

TABLE V.—*Conidia from Poa pratensis*—Continued.

No. of Experiment.	Date.	Plants on which spores were sown.	Age of seedlings.	No. inoculated.	No. of controls.	Observations.				
						Date.	Inoc. seedlings.	Controls.	Blue grass.	
									Inoc. seedlings.	Controls.
76c .	Feb. 2	*Dactylis glomerata*	48	"	"	8	—	—	++	—
						17	"	"	"	"
77c .	2	" "	48	"	"	11	"	"	"	"
						17	"	"	"	"
78a .	2	" "	48	"	"	11	"	"	"	"
						17	"	"	"	"
86b .	17	" "	63	"	"	24	"	"	"	"
						Mar. 2	"	"	"	"
94a .	Mar. 2	*Phleum pratense.*	27	7	"	8	"	"	"	"
						15	"	"	"	"
96a .	5	" "	30	6	"	15	"	"	—	"
						19	"	"	"	"
100c .	8	" "	33	7	"	19	"	"	+	"
						23	"	"	"	"
101c .	14	" "	39	11	"	23	"	"	++	"
						30	"	"	"	"
102a .	14	" "	39	"	"	23	"	"	+	"
						30	"	"	"	"
103b .	15	" "	40	"	"	23	"	"	"	"
						30	"	"	"	"
104c .	15	" "	40	"	"	23	"	"	++	"
						30	"	"	"	"

In the table the record of the observations on the blue grass is repeated. The actual number of experiments with the blue grass recorded is twenty five. Only one was unsuccessful. The blue grass seedlings were from 43 to 96 days old.

Lolium perenne.

English Rye grass is a valuable pasture grass and is grown abundantly associated with both the cereal grains and blue grass. Like the latter it forms a perennial green sward. It is, however, not closely related to either rye or blue grass and all attempts to infect it with conidia from these failed. Mildew has not been reported upon the English rye grass in this country. The seed germinates quickly, the young seedlings coming out of the soil in about ten days. It is favorable for experimentation for the seedlings grow rapidly. They are bright green and very slender. The seed used seemed to be entirely pure.

Festuca elatior and *F. heterophylla.*

Both of these grasses are natives of Europe and are grown as hay and pasture grasses. The tall meadow Fescue is a coarse growing grass. The young seedlings appeared above the soil in about twelve days after the seed was sown. They grew vigorously, becoming rather broad leaved and coarse.

The seedlings of the various leaved Fescue were a little darker green in color than those of *F. elatior.* They were also a little longer in appearing above the soil, about two weeks elapsing after the seed was sown before they appeared.

Spores from rye were sown on both of these grasses while spores from blue grass were sown on *F. elatior* only. No infection occurred.

Dactylis glomerata.

Orchard grass is grown quite extensively for forage purposes. It has the habit of growing in tufts. It is easy to work with as the seedlings grow vigorously and rapidly. They appear in two weeks after sowing. The mildew has not been reported upon the grass in this country. Spores from neither rye nor blue grass were able to infect it.

Phleum pratense.

Timothy is a native of Europe, but was naturalized in America many years ago. It ranks in this country as by far the most important of hay grasses and it is certainly commonly associated with the grains. The seed germinates quickly, the young seedlings appearing in about nine days. The seedlings grow rapidly and are very hardy. No infection occurred from inoculation with spores from either rye or blue grass.

Glyceria fluitans.

Floating meadow grass is found in wet soils and marshes and its damp surroundings should favor the mildew. The young seedlings appear above the soil in ten days. They grow very rapidly becoming quite tall and slender. Only spores from rye were tried on the seedlings of this grass. No infection resulted.

In connection with these experiments the seedlings of rye that were inoculated with conidia from rye uniformly became infected. The same was also true in the case of the experiments in which blue grass was inoculated with conidia from blue grass, no infection occurring in only one case. The controls in every case were free from mildew when the inoculated seedlings became infected except in experiments 87 and 89 where some of the controls near the inoculated leaves had small infected areas on them. In the case of the rye many of the controls in several experiments when examined eight to ten days after the inoculations were made had numerous small infected areas on them. This was doubtless due to some of the spores that were formed on the infected leaves falling upon the controls. The inoculated seedlings also had several additional infected areas on them when the infection of the controls was observed.

These experiments confirm the general conclusion that spores of the mildew from one grass will not infect a grass belonging to a different genus. Marchal, it is true, states that the form on oats is the same as that on *Arrhenatherum elatius.* Salmon, however, was unable to verify this statement.

It is entirely possible that for *E. graminis* at least, there is one, if not more, distinct physiological species for each genus of grasses that contains species which are hosts for this mildew. In many genera of grasses the specialization has undoubtedly gone still further so that there may be a number of physiological forms upon the various species of the same genus. This is Salmon's conclusion in the case of the Brome grasses and my work with the various Poas leads me to the conclusion that there is more than one at least partially differentiated physiological form for this genus also.

Further investigations will probably show that some physiological forms are much more fixed in their characters than others, just as is the case with morphological species. Such special forms will doubtless be found to grade over into each other and we may thus get interesting evidence as to their developmental history.

This work has been done under the direction of Prof. R. A. Harper and I am greatly indebted to him for his kindly criticisms and valuable suggestions.

Madison, Wisconsin,
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THE STATE ADMINISTRATION OF TAXATION IN WISCONSIN.

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I. THE STATE BUDGET.

II. THE VARIOUS SYSTEMS OF TAXATION.
  1. The General Property Tax.
  2. The Corporation Tax.
  3. The Inheritance Tax.
  4. The Suit Tax.
  5. Miscellaneous License Taxes.

The purpose of this paper is to describe the changes in the form and in the powers of the central administration of taxation in Wisconsin since the organization of the territory, but it is only indirectly concerned with the actual workings of the system, and not at all with the economic phases of the subject.

I. THE STATE BUDGET.

It is only a little that the administration has to do with the budget.

During the territorial period the congressional appropriations for "the contingent expenses" of the territory were made upon estimates submitted by the secretary of the treasury of the United States.[1] In 1836 the president of the state senate and the speaker of the house were directetd to prepare estimates as the basis of the secretary's action,[2] but these

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[1] O. L., s. 11.

[2] H. J. 1836, pp. 127, 141.

preliminary estimates were made by the governor apparently during most of the period.[1] No officer was charged with preparing estimates for expenses to be paid out of the territorial treasury.

Since 1848 the secretary of state has been required to make detailed estimates of the expenses to be defrayed from the state treasury as a basis for the action of the legislature.[2] The secretary has based his estimate almost wholly upon the income received by the various departments during the preceding year, though recently the tax commission has recommended that all departments file preliminary estimates with him.[3]

II. THE VARIOUS SYSTEMS OF TAXATION.

Till recent years the general property tax has been the most important source of the state revenue, and legislation has been directed for the most part to this kind of taxation. But with the growth of the corporation tax, the general property tax has practically disappeared as a source of state revenue, and henceforth the central administration will be interested in this tax only in the control, which it has recently obtained, of the administration of taxation by the local authorities. The chief interest in state administration of taxes is now divided between the problems of this central control and the problems of the taxation of corporations. The minor sources of taxation have some interest on account of the peculiarities of their administration.

1. *The General Property Tax.*

A. The Levy of State Taxes.

Of course the general property tax has been levied, in the usual sense of that word, only by the legislature, till 1845 a percentage on the gross amount of taxes assessed by each county, and since that time a territorial or state tax levied in each county with the county tax.[4]

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[1] H. J. 1840–1, app., p. 85; C. J. 1842–3, app., pp. 49–53.

[2] L. June 1848, p. 115, s. 10 (2): R. S. 1898, s. 144 (13); L. 1901, c. 368.

[3] Especially L. 1901, c. 97; Tax Comm. Rpt., 1903, pp. 243–4.

[4] Const. Art. 8, s. 5; L. 1837–8, No. 93; E. g., L. 1845, p. 1, s. 2.

But since 1869, whenever, before the apportionment of the state tax to the counties, it is evident that the appropriations exceed the amount of state tax levied to meet the expenses of the year for which the tax was levied, the secretary of state is required to "levy" and apportion such an additional amount as may be necessary to meet all authorized demands on the state treasury up to the time when the next succeeding tax will be due.[1] For thirty years the validity of this measure seems not to have been questioned, but in 1899 both the governor and the secretary of state were of the opinion that under the constitution[2] the legislature itself is the only tax-levying authority of the state and that this power cannot be delegated; and the secretary therefore refused to make the additional levy called for by the statute under the circumstances.[3]

A somewhat analogous power was vested in a board consisting of the governor, secretary of state, and state treasurer by a law of 1887 which directs that whenever in the opinion of the board the public interest requires it, they may apply the surplus in the treasury, or so much of it as they deem proper, to reduce the state levy each year.[4]

B. The Assessment of Taxes.

Until 1852 the counties assessed the state taxes as they did their own, without any further control by the state adminisration than a requirement of the filing of a duplicate of the county tax and, a little later, also local valuation statistics with the territorial or state authorities.[5]

The inequality of the burden of taxation under the old system had long been a subject of complaint,[6] when the state assumed a greater control in 1852 by establishing a state board of equalization.

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[1] L. 1869, c. 153, s. 1: R. S. 1898, s. 1071.

[2] Const. Art. 8, s. 5.

[3] Wis. (Weekly) State Journal, June 16, 1899; Wis. State Journal, Sept. 28, Oct. 3, 12, 1899.

[4] L. 1887, c. 397: R. S. 1898, s. 1069a.

[5] L. 1837-8, No. 93, s. 2.

[6] Especially A. J. 1852, app., pp. 4-5.

a. The Development of the State Tax Commission.—The first board of equalization consisted of the governor, secretary of state, state treasurer, attorney general, and state superintendent,[1] the lieutenant governor and bank comptroller being added two years later.[2] Beginning with 1858, for the next fifteen years the board was composed of the state senate and secretary of state.[3] This change was induced partly because of the dissatisfaction with the data available for the use of the board in the returns from the counties, and partly by the "anti-republican" nature of the old board. The results of the change seem generally to have been bad. Rings were formed in the senate, and much logrolling took place to the great detriment of some sections.[4] Hence in 1873 a board composed of state officers was again established, the state board of assessment,[5] consisting of the secretary of state, state treasurer, and attorney general.[6] This board was replaced by the present commissioners of taxation in 1901.

The commission, the direct outgrowth of the tax commission of 1897-8 (an investigating body), was established in 1899, "in order to secure improved taxation within the state."[7] It consists of the commissioner, first assistant commissioner, and second assistant commissioner, all appointed by the governor with the consent of the senate, all known to the governor "to possess knowledge of the subject of taxation and skill in matters pertaining thereto," and serving ten years from 1899. Neither the commissioner nor the assistants nor any clerk in the office is permitted to "hold any other office or position of trust or

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[1] L. 1852, c. 498, s. 1.

[2] L. 1854, c. 73, s. 1.

[3] L. 1858, c. 115, s. 26.

[4] S. J. 1860, pp. 817–8; Wis. State Journal, Mar. 12, 1860; April 7, 23, May 5, 1868; Secy. State Rept. 1868, pp. 38–9; S. Proc. in Wis. State Journal, Feb. 4, 1874; Wis. (Weekly) State Journal, Dec. 10, 1878.

[5] The former board had been known as the Board of Assessors since 1870. (L. 1870, c. 144, s. 1.)

[6] L. 1873, c. 235.

[7] L. 1897, c. 340; Tax Comm. Rpt., 1897–8, p. 182; Wis. State Journal, April 18, 1899.

profit, or possess any other business or avocation, or serve on or under any committee of any political party.''[1]

In 1899 the commissioner was made a member of the state board of assessment, presided at its meetings, and assisted the board with his information.[2] Finally in 1901 the old board was abolished, and the commission of taxation became the state board of assessment.[3]

While the board consisted of the senate and secretary of state and met during the recess of the legislature, members received a per diem and mileage the same as the members of the legislature,[4] but the members of the former boards (all ex-officio) were paid no additional compensation for their services. The salary of the present commissioner is $5,000, and that of the assistants, $4,000 each.[5] In 1899 the necessary traveling expenses of the commission were allowed, and the commissioner was authorized to fix the number and compensation of any clerks in the office; but upon the governor's protest against placing so much discretion in the commissioner's hands, the maximum amount for all the disbursements of the office was later fixed by law.[6] However when the commissioners took over the taxation of railroads they were given unlimited power in the appointment of the necessary additional assistants etc., for the purpose.[7]

b. The Assessment of State Taxes; the State Supervision of Local Taxation.—By the law of 1852 the board was to meet annually to equalize the valuations made by the counties, ''to produce a just relation between the valuation of the taxable property in the state.''[8] The action of the various boards has always been annual with the exception of the years between 1859 and 1879, when it was biennial.[9] Since 1870 the

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[1] L. 1899, c. 206, ss. 1, 7.

[2] L. 1899, c. 206, s. 6.

[3] L. 1901, c. 237, ss. 1, 6; State Tax Comm. Rpt., 1900, p. 171; Gov. M. 1901, pp. 9–12.

[4] L. 1859, c. 167, s. 28.

[5] L. 1899, c. 206, s. 7; c. 322.

[6] L. 1899, c. 206, ss. 7, 9; L. 1901, c. 220, s. 2; Gov. M. 1901, p. 8.

[7] L. 1903, c. 315, s. 27.

[8] L. 1852, c. 498, s. 2.

[9] L. 1859, c. 167, s. 29; L. 1879, c. 124.

action has been recognized as assessment rather than equalization.[1]

The matter of getting correct returns from the localities has been the subject of much legislation. Before any central equalization was attempted, beginning with 1841 reports from the counties to the treasurer, auditor, or secretary were required, showing the local valuation of property.[2] After the creation of the first board began a further series of laws to secure proper returns of local valuation to the secretary of state as a basis of state equalization,[3] one of them authorizing the secretary of state to send a special messenger for the required statistics in case of the neglect of the county authorities.[4] But the returns have never been satisfactory.

The board did not even attempt to make an equalization before 1854, and at that time the secretary of state declared the false valuations received made any action on their basis "mere guess work."[5] It was claimed that the board of 1878 was the first body which had before it a complete set of returns from every county, and that theirs was "the first endeavor honestly to live up to the law and equalize in fact as well as in name."[6]

As early as 1861, in a complaint of the inequality of taxation on account of the false returns of property, the secretary of state declared it to be doubtful if a return of all property could be secured unless through the appointment of assessors by the governor or legislature, who by residence and tenure of office would be removed from local influence.[7] The state has not gone to this extremity, but the powers of the present tax commission would seem to exhaust all remedies up to this point.

In 1899 the commission was given "general supervision of the system of taxation throughout the state," but was really limited to making investigations and reporting the results to the leg-

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[1] L. 1870, c. 144, s. 1.

[2] L. 1840–41, No. 8, s. 6; L. 1843–4, p. 6, s. 6; R. S. 1849, c. 15, s. 41.

[3] E. g., L. 1854, c. 73, ss. 4, 6; L. 1881, c. 236, s. 4: R. S. 1898, ss. 1004 sq.; L. 1903, c. 315, s. 12.

[4] L. 1874, c. 43, s. 2: R. S. 1898, s. 1016.

[5] S. J. 1854, p. 510; Secy. State Rpt., 1854, pp. 43–4.

[6] Wis. (Weekly) State Journal, Dec. 10, 1878.

[7] Secy. State Rpt., 1861, p. 222.

islature with recommendations. In making such investigations the commission was empowered to require individuals and corporations to give information, to examine their records, to summon witnesses, etc., and to direct the attorney general to proceed against persons refusing their demands.[1]

In 1901 the powers of the commission were largely increased. In addition to the assessment of state taxes in each county made by the former boards, the commissioner has the following powers:

1. To "exercise general supervision of the system of taxation throughout the state;"
2. To exercise general supervision over the assessors, local boards of review, and the assessment of property in the localities by the county supervisors, " so that equality of taxation shall be secured according to law;"
3. To advise and direct assessors, boards of review, and county supervisors as to their duties under the statutes;
4. To direct that proceedings be instituted to enforce the laws relating to the liabilities of officers, corporations, and individuals for failure to comply with the tax laws; to cause complaint to be made to the proper circuit judge for their removal from office for official misconduct or neglect of duty—in all these cases to require the district attorney to assist in prosecution;
5. To require local officers to report information as to the assessment of property, collection of taxes, expenditure of public funds for all purposes, and any other information the commission may request;
6. To require individuals, corporations, etc., to furnish information concerning their capital, "and all other information called for;"
7. To summon witnesses to appear and testify in any matter deemed material to the investigation of the system of taxation and the expenditures of public funds for state and local purposes. Both the commissioner and his assistants are authorized to administer oaths to such witnesces. Refusals to testify are reported to the attorney general who is required to proceed against the offenders;

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[1] L. 1899, c. 206, ss. 3, 4.

8. To visit the counties for the investigation of the methods of the local authorities in the administration of taxation, and to examine into all cases where evasion of proper taxation is charged, access to all documents of the state and localities being allowed for such purposes; to ascertain wherever the existing laws are defective or improperly administrated, and to investigate the tax systems of other states and countries;

9. To formulate and recommend such legislation as may be found necessary to prevent the evasion of just and equal taxation and for the improvement of the system of taxation;

10. To consult with the governor upon the subject of taxation, and furnish him with such assistance and information as he may require;

11. To transmit to the governor, before each regular session of the legislature, a report showing the taxable property of the state, with recommendations and such measures as may be formulated for the consideration of the legislature; and to hand copies of this report to the members of the legislature.[1]

With the disappearance of the general property tax for state purposes, these powers of the commission are reduced wholly to the control of the local administration by the central administration.

c. The Apportionment of Taxes.—Under the system which existed till 1845, the treasurer of the territory simply demanded the amount of taxes due the territory according to the reports from the counties.[2] Under the present system the auditor or the secretary of state has apportioned and certified the state tax to the counties.[3]

d. The Collection of Taxes.—The secretary of state superintends the collection of all taxes as of all other moneys due the state.[4] The taxes have always been payable to the terri-

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[1] L. 1901, c. 220; c. 237; A. J. 1901, pp. 24–7.

[2] L. 1837–8, No. 93, s. 3.

[3] L. 1845, p. 1, s. 6; R. S. 1849, c. 15, s. 42: R. S. 1898, s. 1070.

[4] L. June 1848, p. 115, s. 10 (6): R. S. 1898, s. 144 (9). From 1859 to 1878 the secretary also, with the advice of the attorney general, was directed to decide all questions as to the construction of the tax laws, subject to an appeal to the supreme court. (L. 1859, c. 167, s. 50; R. S. 1878, s. 4978.)

torial or state treasurer by the county treasurer.[1] Since 1849 the latter has been required to make with his payments to the state treasurer, a statement of all state taxes as well as other state moneys paid to him during the preceding year,[2] and since 1858, when he does not pay the full tax, to file with the state treasurer as affidavit to the effect that he has paid the whole amount received by him.[3]

Until 1858 the counties were very delinquent in paying their quotas of the state tax.[4] From the beginning penalties were enacted against the county treasurer for any neglect to turn over the state taxes,[5] but the delinquency of the smaller localities in their payments to the county treasurer made these penalties of no avail.[6]

For some years previous to 1858 it had been the practice of the state treasurer to retain the school moneys apportioned to the delinquent counties to balance their indebtedness to the state, but that year the treasurer was satisfied that such a procedure was not authorized by law,[7] as was later decided by the supreme court in the case of swamp land funds retained for the same purpose.[8] In 1858 a penalty was enacted against delinquent counties, and the practice above mentioned was legalized, no county being permitted to draw any money from the state treaasury as long as indebted to the state for taxes.[9] The operation of this law was later declared to have been "most happy,"[10] but it was repealed the next year after its enactment, and even the penalties collected were returned,[11] on the ground that by reason of the delinquency of some of the

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[1] L. 1837-8, No. 93, s. 3; L. 1845, p. 1, s. 4; R. S. 1849, c. 15, s. 85; R. S. 1858, c. 18; L. 1859, c. 14: R. S. 1898, s. 1121; Gov. M. 1859, p. 15.

[2] R. S. 1849, c. 10, s. 111; R. S. 1898, s. 715 (5).

[3] L. 1858, c. 152, s. 3: R. S. 1898, s. 1122.

[4] E. g., H. J. 1838-9, pp. 317-8; C. J. 1839-40, app., pp. 249-50; S. J., June 1848, app., pp. 22-4; Weekly Wis. Patriot, Nov. 27, 1858.

[5] L. 1837-8, No. 93, s. 4; R. S. 1898, s. 1123.

[6] A. J. 1858, p. 1300; S. Proc. in Weekly Wis. Patriot, Feb. 26, 1859.

[7] A. J. 1858, p. 1300.

[8] State v. Hastings, 11 Wis. 448 (1860).

[9] L. 1858, c. 152, ss. 1, 2.

[10] A. J. 1862, p. 641.

[11] L. 1859, c. 29, s. 1; L. 1859, c. 67.

towns of a county, the burden was thrown upon those which had already paid.[1]

But the "old difficulty" returned,[2] and again in 1872 a penalty was provided against delinquent counties, with the retention of all moneys due the county from the state except school moneys.[3] The "old difficulty" disappeared.[4]

2. *The Corporation Tax.*

The state taxation of corporations began in 1848 with telegraph companies,[5] and has since been extended to a large number of corporations. The four general methods of the administration of the tax are seen in the "license" system, the taxation of railroads and certain other carriers, the taxation of street railways and electric light and power companies, and the taxation of steam vessels.

A. The License Tax.[6]

This was the first form of the corporation tax used, and it is still used in the case of many corporations. The method employed is practically that of self-assessment. The tax is generally estimated by the state treasurer upon the basis of reports of the required data made by the corporations to him, and paid directly to him. At present all insurance companies are licensed by the commissioner of insurance, the company reporting the required data to him, and sometimes also paying the tax through the commissioner. The tax is enforced by money forfeitures, forfeiture of license, or sale of the corporation's property.[7]

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[1] S. Proc. in Weekly Wis. Patriot, Feb. 26, 1859.

[2] E. g., A. J. 1862, p. 641; Secy. State Rpt., 1866, p. 37.

[3] L. 1872, c. 158: R. S. 1898, s. 1124.

[4] A. J. 1873, app., p. 7.

[5] L. Feb. 1848, p. 257, s. 3: R. S. 1898, s. 1216.

[6] The validity of this "license" system has been upheld as constitutional (M. & M. R. R. Co. v. Board of Supervisors, 9 Wis. 410 (1855); Kneeland v. City of Waukesha, 15 Wis. 454 (1862); Fire Dept. of Milwaukee v. Helfenstein et al., 16 Wis. 136. But see Atty. Gen. v. W. L. & F. R. P. R. Co., 11 Wis. 35).

[7] A general example is L. 1891, c. 422: R. S. 1898, ss. 1222g–j.

B. The Taxation of Railroads, etc.

The license system of taxing railroads was established in 1854 and continued till 1903, when the present system was established. At first the companies simply paid the tax to the treasurer estimated on the basis of reports made to him.[1] Although since 1874 the railroad commissioner had been required to ascertain and report to the treasurer detailed information, which might have been used as a check upon the reports made by the railroads, the treasurer was not required to consider this information before issuing the license, the companies continuing to be "their own assessors and own collectors."[2] After 1899 the approval of the report by the commissioner was required before the license was issued.[3] In case of failure to report as required, the treasurer was to make the assessment without the report and to sell the road for the tax, or the attorney general instituted proceedings for the forfeiture of the franchise.[4]

In 1903 the taxation of railroads was assimulated to the general property tax, and was turned over to the tax commission.[5] Thirty years before the secretary of state had urged that the roads should be taxed by the state board of assessment.[6]

In performing this duty the commission is given access to all records in the departments of the state and localities; is authorized to require local officers to return information, to compel the attendance of witnesses, and to administer oaths. All records of the railroads are subject to examination by the commission. Annual reports are required to be made to the commission by all railroads, but if the report is not made the commission is directed to "inform itself the best way it may" on the matters necessary for valuation. The commission determines the aver-

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[1] L. 1854, c. 74; L. 1860, c. 174.

[2] L. 1874, c. 273, s. 12: R. S. 1898, s. 1795; R. R. Commr. Rpt., 1883–4, p. 13. In 1856, in case of the Wis. and Superior R. R. Co., the governor was empowered, in order to ascertain the truth of the statement of the earnings, to examine the books and papers of the company, and to examine under oath the officers, etc. (L. 1856, c. 137, s. 23.)

[3] L. 1899, c. 308, s. 4.

[4] L. 1854, c. 74, s. 5; L. 1860, c. 174, s. 3; L. 1861, c. 68.

[5] L. 1903, c. 315.

[6] Secy. State Rpt., 1873, pp. 28–30; Cf. State Tax Comm. Rpt., 1903, pp. 182–4.

age rate of taxation on all the property in the state upon the basis of the returns in the office of the secretary of state, and applies this rate to the railroads.[1]

This system of taxation had already been applied in 1899 to certain other carriers, formerly paying a license tax. Under the earlier system, upon the payment to the state treasurer of an amount computed by the railroad commissioner on the basis of reports made to the latter, the treasurer issued the license[2] When the new system was adopted in 1899, the taxation was accidentally put into the hands of the old state board of assessment instead of the tax commission, which took charge in 1903. The procedure is practically the same as in the case of railroads, though the commission has not such large powers for this purpose.[3]

C. The Taxation of Street Railways, etc.

Since 1895 street railways, and since 1897, also electric light and power companies, pay a license tax to the municipality. A proportion of the tax is paid by the municipality to the county treasurer as a state and county tax, and the county treasurer remits the state's share to the state treasurer.[4] Till 1899 the state had no check whatever on the localities with respect to these taxes, but since that time the assessors have reported the names, etc., of railways in their districts to the railroad commissioner, and the railway companies have also made a report to him.[5]

D. The Taxation of Steam Vessels.

Just the reverse of the above method is pursued in the taxation of steam vessels of a certain class by the system adopted in 1901. The tax is paid to the state treasurer on the basis of a statement made to the secretary of state, and the state treasurer pays to the county treasurer the county's share of the tax.[6]

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[1] L. 1903, c. 315.

[2] E. g., L. 1883, c. 353; L. 1899, c. 112, s. 7.

[3] E. g., L. 1899, c. 112; State Tax Comm. Rpt., 1903, p. 9.

[4] L. 1895, c. 363; L. 1897, c. 223; L. 1903, c 197.

[5] L. 1899, c. 308; L. 1899, c. 329; L. 1901, c. 417.

[6] L. 1901, c. 192.

3. The Inheritance Tax.[1]

The central authorities have a strong control of the counties in securing the payment of the state's share of the inheritance tax established in 1903, and to some extent even in the collection of the tax in the first instance.

The tax is paid to the county treasurer,[2] who reports to the secretary of state the amount of tax received, and pays over the state's share to the state treasurer. The receipt given by the county treasurer on the payment of the tax must be countersigned by the secretary of state to be valid in the final accounting of the estate, and holders of securities belonging to the decedent are prohibited from delivering them to the executors without prior notice to the secretary. The county judge reports to the secretary the name of every decedent whose estate is liable for such a tax and upon whose estate an application has been made for letters of administration, and also the valuation of the legacy, etc., and the secretary may apply to the county court for an appraisal of the estate. Composition of expectant estates may be effected under certain circumstances by the county treasurer, but only with the consent of the secretary of state and attorney general. As the last instance of central control, the commissioner of insurance, upon the application of the county court, determines the value of future and contingent estates.[3]

4. The Suit Tax.

The tax on suits in the circuit court was created by the constitution in 1848.[4] Its most noteworthy feature is the difficulty with which it has been collected.

At first the tax was paid directly by the clerk of the circuit court to the state treasurer,[5] but a law of the next year required the clerk to report to the secretary of state the amount of

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[1] The inheritance tax law of 1899, L. 1899, c. 355, was declared unconstitutional in Black v. State, 113 Wis. 205 (1902).

[2] In certain cases it may be paid either to the county treasurer or to the secretary of state.

[3] L. 1903, c. 44.

[4] Const. Art. 7, s. 18.

[5] L. June 1848, p. 19, s. 17.

the tax received by him, and to pay the same to the judge of the circuit court, filing the latter's receipt with the secretary, who deducted the amount from the judge's next quarter's salary.[1] A change was again made by the law of 1855, which directs the clerk to pay the tax to the county treasurer, who remits it to the state treasurer, and to report the amount to the secretary of state. The secretary is to notify the judge of any failure of the clerk to report, and the latter is liable to removal by the judge for such neglect.[2]

The law has never been well obeyed,[3] but the state authorities do not seem to have interested themselves in the matter at all for a great many years.

5. *Miscellaneous License Taxes.*

Beginning with 1852 "hawkers and peddlers" have been required to pay license fees to the state treasurer directly, or through the treasury agent, receiving a license from the secretary of state. The law has been extended from time to time to include other such transients, and various special provisions have exempted certain classes of persons from such payments.[4] Though the validity of the tax had previously been upheld by the supreme court, in 1904 it was declared unconstitutional on grounds other than those advanced in the earlier cases.[5]

In 1866 the secretary of state reported that the law requiring the payment of these fees was not generally obeyed and that the state was overrun by these non-resident dealers.[6] Accordingly the office of treasury agent was established in 1867 to enforce the law. The agent is appointed by the governor and holds office during the pleasure of the governor, and his bond of $5,000 is subject to the governor's approval. As compensa-

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[1] R. S. 1849, c. 10, s. 61.

[2] L. 1855, c. 56; R. S. 1898, sec. 743-4.

[3] E. g., A. J. 1851, app., p. 826; S. J. 1861, pp. 362-3; State Treas. Rpt., 1868, p. 11.

[4] L 1852, c. 386, and many amendments: R. S. 1898, ss. 1570 sq; L. 1901, c. 341; L. 1903, c. 393.

[5] Morrill v. State, 38 Wis. 428 (1875); Van Buren v. Downing, 41 Wis. 122 (1876); State v. Whitcom, 99 N. W. 468 (1904).

[6] Secy. State Rpt., 1866, pp. 38-9.

tion he receives the penalty assessed for neglect to pay the license fees and a certain percentage of his collections. The agent may appoint an assistant agent and assign his duties.[1] For some years the agent approved his assistant's bond but later it was made subject to the governor's approval.[2] Either the agent or the assistant may appoint special agents to aid them in some of their duties.[3]

The treasury agent is directed to superintend and enforce, if necessary, the collection of the license fees, and both he and the assistant and special agents have large powers for this purpose.[4] Since 1878 the secretary of state has been expressly authorized to direct the agent in enforcing the license laws.[5] Recently the attorney general has been required to advise the agent as to the discharge of his duties, and whenever the agent deems it necessary, the attorney general must assist in actions brought for the collection of forfeitures.[6] The report of the treasury agent, required since 1889, is made to the governor.[7]

Of course since the decision of the court in 1904 against the validity of the license, the agent's usefulness has disappeared.

A few other license taxes must be mentioned. Sellers of bankrupt stocks, etc., have been required, since 1891, to pay fees to the state treasurer, who issue licenses to them,[8] and since 1901 private employment agencies have been licensed in the same way by the secretary of state.[9] For a few years, upon the payment of the required fees to the secretary of state, the secretary issued to non-residents hunting licenses, countersigned by the state fish and game warden, but since 1901 the license has been issued by the state fish and game warden, countersigned by the secretary of state.[10]

Madison, Wisconsin, Feb. 1, 1905.

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[1] L. 1867, c. 176: R. S. 1898, ss. 1578–1582.

[2] L. 1872, c. 177, s. 3; R. S. 1878, s. 1579: R. S. 1898, s. 1579.

[3] L. 1870, c. 72, s. 15: R. S. 1898, s. 1580.

[4] L. 1867, c. 176, s. 1; L. 1870, c. 72, s. 15: R. S. 1898, ss. 1579–80.

[5] R. S. 1878, s. 1579: R. S. 1898, s. 1579.

[6] R. S. 1898, s. 161 (5).

[7] L. 1889, c. 172: R. S. 1898, s. 1579.

[8] L. 1891, c. 443, ss. 1–5: R. S. 1898, ss. 1584d–g.

[9] L. 1901, c. 420, s. 10; L. 1903, c. 434, s. 9.

[10] L. 1897, c. 221, s. 2; L. 1901, c. 358, s. 3.

THE PARTS OF SPEECH IN THE CHILD'S LINGUISTIC DEVELOPMENT.[1]

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1. SENTENCE WORDS.

A number of students of infant linguistics, as Holden,[2] Humphreys,[3] Tracy,[4] *et al,* have endeavored to determine the relative frequency of the several parts of speech in the child's language during successive periods in his early linguistic development. They have made lists of all the words spoken by a number of children between the ages of fifteen months and three years approximately, classifying them according to the standard grammatical categories. Following this method of

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[1] It should be explained that this essay comprises a short chapter abstracted from a larger study on the Psychology of Linguistic Development, which it is hoped may some time later be brought to the light. It will be presented in two parts: 1. The Non-reflective Processes in Linguistic Development; II. The Reflective Process in Linguistic Development. The present essay is Chapter III of Part I of this study; and the chapters which precede and follow it should, if space permitted, be given in order that it might have the proper setting. As it is, however, I must be content with saying simply that the first two chapters treat of Pre-Linguistic Activity, and the Earliest Reactions upon Conventional Language. The chapters which follow the present one in Part I treat of the Development of Inflection in Early Language; the Order of Words in Sentential Construction; and the Development of Verbal Signification. Part II treats of the psychology of the various phases of linguistic instruction in the mother tongue and in foreign language; and it discusses current theory and practice at home and abroad in respect of language training.

[2] On the Vocabularies of Children Under Two Years of Age; Trans. Am. Phil. Assn. 1877, pp. 58 *et seq.*

[3] A contribution to Infantile Linguistics; Trans. Am. Phil. Assn. 1880, pp. 5 *et seq.*

[4] Psychology of Childhood, Chap. V.

treatment Tracy has calculated that of five thousand four hundred words employed by twelve children from nineteen to thirty months of age, and reported by several investigators, 60 per cent are nouns, 20 per cent are verbs, 9 per cent are adjectives, 5 per cent are adverbs, 2 per cent are prepositions, 1.7 per cent are interjections, and 0.3 per cent are conjunctions.

Now, it will be apparent upon a little reflection that this method of treating the child's vocabulary is quite external and artificial. The classification is based upon what may be called the structure of words viewed *ab extra,* rather than upon their function in the child's expression. Tracy, and all who use his method, take a formal or logical or grammatical, not a pyschological point of view. To illustrate the principle in question, when K,. at eleven months, says *ha* (hat), she always sees the object and thrusts her arms toward it, indicating plainly enough that she wishes to reach it. The word, if her mutilated copy can be dignified by such a term, is uttered in an impulsive or perhaps interjectional way; and all her expressions show that she has active desires with reference to the thing designated. She is not simply naming it in any formal, logical, or purely denotative manner. Looked at from this standpoint the word is seen to be more than a mere noun; it does duty for an entire sentence in a highly generalized form.[1] It is the "undifferentiated linguistic protoplasm" out of which in due course various sentential organs and members will make their appearance, according to some such general method of differentiation, possibly, as a complex animal organism like the chick, for instance, grows out of the undifferentiated germ cell contained in the egg. So far as I can make out, K employs her word *ha* (and I speak of this as typical of all the words she uses at eleven months), to convey the notion, "I want that hat;" or "Take me to the

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[1] Compare, among others, Sully: Studies of Childhood, p. 171; Lukens: Preliminary Report on the Learning of Language, Ped. Sem., Vol. III, p. 453–455; Dewey: The Psychology of Infant Language, Psychol. Rev., Vol. I, pp. 63–66; Egger: *Observations et reflexions sur le development de l'intelligence et du language chez les enfants,* Paris 1877; H. Ament: *Die Entwickelung von Sprechen und Denken beim Kinde* (Leipzig, 1899), p. 163; Meumann: *Die Enstehung der ersten Wortbedeutungen beim Kinde* (Leipzig, 1902), p. 31.

hat;" or "I want to put that hat on." I may add that I think her attitude is not expressed by "See that hat" merely, for she is exceedingly dynamic with reference to it. She is not content to look at it simply or to induce me to look at it; she must do something with it, and her modes of expression are calculated to affect me so that I will aid her in attaining her ends. It seems to me, again, she does not have the attitude indicated by "May I have the hat?" or "I wish I could have the hat," for she does not yet recognize clearly any power or authority to which she must thus appeal in gaining her desires. She is not pleading; she is demanding or commanding. Her attitude is rightly expressed, I think, by the sentence, "I *want* that hat, and you take me over there to it." But the special point I wish to impress is that her word *ha* denotes more than a mere substantitive relation with the object; it denotes, in a general way of course, all that can be indicated, though in a more particular and definite manner, perhaps, by the grammatical elements which in adult analytic speech we designate as noun, verb, pronoun, adjective, and preposition.

Sometimes the adult reverts to the infantile method of linguistic expression, and makes single words do for whole sentences. For instance, he says simply "hat?" to the waiter in the restaurant, at the same time looking up at the object which hangs where he can not get it, and intoning in a characteristic manner. This single word, used in this special situation, and supplemented by gesture and characteristic vocal modulation performs the offices of an entire sentence. The psychology of the process is clear enough; the waiter has learned from previous experience that such a tone of voice and such a pose always denotes a need, and the one word localizes the need, so to speak. The notion expressed in conventional language by "I want my" may be and in this instance is indicated plainly by characteristic motor attitudes; indeed these attitudes could in this case express the entire thought without the use of any word. If the situations we encountered in life were never more complex than in this instance, it seems hardly necessary to say that man would not have invented parts of speech. Primitive races, as

Romanes,[1] Whitney,[2] Sayce,[3] Muller,[4] Powell,[5] Brinton,[6] Bosanquet,[7] and other students of primitive languages have pointed out, get along with single-word sentences. It seems to be well established that linguistic evolution on the phylogenetic side has proceeded by continual differentiation of the primitive sentence this differentiation resulting in our parts of speech and in their varied infected forms. So the infant's expression, on the verbal side, is a highly undifferentiated one; and the process of development consists, for one thing, in continuous differentiation with specialization of function,—just such a process in principle as we see illustrated in the evolution of language in the race.[8] This method of development—continual differentiation with specialization of function—has universal validity in mental ontogeny, holding as well for linguistic as for other activities.

It is apparent why, classifying the child's vocabulary *ab extra*, we find that three-fifths of his words are nouns, the names of things, as Mrs. Moore,[9] Mrs. Hall,[10] Kirkpatrick[11] and others maintain. It is easy to overlook the pronominal, verbal, adjectival, adverbial, prepositional, and conjunctional function of the first words; as I have intimated, we unconsciously infer this function from the child's attitudes, gestures, facial expressions, into-

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[1] Mental Evolution in Man, p. 294.

[2] See The Encyclopaedia Brittanica, 9th edition, Vol. XVIII, pp. 766–722, article on philology.

[3] Ibid., Vol. XI, pp. 37–43, article on Grammar.

[4] See his Science of Thought.

[5] See, among others of his writings, his essay on the Evolution of Language; Trans. of the Anthropological Soc. of Washington, 1880, pp. 35–54.

[6] Essays of an Americanist, pp. 403, *et seq.*

[7] Essentials of Logic, pp. 82–86.

[8] LeFevre (See his Race and Language, p. 42) has attempted to show that in phylogenesis all the grammatical categories have developed from the primitive cry. The cry of animals, even, contains the roots of human speech. There is the cry of need which gives rise in time to our interjection, and later to the elements of the sentence. The warning or summoning cry in turn gives rise to our demonstrative roots, and is the origin of the names of numbers, sex, and distance.

[9] The Mental Development of a Child; Psych. Rev. 1896 (Mono. Supp. No. 3).

[10] First 500 days of a child's life; Child Study Mo., Vol. II, p. 607 (March, 1897).

[11] Fundamentals of Child Study, p. 236.

nation, and so on, and we disregard the part the interpreter plays in reacting upon infant speech. But viewed from the standpoint of the child's use of his words in his adjustments, it is evident that they are never at the outset merely nominal in function.[1] Mrs. Hall thinks objects are at first apprehended as wholes, without regard to their qualities or their action, but this seems extremely doubtful, to say the least. It appears rather that the qualities of an object, as food, for instance, will be uppermost in the child's consciousence in his reactions upon it; and in naming it at any time he will really, so far as his own mental content is concerned, be designating these qualities of the thing and not the thing in itself, whatever this may be. To illustrate, S. at twelve months liked buttered zwieback, and whenever he saw any on the table he would call out *bock, bock,* though he did not care for the plain variety. Surely his reaction—or in other words—his expression, must have been incited by and had reference to the peculiar gustatory quality of this special article. Indeed, the child's mental states must usually if not always be concerned primarily with the sensory effects of objects, which would occasion a predominant adjectival attitude toward them. In the course of development one's experiences of an adjectival character with any object will slowly become generalized into what we mean by the term object; and then when we refer to it we have in mind first this generalized something which we may simply designate, and then go on to specify certain particular experiences we have had or should like to have with it.[2]

But the young child's attitude must always be special and qualitative, not general and nominative. And at the outset the actional is really but a phase of this general qualitative attitude. When H. sees the kitten running after the ball, or her father taking gymnastic exercise, or any thing else in movement, she indicates plainly that it is the actional characteristic of the thing

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[1] Compare with this statement Dewey's view, Psychol. of Infant Lang., Psych. Rev., I, pp. 63–66.

[1] I do not mean that we can form a notion of a thing apart from any of its qualities, states, or actions, but nevertheless with repeated experience with an object we seem to gain a kind of sense of its existence independent of any particular quality, state, or action. Doubtless this sense is for the most part verbal merely.

which attracts her. She does not of course abstract the action from the object and regard it as a thing apart; but she is affected differently by the object when it is at rest from what she is when it is in motion. The conception of action as such arises only very gradually as a generalization upon a body of experiences, wherein particular objects are seen to be capable of a variety of actions. This results in establishing the feeling that there is a something constituting an object which is not displayed in its particular activities. In some such way object, action, quality are differentiated; and our analytic language aids in the differentiation, and tends to make it permanent. The effort to employ differentiated speech imitatively is a great stimulus to the definition of elements in one's original undifferentiated ideas.

2. NOMINAL AND VERBAL FUNCTION IN EARLY SPEECH.

It is probable, as I have intimated, that the child's early interests center entirely in things as qualitative and dynamic; and, confining our attention here to the development of nominal and verbal function, we see that it is only upon a multiplicity of experiences that the child can conceive of any object as distinct from its various dynamic conditions. So that in the young child's consciousness noun and verb, viewing the matter functionally, cannot exist independently; the use of substantive terms, speaking grammatically, always implies predicative characteristics. When the child makes his own terms they always denote objects acting; just as do individual terms in primitive languages. Only in our anayltic adult language, which has been developed to express intricate and highly differentiated intellectual content—only in this language are substantive and predicate function more or less completely differentiated. Now, when the child copies the forms of this highly differentiated language, we may argue that he must have back of them the same differentiated thought as the adult has, but in this assumption we are quite likely to go wide of the mark.

In illustration of this point, take a case like the following. I give K. the term "runs" for her brother cutting across the lawr I repeat it on several occasions, and I find that soon she wil point to the brother running and exclaim, *uns!* What is the mental content back of such an expression? Manifestly her attention is engaged with this object in certain continually changing attitudes; she can not be concerned with the action as an independent thing. However, as her experiences with her brother running and other objects running increase she will gradually generalize this activity as a thing or quality characteristic of these objects. But these same objects present themselves from time to time under other and different conditions, each of which will in due course be generalized as special characteristics; and on the linguistic side, if she would express any particular characteristic of the objects, she finds that she must have some means of designating them, without reference to any special attitude or quality, and then she must have some means of designating the special characteristic in question. If these objects always appeared in the same rôle she would not need to have one term for substantive and another for predicate in describing her experience with them.

Some one has said that, viewed *ab intra,* the child's nearest approach to the use of a noun pure and simple is found in those expressions, which from one point of view may be regarded as exclamations or even interjections. To illustrate, S. hears a barking dog at a distance, and he exclaims, bu! bu! (dog). He makes no effort to get the object, or to get away from it. His eyes, his intonations, his bodily attitudes all show surprise and wonder, however, but with no tendency to definite action. Now, in this expression is he simply *naming* an object—either the dog, or the barking as an independent auditory thing? The strict nominal attitude, it will be agreed, is a purely intellectual one; but in this case the child experiences lively emotion, though for the moment it does not issue in adaptive action. Reaction is held in check for the time being; but nevertheless the individual is in a dynamic attitude toward the object. He is on the *qui vive* to detect what should be done in the premises. If one should attempt to express his attitude in a sentence, it would probably be something like—"I wonder what that noise means?" or "That's the dog; what's he going to do?", or "I can tell that's the dog making that racket; will he be likely to do me any harm?"

It should be added that as development proceeds the individual acquires a more and more impersonal relation toward many objects and so in his speech he may reach the point where he can simply designate them or name them; that is, he can employ the substantive in its grammatical function strictly. Again, a child early finds pleasure in the ability to recognize objects, as Groos has pointed out, and he always wishes to have others share his achievements with him, so he may and probably does often employ his words for the purpose of winning the applause of the alter, and not for the purpose of imparting an idea by naming an object, or predicating anything about it. That is, he uses his terms in a simple denotative way, without attempting to express his experience with the objects denoted.

Before the completion of the second year usually, and in some cases as early as the eighteenth month, the child begins to express himself in eliptical sentences, as, giving two of H's expressions, "doggie-high" (the dog is jumping over a high fence); "Nann-come" (I want Anna to come and help me). Now, are the differentiation of thought which we are making the basis of differentiation in speech? Viewed from without they appear to be; but in reality they are probably often mere mechanical imitations, with no subjective differentiation to correspond to the external differentiated form. I have often said "doggie-high" to H., and she may be and probably at the outset is just copying my words. In her own mental processes there may be but little more differentiation with respect to this particular situation than when she employed the single word "doggie" in reaction thereupon. Children from a year and a half on, constantly illustrate this principle in their speech for a number of months. They learn, as a matter of mechanical imitation, an expression like "birdies fly" and they use it not only when they see a bird flying, but also when the see it sitting on a limb or picking worms from the ground. That is to say, the term *fly* may not denote a clear and definite particularization in the child's thought though he uses it freely enough. It will not carry true verbal function until he employs it for the purpose of indicating a particular aspect or attitude of birds and other objects, and which he can and does distinguish from all other attitudes or aspects.

Then again, such an expression as "birdie fly" in the child's speech may be regarded as a single term describing a bird in certain attitudes. The child is not aware that he is using the substantive and a predicate; he imitates as a unity[1] an expression for a general situation, which expression in adult speech denotes differentiation into object and action. The only way we can tell for sure when substantive and predicate have become differentiated in the child's speech is when he uses them appropriately in situations where he could not have imitated them just as he employs them; as when, dropping some bits of paper over the hot-air register he sees them sail upward and exclaims, "paper fly." Here action is apprehended apart from the special thing with which it was originally connected, and a beginning is made in regarding it as a characteristic that may be possessed by many different things. In due course "flying" or "to fly" will denote a certain kind of thing conceived of as having existence more or less apart from objects as such. The same may be said of "running," "jumping," "shouting" and so on *ad libitum.* It should be added that only very slowly and as a result of a great variety of significant experiences does the conception of action as such become differentiated from the conception of things as such; and the differentiation always comes about through a series of objects performing in the same general sort of way as, for instance, boys, dogs, horses, etc., running. This "general sort of way" is of course always very close in consciousness to objects; but yet, and especially when a particular verbal symbol is used to designate it, it may be felt as having a certain degree of individual existence. Doubtless, though, persons differ greatly in respect of the extent to which action and quality become dissevered, as it were, from things, and attain to a measure of independence in the mental processes. It may be added that there can never be complete severance of relationships and

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[1] Preyer observed his son Axel at twenty-seven months saying *magenicht* (*mag es nicht*) and *tannenicht* (*kann es nicht*). Any observer may notice the same phenomenon and often quite late in linguistic development, after the child has been in school for several years.

dependency in things of the mental commonwealth any more than there can be in things of the social commonwealth.

The prominence which some grammarians ascribe to the verb in linguistic expression may warrant its receiving a little special attention at this point. Before the twenty-fourth month, as a rule, the child uses sentences of two or three words, but the verb is quite often omitted, and from my observations I should say that the novice can get along very handily without it. To illustrate, M. at twenty-five months will say, "Mamma—milk," (Mamma, I want my milk; or Mamma, have my milk brought in). Taking my glasses in her hand she will say, "Baby—nose" (I want to put them on baby's [my] nose). Watchng her nurse prepare her bath, she will repeat many times, "Baby—bath." One may count instances of this kind literally by the hundred every day in the life of an active child from his second to his third or fourth birthday. The copula is quite generally omitted in the beginning. A three-year old will say "My—(or *me* or perhaps *I*) hammering; (I am hammering); "Me—running;" "Me—playing horse" and so on *ad libitum.* So he will ask, "Where—papa going?", "Where—papa been?", "Where—my book?", "My dog—running?", and so on. Helen Keller says in her Autobiography that when she was seven she used such expressions as these: "Eyes—shut; sleep—no," (Their eyes are shut"—speaking of puppies—"but they are not asleep".) "Strawberries—very good," and so on.

It is not difficult to understand why the child should thus do violence to the logic of our sentences. He can convey his limited range of thought adequately without the copula; being an adept at gesture and intonation he can make these latter discharge the office of the former. Of course his expressions always relate to very definite concrete experiences, so that he can make himself intelligible even with an imperfect handling of our linguistic tools; but when he comes to deal with more abstract situations, where every detail of the thought to be conveyed must be suggested by his own expressions, receiving no aid from immediate conditions and occurences, as the child's expressions come gradually to be concerned with complex experiences remote in time and place, then he will feel the need of having command of a larger and larger assortment of linguistic symbols, and of employing them in the precise conventional manner, else he can not make himself understood. Here again the child and primitive man are on a level, as Powell, Romanes and others have pointed out.

I think that so far as actual need is concerned, the child could go on for a long distance, say up to the sixth or seventh year ordinarily, neglecting the verb and particularly the copula in his sentences; but with the logical forms of the adult constantly ringing in his ears he comes to adopt them as a matter of convention at the outset, and not because he feels they are of any special service to him. The parent and governess and teacher are incessantly putting the standard forms before the novice, and forcing them upon his attention, and as a consequence he abandons his own original, abbreviated, gesture-symbol forms, and takes up with the conventional models. Just observe a child saying, for instance, "Doggie—high" (The dog jumps, or is jumping, over a high fence) and notice the parent repeating after him "Doggie jumps high," and asking the child to follow suit. This is going on incessantly in the first years of language learning; if the parent is not dictating conventional forms, then the brothers and sisters and playmates are. Of course, the conventional forms are sailing about the child all the time, even though the speakers are unconscious of his presence, and it is inevitable that he should in time come to imitate these forms in a more or less sub-conscious, mechanical way. So the child is not let alone to do as he chooses linguistically; the social milieu resorts to various devices to get him to abandon his primitive linguistic forms before he feels the need of it. Not only are the standard usages constantly thrust into his ear by all charged with his care and culture; but the people around him make generous use of ridicule to hasten his progress in adopting the standard modes. Observe an eight-year old boy making fun of his three-year old brother for some of his childish phrases, and the importance of this force in urging the child to abandon his original expressions, though they serve him well enough, will be appreciated.

3. INTERJECTIONAL[1] FUNCTION IN EARLY SPEECH.

Thus far I have spoken only of nominal and verbal function in the child's earliest sentence-making. Perhaps I should have begun with the interjection, since this, viewed from one standpoint, is the first part of speech to appear. It may be observed, however, that Meiklejohn[2] and others say the interjection is no real part of language, since it does not enter into the organism of the sentence. But the observers of infantile linguistics have retained the interjection as a part of speech; and, according to Tracy's Summary, the vocabulary of the average child of about two years contains less than two per cent of interjections. Salisbury[3] maintains that in the vocabulary of his child at thirty-two months there were only five interjections out of a total of six hundred forty-two words. At five and one-half years there was but one interjection out of a total of fifteen hundred twenty-eight words. The table given by Kirpatrick[4] shows about the same prenomenon as Salisbury's. Now, these classifications are made strictly *ab extra,* following the formal grammatical categories. But, regarding the matter psychologically, there is an interjection element in most of the child's early words, as Mrs. Hall[5] appears to have observed. She maintains that the language of her child from the two hundred thirty-third to the three hundred fourteenth day was an "interjectional onomatopoetic race-language."

I may illustrate the point in question by citing B's use of Kee (kitty). Whenever he uttered it, in the beginning, there was always something of the "Oh!" quality about it. The kitten was for some weeks a fresh surprise every time he beheld it, and he used his word with much feeling. He might with pro-

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[1] I do not here distinguish between interjectional and exclamatory function, though in strict grammatical treatment this should doubtless be done. Professor Owen makes this distinction. The interjection is a sentence element, though it is not strictly a part of the sentence. The exclamation may be expressionally self sufficient.

[2] See his English Language, Part I, p. 60.

[3] Educational Review, Vol. VII, pp. 287–290.

[4] See his The Fundamentals of Child Study, p. 236.

[5] *Op. cit.* p. 601.

priety have used "Oh!" in place of *kee.* One who follows a child about as he learns new objects cannot escape the conviction that his expressions all have for a time at least an interjectional quality, as the grammarians use the term. It is interesting to note in this connection that anthropologists, as Aston,[1] e. g., maintain that human speech originated in certain natural cries, —hisses, shouts, grunts,—and these in time became interjections. Interjections were in the beginning then the only parts of speech; all others were included in them. Whether this position can be defended or not, it is at least evident to me that interjectional speech comes very easy to the young, and it is prominent up until the adolescent period. One may hear children (boys especially, I think), from five to twelve incessantly using expletives such as *Gee Whiz! Giminy Crickets!* and so on through a long list. They are all employed, it seems, in expression of strong feeling, or to emphasize a thought put forward in conventional fashion; and the child's attitude is in some measure interjectional, even if he does not use the particular forms recognized by formal grammar. He can use "horse" with interjectional content and function about as readily and effectively as "Oh!" or "Whew!", or "Hurrah!" In the course of development this exclamatory or interpectional coloring of the child's language gradually declines after the age of seven or eight, say, so far as ordinary speech is concerned, though throughout the whole period of childhood, and to a less extent during youth, interjectional function is much in evidence. One result of development is to gradually confine interjectional function to the conventional terms, whereas in the beginning, as I have said above, every term may have a greater or less degree of interjectional quality.

4. ADJECTIVAL AND ADVERBIAL FUNCTION IN EARLY SPEECH.

Let us glance for a moment now at the place of qualifying and particularizing terms in early linguistic activity. The term modifier suggests differentiation in mental content, and we should not expect to find limiting terms and phrases employed, intelli-

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[1] See the London Journ. Anthr. Inst., Vol. XXIII, pp. 332–362.

gently at any rate, until the child's thought had attained a considerable degree of complexity, so that he might feel the need of some particularization in his expression. Of course, the child's appreciation of particular properties of objects is implicit in his reactions upon them,—in his attitude toward his kitten for instance,—long before he employs qualifying terms. He shows that he regards his kitten as "nice," for illustration; but still the notion of *niceness* as a general attribute is not dissected out, so to speak, from the mass of impressions in which it is imbedded. Then the right use of a modifying term requires the generalization of numerous experiences, all of the same general effect. So two processes must go on *pari passu* in order that the child should feel the need of verbal instruments to function as modifiers. In the first place, there must take place continual differentiation in the body of general impressions which together constitute any particular object; and in the second place, there must be constant generalizing of similar experiences with objects, giving certain types of experience which are designated by modifiers and attached to objects according to the type of experience which they yield. Of course, qualifying and particularizing terms may very early be used which have the outward aspect of modifiers, but inwardly they do not function as such. They are the resultants of mere mechanical imitation. Take, for example, H. who at two and one-half years would say, when running to greet her father returning to the house, "nice papa." She had been taught this formula, and it probably was the expression of no different mental content from when she said simply "papa." So she would ask for a "nice story;" but what she wanted was *a* story, not some special kind of a story,—a *nice* as distinguished from some other sort of a story that she had heard. She did not employ *nice* as a particularizing term. Again, I say in the presence of S. at nineteen months, "nice mamma," at the same time patting her head. He imitates my action and words, but manifestly he uses both words as a single term. Possibly the patting suggests to him some of the mother's special qualities denoted by *nice,* but even so his conception must be extremely dim and undefined. Now, if a friend should happen in while he was caressing his mother and using his new term, it would be thought that he was employing a modifier in an intelligent manner simply because in grammatical form it resembled such. It should be noted, however, that the use of a modifying term even mechanically does tend to draw attention upon certain characteristic qualities of the object in question; so that mere formal imitation often ends by hastening the intelligent use of terms, a point to be worked out in detail later.

The principle is that at the outset the child views the kitten, to keep to our illustration, in a certain very concrete, totalized way, without differentiating the notions of niceness, of gentleness, of playfulness, and so on. But as experiences with the kitten and other domestic animals (including human beings possibly) increase, these ideas gradually gain a certain degree of independence or individuality. The attribute denoted by *nice* for instance, is, of course, always experienced in connection with some definite thing; but as the number and variety of such things are augmented, the characteristic of affording pleasure of a special sort, to which is attached the conventional symbol, *nice*, being common to all, it acquires a kind of existence apart from the particular things which occasioned it. When this stage is reached the child can use the modifier in an intelligent manner. He can say, "I have a nice doggie," and the adjective indicates that a particular characteristic of his dog has come to clear conscousness in his reactions. He appreciates the quality as such, too, for he can employ the term appropriately in reference to other objects where he could not have mechanically imitated its use. *Nice* then has become a true particularizing term; and the principle is universal in its application to the natural history of all modifiers. Of course, a term like the one in question is incessantly changing in its content and in the range of its application. As development proceeds extensions are made here, and excisions there. Experience is all the time at work remodelling it; and just what is accomplished depends upon the peculiar character of the experiences. Here is a home where the children hear the term applied frequently under a variety of circumstances; both physical and spiritual characteristics are designated by it. But here again is a home where the term is used infrequently; the members of the family rarely take the attitudes toward things denoted by this term. The children from these two homes will have quite different bases for the employment of this adjective; and the principle applies to the developmental history of all qualifying terms.[1]

It may sound like a commonplace to say that the adjectives which are earliest used relate to the impressive characteristics (depending upon the child's peculiar experience), of the objects with which he has direct, vital relations. The peculiar characteristics of different articles of food are among the very first to become differentiated, and designated by separate terms,[2] so that the adjectives appearing first in the vocabulary are such as "nice," "sweet," "bad," "hot," "good," "cold," and the like. Some of the terms descriptive of the child's experience with food apply also to experience with other objects, and it happens that these special terms become more prominent than any others. Large things early impress the child deeply, and his social environment intensifies his natural tendency in this respect by laying special emphasis upon big things in stories, and in all representations and descriptions of the child's surroundings. So "big," "great," "awful," and the like, early acquire prominence in his vocabulary, as do "little," "small," "tiny," and similar terms. So if one should go through with all the types of experiences of the child at different stages of his evolution he would find that intelligent adjectival function depends directly upon the degree to which particular attributes of objects become differentiated from their general characteristics because of the new relations which the individual, as an inevitable consequence of his development, comes constantly to assume toward them. Terms denoting abstract moral qualities in things appear in the vocabulary last of all, unless such terms are imitated in a mechanical way.[3] Of course, it is utterly impossible to tell just

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[1] I discuss this matter in detail in the chapter on The Development of Verbal Signification.

[2] It will be appreciated, of course, that long before the child uses conventional terms to denote the qualities of his food, for example, he indicates his appreciation by gesture and facial expression with characteristic interjectional expression of rich variety and complexity.

[3] All observers of child linguistics give instances in illustration of this principle; but see Chamberlain (Ped. Sem., 1904, Vol. XI, p. 278).

what is the extent and content of a term as the child employs it at different stages in its developmental history; but still the evidence all indicates that abstract moral qualities are not appreciated until relatively very late, so that the terms designating them are not intelligently handled until the later stages of development. This is not to say that adjectives denoting moral qualities in adult speech are never used early; indeed such terms as "good," "bad," "horrid," "ugly," "mean," "nice," "naughty" and the like are applied to persons as early as the third year; but they are always used in a very concrete, even physical way. The young child has had some unhappy *physical* experience with his playmate, and he calls him "bad," or "ugly," or "mean," or "horrid;" but as he develops he will be apt to use these terms to denote more and more general attitudes of persons; to designate "qualities of heart," as well as, or perhaps rather than, mere muscular traits.

In her eighth year, H., who had been read to a great deal, and who had herself at that time read twenty-five books of classic fairy-tales and fables and myths, and nature stories, and even a few novels which her parents were reading,—with this linguistic experience H. occasionally used in her conversation such a term as "excellent" or "genuine." She would say, speaking of a character in one of her books, he was an "amiable" or "genial" or "excellent" person. Now, when I would test her understanding of one of these terms I would find that she had in mind some definite act described in one of her books, and she had remembered this term as applied to the particular character in question, and had seized upon it without any adequate idea of its significance. When I ask her to apply one of these terms to her playmates she shows that she has but a slight and very hazy notion of its precise connotation. Of course, she has only a very general and quite incomplete idea of the qualities denoted by "virtuous," say. It will require the experiences of many more years before she can use this term with a true sense of its significance, as this has been determined by racial usage. In the mak-

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His child, in her third year, used the word "sinecure" without the slightest idea of what it meant. See also Hall: The Contents of Children's Minds on Entering School. (Heath & Co., Boston.)

ing of the term in phylogenesis some such stages have been passed through, in growing from concrete and physical to more and more general and abstract reference, as the child passes through in his acquiring the ability to employ the term correctly and efficiently to connote moral quality.

What has been said of the development of adjectival function applies practically without modification to the development of adverbial function. The only word needing to be added here is that the adverb appears considerably later than the adjective, and even when learned it is used much less frequently, as all vocabularies indicate. According to my observations, the term earliest used adverbially is one denoting place,—*here*[1] in "here I am." *There* and *where* are used early also. Mrs. Moore[1] thinks these antedate all parts of speech except interjections and nouns. But unless under exceptional conditions of training, it is probable that with the possible exception of "here," "up," "down," "there," and "when," they appear later than the more concrete adjectives relating to quality of food and prominent characteristics of dogs, playthings,[2] etc. As we might expect, adverbial function at the outset is confined to the immediate, concrete, physical needs of the child, and relate to time and place principally. S., in his fifteenth month, being on the second floor and in his father's arms, points to the stairs, at the same time urging his body in that direction, and says, "dow" (down). So he will point to objects and say "dā" (there), "uh" (up), and "hē" (here), "more," "out," "now," "where," "away," and possibly two or three other adverbs are found in the vocabularies of children before the close of the second year, though they are not always used with precision, according to the traditional standards.

5. PREPOSITIONAL AND CONJUNCTIONAL FUNCTION IN EARLY SPEECH.

From what has been said in previous sections, it must be apparent that the part played by connective terms in adult speech is carried to a large extent by gesture in child linguistics. Of

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[1] *Op. cit.*, p. 129.

[2] Cf., Hall, *op. cit.*, pp. 604–606.

course, connective function is almost wholly lacking in the infant's expression, since his mental content is not sufficiently differentiated to require connective terms; or at least he can get on very well without them. When a boy of seventeen months says "My—go—snow" (I want to go out into the snow) and a little later when he comes in, exclaiming "My—come—snow" (I am coming in from the snow),--here the child is not in any explicit sense aware of the difference between going *to* the snow and coming *from* it. In his own thought *snow* occupies the all important place. His attention is filled with his experience in the snow. In the first instance he longs to have these experiences repeated, and his sentence, "my—go—snow," will reveal his desires completely and definitely to his care-takers. In the second instance his "my—come--snow" also meets the needs of definite expression; here his impulses concern the imparting of his experiences to his care-taker; and these experiences do not include to any appreciable extent the relation expressed by the preposition in adult speech. The verbs—"go" and "come"—used in this special connection, include the idea of prepositional relation, so to say,—a principle exhibited in all primitive language, according to Müller, Sayce, Powell, Romanes, and others. With the child's relatively undifferentiated experience, and with his facility in gesture, as I have suggested, he may readily convey his notions without such relational terms, and this is why he never employs them at the outset of sentence making. As experiences multiply and become ever more complex, and there arises an urgent need to express precisely experiences remote in time and place, gesture is found sooner or later not to be definite enough, and then prepositions will begin to find their way into the child's vocabulary. It may be added by way of qualification that the imitative tendencies of the child lead him often to adopt connective terms before he has real need for them, but it is probable that such terms are not imitated as readily as those expressive of concrete elements of experience. However, mechanical imitation must be reckoned with, and if it were not for this the average child would, I think, leave relational terms locked up in the other parts of speech somewhat longer than he usually does.

One cannot easily detect the emergence of prepositional elements in early speech. Their individuality is at first not at all marked or distinct. It is as though they were still a part of the organism in which they were originally imbedded. H. at nineteen months says, as a typical expression, "Papa—go—ŭ—University," the *u* here being evidently a mutilated form of the preposition "to". At the outset it was lacking altogether; but by the twenty-sixth month it had become differentiated completely from the verb. We catch it here in the nineteenth month in its embryonic form; and so far as I have observed, all prepositions have a similar history, which seems to be much the same in principle in phylogenesis as in ontogenesis. Powell,[1] commenting on prepositional function in the Indian language, maintains that prepositions are often intransitive verbs. When an Indian says, "That hat table on," we are to consider the "on" as an intransitive verb which may be conjugated. "Prepositions may often be found as particles incorporated in verbs; and still further, verbs may contain within themselves prepositional meanings without ever being able to trace such meanings to any definite particles within the verb . . . Prepositions may be prefixed, infixed, or suffixed to nouns, i. e., they may be particles incorporated in nouns."

In some children's vocabularies "up" and "down" are given as about the first prepositions to appear, and they are said to be used properly by the eighteenth month, or so. The child says, "up-stairs" (I want to go upstairs) and "down-stairs." Now, as I have observed the early use of these words, I should say they were not employed with exclusive prepositional meaning at all. In the beginning the child says simply "up," and makes this expression definite by extending his arms upward, by straining upward with his body, by looking upward, and by so employing his voice as to leave no doubt respecting his desires. I should say his word really denoted the *place* he wished to reach, and the *method* of reaching it, although neither of these elements would be focal in consciousness in the sense in which we can imagine they might be in the case of an adult who sat

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[1] *Op. cit.*, p. 46.

down and reflected upon getting up stairs. The child's consciousness might be said to be motor rather than ideal when he is expressing himself in this way. The word is just one phase of a general motor excitement, and it is impossible that it should be used with precise prepositional value. Before this word can be employed as a preposition merely, a number of other words will need to be used with it in the sentence, each to carry phases of the meaning which it now carries alone.

In her twelfth month, K. would throw objects from her high-chair to the floor, and would exclaim "down!" To my mind, this term denoted mainly the racket made by the objects when they struck the floor. Prepositional relation was surely not a prominent element in the child's consciousness on such occasions. A little later she would take an object in her hand, and at the moment of releasing it she would exclaim "down!" and blink, evidently anticipating the noise to follow, which was the thing prominent in consciousness. Later on she would use the term when she wished to get out of her high-chair, but here, also, it had much more than prepositional meaning. Her consciousness could be expressed in adult language by the following, perhaps: "Unfasten me so I can get onto the floor and play." It is improbable that the child uses such words as "up" or "down" with strict prepositional meaning, or adverbial meaning either, before his third year at the earliest, and I should prefer to make it a year later. In the fourth year one may hear expressions like the following: "I am going *down* the street;" "I climbed *up* the stairs," etc., in which we doubtless have examples of genuine prepositional function. The original terms "up" and "down" have persisted, but much of their early meaning has been drawn off from them, and loaded on to other terms in the sentence; and such is the history of other prepositions, as *on, in,* etc.

It should be pointed out that there are prepositions which are never used except with prepositional meaning pure and simple. They describe relations which the child does not apprehend until he has made good headway in differentiating the parts of speech, and constructing the sentence. Before such terms as *toward, among, against, notwithstanding,* and the like are employed he has abandoned his primordial sentence-words, and in their place he uses sentences with substantive, predicate, and modifiers, so that any one word now carries special, differentiated meaning. When S. at four says: "I throw it *toward* the house," he shows that he has reached the prepositional plane, so to speak, in linguistic development. It may be added that the principle here in question applies to development in respect of all the parts of speech. To illustrate with our adjective *nice,* already often cited, this is first used as a sentence-word; but a term like *virtuous* is never employed until the sentence-word period is outgrown, and the word carries adjectival meaning alone.

It will not be necessary to dwell long upon the proposition that conjunctions as such appear relatively late in the child's language. It is probable that the primitive sentence-word carries conjunctional function, but to a very limited extent, I should say. It is questionable whether the thought of a child of two is integrated to the degree required for the intelligent use of the conjunction. The central processes relating to any situation are, relatively speaking, fragmentary, disjointed; or better still, unconnected, or non-integrated. Now, development results in the gradual integration of elementary processes, and this makes necessary the use of the conjunction in expression. Probably the earliest sort of integration has reference to objects acting simultaneously in the same way. In the beginning the child will say, "Baby-go-stairs" (Baby is going upstairs); "papa-go-stairs" (papa is going upstairs). But before the completion of the second year, one may hear this expression, "Baby ă papa going upstairs." This example is typical of much that may be heard as early as the twenty-fourth month. Judging from my own observations I should say that objects acting simultaneously and congruently are coördinated considerably earlier than are the acts they perform, or the qualities predicated of them. One may hear children after the third year say, "My run and fall and get up again," and "Mamma nice and good," and the like; but such expressions appear later than the first type mentioned. It is probable that two objects acting in the same way fuse in the child's thought more readily than succeeding actions or co-existent qualities of the same object. Baby and papa, going upstairs together, are apprehended in a single act of attention so they tend to stick together in representation, and in expression they require to be named together. In attention they gradually fuse into a unity which ultimately will be expressed by *we;* but in the child's speech the old habit of naming each object separately persists, and so he must use "baby" and "Mamma" The use of the *and* shows the growth in coördination or integration of the two objects. Now, there is not quite the same necessity for coördination in successive actions performed by the same object, though, of course, with development they tend constantly toward integration, and by the fourth year it is plain that coördination has been achieved. The child then joins with the conjunction two or three of his own acts, as well as those of his parents, his brothers and sisters, his dog, and so on. And what has been said of the coördination of actions applies also without modification, I think, to the coördination of attributes.

The first conjunction appearing in the child's speech is unquestionably *and.* As for the order of the appearance of the other conjunctions one can not speak with certainty. Probably *or* is the second to be used with strictly conjunctional meaning. The child says, "Baby have apple or peach?" This expression was forced upon H. early because of her being required to choose between eatables, the parent saying: "Take this *or* that." The child early hears *or* used a great deal,—"Hurry *or* I will go;" "look out *or* the baby will fall;" and so on *ad libitum.* Of course, the central processes required for its intelligent employment are quite a bit more complex than in the case of *and;* and I think it is apt to be employed as a result of mere imitation at the outset.

The general principle holds, that the appearance of any conjunction depends primarily upon the complexity of the thought which it is employed to express, though imitation is always a disturbing factor, leading to the mechanical use early of a term much heard from the lips of parents and others. *Because* is such a term, I think. Quite early one may hear the child saying, " 'cause I do," " 'cause I must," " 'cause I want to," and so on; and it is probable that his thought is not complex enough to really demand these expressions.

I have endeavored to determine just when such words as *except, although, unless, lest, in order that, nor, whether—or,* and so on, appeared in the vocabularies of my children, but I find I cannot speak with certainty about the matter. Of this I am confident, however, that none of these terms is employed with precision before the fifth year. V. at six and a half does not. use one of them intelligently, so far as I can detect But H. at nine uses them all fluently. She has read much, and has been much read to; and it is probable that these terms have forced themselves into her vocabulary mainly because of their prominence in her reading. She has heard them in the speech of the people about her, and she has been correctly interpreting them for years; but they have all played a minor part in her consciousness of spoken language. So far as auditory language is concerned, relatively unimportant elements are swallowed up in wholes of greater prominence; but they are likely to gain some measure of individuality when reading is begun, though they are at the same time likely to lose it again as the reader gains in facility in grasping and interpreting larger and larger language units. It may be added that a child of five seems to be able to express himself definitely and fully enough without resorting to any of these conjunctive aids that imply quite complex ideational integration. If he did not find these terms ready to hand, and if they were not continually impressed upon him, I think he would not miss them, at least not until he should be placed in situations where he would be required to express involved thought very connectedly and precisely.

6. PRONOMINAL FUNCTION IN EARLY SPEECH.

The absence from early speech of anything which could be called a pronoun has attracted the attention of all students of linguistics, and of psychologists and philosophers as well. Philosophical literature is full of speculation concerning the development of self-consciousness in a child, indicated by his use of the personal pronoun. The philosophers, many of them, have said that the child does not distinguish self from others, the ego from the alter, until the terms "I," "my," "mine," "you," "yours," "he," "him," "his," begin to appear in his vocabulary, which most observers have found to be somewhere about the twenty-fifth month, though a few have not noticed it until the beginning of the third year. Ament detected it in the twenty-first month, Schultze in the nineteenth, and Mrs. Hall even in the seventeenth. It is suggestive in this connection to note that primitive languages show great confusion in the use of the pronoun. Brinton[1] maintains that in aboriginal American languages there is no distinction between persons in the pronoun; "I," "thou," and "he" are not discriminated, a single monosyllable serving for all persons, and also for both singular and plural numbers. In some American languages, however, there is a great variety of pronouns, used to denote not only person and number, but various conditions and aspects of the person or persons designated, as that they are standing, sitting, or lying, alone or with others, moving or stationary,[2] and so on. According to Powell,[3] "The Indian of today is more accustomed to say *this* person or thing, *that* person or thing, than *he, she* or *it*. Among the free personal pronouns the student may find an equivalent of the pronoun 'I,' another signifying 'I and you,' perhaps another signifying 'I and he' and one signifying 'we,' more than two, including the speaker and those present, and another including the speaker and those absent. He will also find personal pronouns in the second and third person, perhaps with singular and dual forms." The pronouns are not in all cases completely differentiated in these languages, but are incorporated in the verb as prefixes or infixes or suffixes, and as such they designate the person, number, and gender of both subject and object, and in the conjugation of the verb they play an important part.

How is it now with the child? Is pronominal function in his

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[1] See his Essays of an Americanist (Philadelphia, 1890), p. 396.

[2] Powell says that in Indian languages genders are not confined to sex, but are methods of classification primarily into animate and inanimate, which are again classified according to striking characteristics or attitudes or supposed constitution.

[3] *Op. cit.*, p. 43.

case discharged by the verb or some other part of speech? Does he employ a single term for all persons and things? What need gives rise to the differentiation of special words to carry pronominal function? In discussing these questions it should be said at the outset that from the very beginning the child in his reactions distinguishes himself from others and from things. Of course, he does not make this discrimination reflectively; but nevertheless he does not confuse himself with foreign objects when he is in need of food, say; though, as President Hall[1] has shown, he may not recognize his fingers and toes as his own. But when he is hungry he does not give his food to another, thinking that the other is himself. As early as the sixth month he exhibits in his reactions a certain realization of the opposition between ego and alter, for he will squall if another takes his bottle, or even if the mother shows, overt partiality for some other child. This appreciation is very keen at a year and a half; though the child does not yet use terms that denote distinctions in persons. I mention these obvious facts merely to suggest how far astray some persons have gone in assigning the birth of the ego to the period when the personal pronoun first appears. Their treatment of the matter has been purely *a priori* and metaphysical. They have reasoned that because "I" denoted self, the self must be in consciousness as a distinct object when the term is used, and if it can not be used the self can not be conceived of as a distinct entity; but they have overlooked the fact that for months before the child uses the pronoun he has been using other modes of expression which show clearly that his personal self exists in consciousness as a thing apart from all other things.

What then are these modes? First of all, gesture, grimace, pantomime, intonation. When a vigorous year-old child wishes to be taken in your arms, no one who sees and hears him can doubt that his discrimination between ego and alter is very clear. All that can be denoted by "I" is exhibited by the child, though in a generalized, consolidated, impulsive, instinctive, nonreflective fashion. Again, when you see a child of this age

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[1] See his Some Aspects of the Early Sense of Self. Amer. Jour. of Psych., April, 1898. Vol. IX, pp. 321–395.

scream and strike at his brother who appropriates his food or playthings, you cannot doubt that he possesses a rudimentary, undifferentiated sense of "mine." When, again, this same child offers his father a taste of his sugar-lump, and exclaims "Ndobbin," "ndobbin" with interrogative intonations, he is certainly *acting out* the question "Will *you* have some sugar?" The *you* as contrasted with *I* is involved in the child's action, though he can utter not a syllable to denote the distinction. Further, when the child's brother performs tricks for the babe, and the latter turns to the father or other person, and pointing at the brother laughs at him and gabbles about him,—in a reaction of this sort the idea of *he,* or possibly *it,* is clearly involved. There is a third person in the case, who is not now in vital relations with the speaker and listener. He is being talked *about,* not *to.* In this latter situation the child shows in his reaction, not reflectively, an appreciation of all three persons in their grammatical relations to him, so to speak.

We have seen how, in the course of expressive development, verbal symbols come gradually to take up the function which had been originally discharged by gesture and pantomime; and the principle obtains in respect of pronominal as of all other forms of linguistic function. In the beginning the child designates persons and things by gesture, and pronominal function in this stage might be said, perhaps, to be *demonstrative.* Even when he wishes attention turned upon himself he indicates it by characteristic bodily attitudes and contortions and vocal demonstrations, saying, in effect, "*this* person needs your help." But as development proceeds, *demonstrativeness* in linguistic function declines, and pure symbolization increases; and in the matter of pronouns it results that terms introduced which not so much point out or demonstrate as name or denote merely. This is true, of course, of racial as of individual evolution; to the primitive mind things must be made very objective, concrete, explicit, but with mental development simple suggestion becomes continuously more efficient. In other words, language becomes ever more *abstract,* which means relieved of direct, concrete reference.

In his pronominal evolution the child passes first from the pantomine to the nominative stage, he gives its name to everything to which he alludes, including himself. If his elders address him as "baby" then he always uses this term when referring to himself in any way; or if his proper name is used, then he employs this on all occasions. So he says,—a phenomenon observed by every student of the matter, I think,—"Baby wants baby milk;" or "Baby hurt baby hand," and so on *ad libitum.* In the same way he says, addressing his father, "Papa take baby," usually, I may add, in the imperative mode. Similarly, when speaking of his brother, he will say, "Stanley is putting on Stanley coat." V. continued in this nominative stage until he was past five years of age; then with great swiftness he went over into the pronominative stage. Within his linguistic range, he used pronouns with considerable freedom, accuracy, and efficiency by the time he was six and a half, though he still got the cases of his personal pronouns mixed at times, and he could not use the relatives according to the prevailing standard; his *whats* and his *thats,* for instance, gave him trouble. H. and S. were well into the pronominative stage by the time they were three and a half; and by six they had overcome all their difficulties.

Why does the child pass through the nominative on his way to the pronominative stage in linguistic function? To begin with, the *name* of a person is far more definite and uniform than his *pro-name,* and so all persons in speaking to the child use the former and avoid the latter, as Preyer[1] has already pointed out. To illustrate: In addressing my child I say, "*Papa* wants this or that;" or "*Papa* will do or did this or that," and so on *ad libitum.* The mother speaking of the father in the presence of the babe says, "*Papa* loves *baby;*" or "*Papa* has come home," and so on. Now, everyone who mentions the father when the babe is concerned uses this term invariably; and the same is true in principle of the baby himself, and the mother and brothers and sisters, and every object mentioned. If the pronoun were used, see what confusion (from the learner's standpoint) would result. When I referred to myself I would designate myself

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[1] The Development of the Intellect. (Trans. by Brown), p. 202.

by "I" or "my" or "me;" when the mother addressed me directly she would designate me by "you" or "your's;" when she spoke to the babe about me she would use "he" or "him" or "his." Here are eight symbols for the same object, looked at from the child's standpoint, and it would be a long story to tell how he could orient himself with reference to each and all of these terms for the same individual. To present the matter in a sentence here,—the individual must reach a stage of development where he can organize a variety of experiences around a common center before he can comprehend or use intelligently our system of personal pronouns. As you watch him pushing forward in integrating ability, you see him adopting first one form and then other forms of the pronouns. At the outset he makes his one form do duty in all cases. "*Him* is a nice boy;" "*Me* wants to go to *him's* (or perhaps *he's*) house," are illustrations. We shall go into this in greater detail in the chapter on Inflection; but it may be noticed here that the young child cannot readily accommodate himself to the notion of having different forms of his words apply to the same unchanging thing, unchanging so far as he can see. This leads parents, more or less intuitively, to avoid the pronouns in speaking to young children, and this has the effect to retard the appearance in the vocabulary of pronominal forms.

Then the pronoun, as employed in conversing with a child, lacks individually, warmth, color. Try talking to your year-old child in pronominative terms, and see how much weaker is your speech in personal suggestiveness. On the other hand, to continue the nominative stage too long is equally objectionable; it seems silly, babyish, ineffective. The opening mind needs to be assissted in its grasp of things by all possible concrete aids; but once it has got a hold it knocks out the ladder by which it has ascended. This, I take it, is a principle of universal validity in mental development, and is one of the forces incessantly at work transforming the individual's interests and abilities.

This will be the best place, perhaps, in which to glance at the forms of the pronouns which are used most frequently at first. I said above that one form of the personal pronoun is often made to do duty for all cases; but what is this form? Mrs.

Hall's[1] boy used *his* first; Rzesnitzek[2] says that the possessive form *mine* is first used, while von Pfeil[3] thinks that the pronouns denoting second person are first mastered; then those denoting third person, and last of all come those denoting the first person. In Chamberlain's[4] account of the linguistic development of his child, "*I*" and "*my*" appear very frequently after the beginning of the third year, but the other forms are not in evidence. Preyer[5] observed that his son, Axel, in his thirty-second month used "I" meaning by it "you." In the thirty-third month came such expressions as "*das will ich!*" "*das mocht ich.*" However, before this, in the twenty-ninth month, the objective form of the third person was used, "*gib mir,*" and "*bitte heb mich herauf.*" The boy often used the third person, though, in designating himself, as when the father would ask "*Wo ist Axel?*" the latter would respond "*da ist er wieder.*"

These citations will perhaps suffice to indicate that there is no certain and invariable order followed by all children in the employment of the personal pronouns. In my own observations *my* has been the first form to be adopted. In every case it came before *I*. It was used in such relations as the following: *My* want to do this or that; *my* feel bad; that is *my* pencil or apple, or what not; take *my* to bed or out of doors. The form *mine* came considerably later than *my,* and *I* still later. To my mind the situations involving the use of *my* are more concrete, more obvious than those involving *I,* and it seems reasonable that it should first appear, and once it gets started it will serve for *me, mine,* and *I* for a time. The use from the beginning of all forms of the pronouns, as given in some of the vocabularies, appears to me very remarkable, and quite in contrast to the child's usual method of procedure in similar situations.

Why does the child not settle upon one form permanently? For the very effective reason that his social environment will not

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[1] *Op. cit.,* p. 606.

[2] *Zur Frage der Psychischen Entwickelung der Kindersprache* (Breslau, 1899), p. 35.

[3] *Wie lernt Mann eine Sprache,* p. 5.

[4] Studies of a Child; *Ped Sem.,* Vol. XI, pp. 264–291.

[5] *Op. cit.,* p. 202.

permit him so to do. His parents, once he gets to using pronouns at all, keep putting the conventional forms before him whenever he uses a form incorrectly; his brothers and sisters and playmates make fun of him for his lack of conformity to environmental standards, and the teacher tries to habituate him in the use of the standard forms, and gives him rules for his guidance. These are all powerful corrective forces, and no child can long resist them, except in respect of the least important matters. Then simple imitation, where the child more or less unconsciously copies the models in his environment, is of immense importance in leading him to appropriate the various forms employed about him. It is suggestive to note in this connection that when an adult tries to write or speak a foreign language with which he is not very familiar he experiences much trouble in mastering the cases of his pronouns. If he is just a novice one form will answer for all cases, and one of the difficult tasks in using a foreign tongue with ease is to gain facility in employing the right form of the pronoun in different situations; and of course, this principle applies to other parts of speech than pronouns.

ON THE NATURE OF THE PROCESS OF OSMOSIS AND OSMOTIC PRESSURE WITH OBSERVATIONS CONCERNING DIALYSIS.*

LOUIS KAHLENBERG.

INTRODUCTION.

A brief but excellent outline of the history of the development of our knowledge of osmosis up to 1877 is given by Pfeffer in his well-known monograph, "Osmotische Untersuchungen." The great importance of osmotic phenomena in physiological processes was clearly recognized as early as 1826 by Dutrochet, and for half a century later osmotic investigations were conducted very largely, though not exclusively, in the interests of physiology. Precipitated membranes were first used by the botanist, Moritz Traube,[1] in 1865, and these were employed by Pfeffer in his researches above mentioned. Special interest in osmosis has developed since 1887, when by using Pfeffer's data of osmotic pressure measurements van't Hoff sought to show that the simple gas laws hold for dilute solutions. Since the latter date, so-called semipermeable membranes have been used almost exclusively in osmotic investigations. These membranes usually consisted of ferrocyanide precipitates of some heavy metal, copper ferrocyanide being the favorite precipitate for osmotic work, though the ferrocyanides of zinc and nickel were occasionally employed, as were also a few other precipitates.

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[1] Centralblatt f. medic. Wissenschaften, 1865.

In this later work effort in two directions is clearly discernible. (1) Those who have been favorable to van't Hoff's hypothesis of solutions (based on the analogy between gases and solutions) according to which the osmotic pressure, so-called, is due to the bombardment of the semipermeable membrane, by the dissolved molecules, have sought either to compare osmotic pressures of aqueous solutions with each other, or to measure directly the osmotic pressure of certain solutions in the hope of securing data to uphold the theory. It is true, however, that considering the vast importance of direct measurements of osmotic pressure for the van't Hoff theory of solutions, but little effort has been made to measure osmotic pressures directly. This has come about very largely because of the attitude taken in the matter by the main adherents of the van't Hoff theory, who voiced and continually supported the dogma that the osmotic pressure is necessarily independent of the nature of the membrane if it be semipermeable; and that since it is very difficult to measure osmotic pressures directly, it is better to content one's self with the so-called "indirect" measurements of osmotic pressure, namely, with a computation of the latter from vapor tension, freezing-point or boiling-point observations on solutions, which, be it remembered, involves the assumption that the gas laws hold for solutions. And so we have the rather remarkable situation that direct measurements of osmotic pressure, and indeed the general investigation of osmosis, has not only been neglected by the chief advocates of the gas theory of solutions, but they have in addition through the attitude they have taken actually discouraged work in this direction. They have even claimed to have proven by thermodynamics that the osmotic pressure must be independent of the nature of the membrane provided the latter is semipermeable. The assumptions made in such "proofs," and the fact that there is in reality no such thing as a semipermeable membrane in the strict sense of the word, have been passed over lightly. (2) Quite a different direction in the investigation of osmotic phenomena has been taken by those who have held van't Hoff's conception of the nature of osmotic pressure to be untenable. These men have continually brought forward experiments, of a qualitative nature to be sure, showing that the hypothesis of van't Hoff can not be held and that no special stress is to be laid upon direct measurements of osmotic pressure, which they have consequently not attempted to make.

In his efforts to measure osmotic pressures directly, Tammann[1] came to the conclusion that it is not possible to obtain reliable, concordant results by means of the method adopted by Pfeffer,[2] which he consequently abandoned entirely and turned his attention to comparing the osmotic activity of various solutions with one another. Attempts at direct measurements of osmotic pressure have again been taken up recently by H. N. Morse[3] and his coworkers. They have measured the osmotic pressures that are developed when aqueous sugar solutions are separated from water by means of precipitated membranes of copper ferrocyanide. The method they employed is essentially that of Pfeffer, with the exception that they prepared the membranes with the aid of electrolysis. Enough can hardly be said in praise of the care and perseverance exercised by Morse and his assistants in this work, and yet they have neglected a very essential point in their determinations as will appear from considerations given below, and consequently their experiments are not conclusive in establishing, as they suppose, that the gas laws hold fairly well for the osmotic pressures of aqueous sugar solutions, using copper ferrocyanide membranes. Furthermore, attempts to generalize from the data collected by Morse and Frazer on aqueous sugar solutions, as to the behavior of all solutions taking no consideration of the membranes employed, are quite unwarranted. Moreover, while according to the work of Morse and Frazer and also according to Flusin[4] the aqueous sugar solutions show osmotic pressures in approximate conformity of the gas laws, the Earl of Berkeley and E. G. J. Hartley[5] have found materially higher pressures than those deduced

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[1] Wied, Ann. 34, 299 (1888). See later attempts by new method Zeit, Phys. Chem. 9, 97 (1892).

[2] Pfeffer, be it remembered, worked solely in the interests of physiology and for his special purpose, his experiments were quite sufficient.

[3] Amer. Chem Jour. 34, 1 (1905).

[4] Compt. Rendus 132, 1110 (1901).

[5] Proceedings Royal Society (London), 73, 436 (1904).

from the gas law, even though their membranes, which were also prepared with the aid of electrolysis, were admittedly not perfectly tight.

Among the many opponents of the van't Hoff theory of osmosis may be mentioned Lothar Meyer, Raoult, Fitzgerald, Pickering, Quincke, Dieterici, J. Traube, Battelli and Stephanini. The opponents of the van't Hoff idea have generally held that the so-called osmotic pressure is an ordinary hydrostatic pressure brought about by entrance of liquid into the osmotic cell. Concerning the reason for this entrance of additional liquid into the osmotic cell there has, however, been difference of opinion; some holding that it is due to attraction that is essentially chemical in character; others that it is due to capillarity; and still others that it is caused by surface tension. The latter view has lately been prominently brought forth by Traube,[1] whose claim is that "The difference in surface tensions determines the direction and velocity of the osmosis." His idea is that the main direction of osmosis is always toward the liquid having the greater surface tension. Again, Battelli and Stephanini[2] also express the opinion that difference in surface tension is the cause of osmosis. But on the basis of their experimental work, they modify Traube's contention to the effect that the main osmotic current is not always toward the liquid of higher surface tension, but that the process always proceeds in such a direction as to tend to equalize the surface tensions of the liquids on the opposite sides of the septum. Very recently Barlow[3] has also brought forward cases which are not in harmony with the theory of Traube.

In the present investigation the main purpose has been to inquire into the nature of osmosis and osmotic pressure, and to test whether the latter really follows the gas laws. To this end a considerable number of osmotic experiments were performed in which liquids of similar and also of very different character were separated from each other by different membranes. In this work observations were taken as to the direction of osmosis with change of membrane, with change of solvent and also with

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[1] Phil. Mag. (6) 8, 704 (1904).

[2] Atti della Reale Accademia dei Lincei 14, 3 (1905).

[3] Phil. Mag. (6) 10, (1905).

change of solute. The effect of temperature was considered. The degree of permeability of the membranes for various substances was noted, with the result that membranes as semipermeable as any known hitherto were found, which are yet not precipitated membranes. Furthermore it was ascertained *why* the membranes were semipermeable in some cases and not in others. It was consequently possible to foretell for which substances the membranes were permeable and for which substances not permeable. Further, this work has cast light on the process of dialysis. It has been possible to separate different dissolved crystalline substances from each other by dialysis, and also to separate dissolved crystalline from non-crystalline bodies by having the *non-crystalline substances pass through the septum* and the crystalline ones remain behind in solution in the dialyzer. The direct measurements made with the semipermeable membranes employed, moreover, showed that the gas laws do not hold at all in these cases. It was also found that in making direct measurements of osmotic pressures it is necessary to stir the liquids separated by the membrane, a very important fact which has been entirely overlooked in all osmotic pressure determinations hitherto made. The necessity of such stirring was really accidentally discovered, as will appear from details given below, after having worked in vain for over a year trying to get reliable, concordant results without stirring. The discovery that agitation of the liquids is essential in osmotic pressure measurements is of paramount importance in deciding as to the nature of the osmotic process.

In the presentation of the experimental work which now follows, the experiments will not be described in the order in which they were actually performed for the reason that greater clearness will be obtained by detailing the results in the light of the theory which was gradually evolved in the course of the investigation.

A TYPICAL CASE OF OSMOSIS.

If chloroform A, Fig. 1, be placed in a glass tube and a layer of water B be poured upon it, and again a layer of ether C be carefully poured upon the water, and the whole be allowed to stand, there will eventually be but two layers A′ and B′, Fig. 2. An examination of A′ shows that it consists of chloroform and

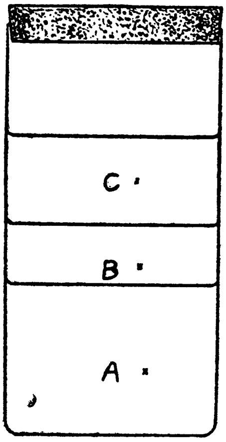

Fig. 1.

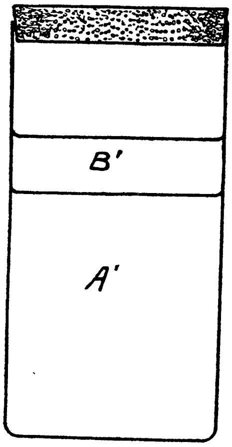

Fig. 2.

ether saturated with water, whereas the layer B′ consists of water saturated with ether and chloroform. In the change which has taken place, one layer, that of the ether, has gradually disappeared, and the lower layer has greatly increased in bulk and lifted the aqueous layer to the top. The explanation of this phenomenon is evident. Ether dissolves very readily in chloroform, but in water it dissolves much less readily; again, chloroform and water hardly dissolve each other at all. In the arrangement we have in Fig. 1, the aqueous layer B dissolves ether, and in turn the ethereal layer C takes up some water. When the ether has gone into B until it touches the chloroform layer A, the latter extracts ether from the aqueous layer B. Thus the upper part of the chloroform layer A becomes enriched with ether, whereas the lower part of the aqueous layer B becomes depleted in ether. The latter depletion is made good by a continuous supply of ether from the upper parts of B, which are in turn supplied with ether from C. Again, the ether in the upper layers of A gradually diffuses into the more distant parts of A. This process of the transportation of the ether in C through B into A proceeds, then, until the supply in C is exhausted. It is to be noted, however, that since chloroform is more soluble in water containing ether than in pure water, the aqueous layer B will take up more chloroform after becoming charged with ether. Thus some of the chloroform is making its way upward into the aqueous layer B, from which it also passes in part into the ethereal layer C.

It is clear then that the ether of layer C is making its way through the aqueous layer B into the chloroform layer A, and that on the other hand the chloroform is passing into the aqueous layer B charged with ether and further into C. Thus we have currents of ether and chloroform going in opposite directions. The movement of the chloroform as to quantity is, however, extremely slight as compared with that of the ether; and so the movement of the latter practically predominates and the ethereal layer is finally absorbed. It must be borne in mind, however, that water, being somewhat more soluble in chloroform charged with ether than in pure chloroform, will to a slight extent go into the layer A as it becomes enriched with ether; and, as has already been remarked, some water also passes into the layer C, for water is somewhat soluble in ether. When the change is complete, we have, as stated above, a lower layer consisting of chloroform and ether saturated with water, and an upper layer of water saturated with ether and chloroform.

The rate at which this process goes on depends on the temperature and upon the pressure, but also upon the thickness of the aqueous layer B and the area of the surface of contact with the ethereal layer C and the chloroform layer A. It is further clear that the process would be hastened if each of the layers A, B, and C were continually stirred, for by so doing the slow processes of diffusion would be aided and the changes in concentration which take place in the layers where they are in contact with one another would be lessened.

Summed up then, the observed change goes on because ether is soluble in water, but much more readily soluble in chloroform, so that latter is able to extract ether from the aqueous solution of ether, B. The chloroform—ether solution in A may be regarded as a solution of chloroform in ether, especially after a considerable quantity of ether has accumulated in it; and since but very little chloroform passes into the aqueous layer B, the latter is practically permeable only for ether, and therefore this aqueous layer B acts as a *semipermeable septum.* It permits ether to pass from C to A, but allows very little, practically no chloroform to pass into C. It is clear further that as A becomes richer and richer in ether, the tendency for more ether to enter A from B becomes less and less. Ether dissolves more readily in chloroform than in water because ether and chloroform have a greater mutual attraction for each other than have ether and water; it is for this reason too that chloroform is able to extract ether from an aqueous solution of the latter. As ether accumulates in A, however, the power of this layer to extract ether from the aqueous layer B diminishes, because the attraction or affinity of chloroform and ether for each other becomes more and more satisfied. Finally, suppose we prevent the supply of ether in C from becoming exhausted by adding some as may be required, a point will be reached where compartment A has become so rich in ether that the attraction or affinity of this chloroform—ether solution for additional ether has decreased to such an extent that it can no longer extract further ether from the aqueous layer B. In other words, when the chloroform solution in ether, A, has become so dilute that its attraction for additional ether just equals the attraction of water for ether in the aqueous layer B, the process is arrested. The point at which this occurs would clearly vary with the temperature and also with the pressure.

If in Fig. 1 compartment A be filled with carbon disulphide instead of chloroform, the process would go on as before in a similar manner and for perfectly similar reasons. Indeed, any liquid which in itself does not mix with water, practically speaking, and yet has a greater attraction for ether than has water, would serve in place of the chloroform. The rapidity with which the process proceeds and the final point of equilibrium reached would, however, also be a function of the nature of the substance so employed. If in Fig. 1 the ether in C be replaced by an oil, say olive oil or a hydrocarbon oil, retaining the water in B and chloroform in A, nothing will take place,[1] for these oils are not appreciably soluble in water, that is to say the attraction or affinity existing between them and water is insufficient to overcome their cohesions to the extent necessary to cause a fusion, a blending, an interpenetration of their masses. Again, if the water in B were replaced by a liquid in which neither ether nor chloroform are appreciably soluble, nothing would take place. Further if the chloroform A be replaced by a liquid which does not dissolve water and has less attraction for ether than has water, nothing will take place. *It is clear then that the process under consideration proceeds because of the specific nature of the septum B and also that of the two liquids that bathe it.* It should also be emphasized in this connection that while it is essential, as stated above, that the layer B, the water, must be capable of dissolving C, the ether, it is further necessary that this solubility be restricted in character, as it is in fact, otherwise the layer B, would not be distinct from C and would become so rich in ether that the boundary lines between A and B would also disappear.

Returning now to the original experiment Fig. 1 in which ether, water and chloroform are in A, B, and C respectively, let us imagine the aqueous layer B as quasi solid and also immovable, i. e. attached firmly to the sides of the glass tube, nevertheless otherwise retaining its original properties. This would make A a compartment whose volume remains fixed, and as the ether enters it from B, for reasons already detailed, a hydrostatic pressure would be produced upon the sides, top and bottom of A; and as this pressure develops, it would become more and more difficult for additional ether to enter this compartment. Finally, if the walls of this compartment did not give way, a point of maximum pressure would be reached. At this point, at the temperature of the experiment, the affinity of the

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[1] If olive oil be dissolved in ether and this solution placed in C, whereas water and chloroform are retained in B and A respectively, the ether would pass through the water into the chloroform leaving the olive oil behind in C. We have here an illustration of what may be called selective action on part of the membrane (the aqueous septum B) in which property biologists are particularly interested. The explanation is obvious. Water dissolves ether appreciably, but not olive oil, so the latter is left behind.

ether—chloroform solution in A for additional ether is insufficient to extract further ether from the aqueous layer B. In other words, the affinity of water for ether aided by the hydrostatic pressure developed in A (which militates against the ether passing into A) just balances the affinity of the chloroform—ether solution in A for additional ether. And yet the case is, after, all not quite so simple, for it must, be remembered that chloroform is somewhat soluble in water saturated with ether at atmospheric pressure; now this solubility is increased with increase of pressure, so that as the hydrostatic pressure in compartment A increases due to the influx of ether, the outflow of chloroform is continually slightly increasing, which tends, of course, to relieve the pressure. The actual final maximum pressure reached is therefore determined by the relative influx of ether into compartment A and the outflow of chloroform from that compartment. When this outflow is practically nil, or at any rate very slight, we should be dealing with a so-called semi-permeable membrane; when the outflow is not a negligible quantity, as in the case of most septa, the final maximum pressure attained is materially influenced thereby.

Now, it is easy enough to imagine the aqueous layer B firmly held in place as we have done, but to realize this experimentally presents great difficulties. It may, however, readily be demonstrated that such hydrostatic pressure is actually produced, without to be sure making an attempt to furnish anything more than a qualitative proof that such pressure is really formed. The apparatus used for this purpose is shown in Fig. 3. In this glass tube, D represents mercury, A, chloroform, C, ether, and B is a slice of an excellent piece of cork which had been kept under boiling water for some time so as to drive the air out of it and thoroughly inject it with water; during this process much soluble material was also extracted from the cork. The cork thus thoroughly soaked with water was firmly pushed into position. It is, of course, somewhat difficult to avoid having air bubbles just above and below the cork, but by careful manipulation it is possible to secure the arrangement as shown in Fig. 3. The chloroform and ether used in this case were each first saturated with water, so that they would not unduly rob the cork of its water content, which was necessarily small enough to begin with. On allowing the apparatus to stand, the mercury rises in the tube as indicated, showing that pressure is produced on the walls of A. The mercury rose until the cork either broke or began to slip upward, the experiment being repeated three times. Using a cork not soaked in water no pressure was obtained. It is quite probable that a cork might be so fastened in place by the aid of mechanical contrivances that an ordinary tube would give way before the cork would move; but since the maximum pressure could not be measured in this manner, the qualitative demonstration of the presence of the pressure was deemed sufficient. The experiment just described may be performed also in the form illustrated in Fig. 4, which is more like the usual arrangement employed in osmotic experiments. In this figure, the liquid B in the bottom of the bottle is ether; the end of the glass tube is closed with a tight fitting slice of cork A saturated wtih water; in the tube above the cork is chloroform, C; and above the chlo-

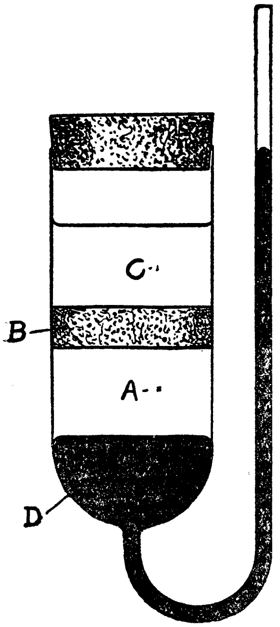

Fig. 3.

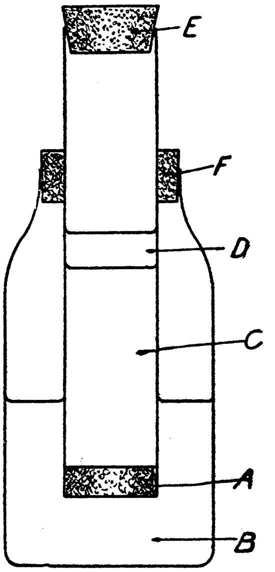

Fig. 4.

roform is water, D. At E there is a cork collar which holds the tube in place. This collar does not fit perfectly tight, yet it minimizes the evaporation of the ether. The cork E is rather loosely inserted; its purpose is simply to prevent undue evaporation. This was also the purpose of the layer of water, D, which to be sure also kept the chloroform saturated with water. Both the ether and chloroform used were saturated with water to begin with as in the previous case. With this arrangement the layer C increased in bulk, the column rising until the layer D touched the cork E. The experiment was continued for two weeks, the cork at A being rather thick caused the change to progress slowly.[1]

This case of ether, water, chloroform, Fig. 1, has been thus described in detail because it illustrates all the essential points to be taken into consideration in the study of the osmotic process. We have seen what conditions are necessary for the process to proceed at all, what are the various factors that modify the rate of the change, and what causes the so-called osmotic pressure. It has further been shown under what conditions we get a so-called semi-permeable membrane, and it is evident that an absolutely semi-permeable membrane exists only in theory. On the basis of his elaborate experimental researches, Quincke[2] has also arrived at this conclusion. He stoutly contends that there really is no such a thing as a semi-permeable membrane and that a theory which postulates such can not be maintained. His words are, "Ich bestreite, dass eine halb durchlässige Membran existirt. Damit fällt aber auch der osmotische Druck, dessen Theorie die Existenz einer halbdurchlässigen Membran voraussetzt."[1] As stated above, there is in general always an outflow from an osmotic cell as well as an inflow, and when the former is extremely slight as compared with the latter, the membrane is *called* semi-permeable; but commonly the outflow is quite sufficient to demand consideration. At any rate, the osmotic pressure is always the resultant of inflow and outflow caused by the attractions that come into play,

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[1] Compare in this connection a similar experiment of Nernst, Zeit. Phys. Chem. 6, 35 (1890).

[2] Drude's Ann. 7, 682 (1902).

which attractions are to the mind of the writer the same as what is commonly called chemical affinity, and consequently such so-called osmotic pressures may under suitable conditions be very great indeed, while under other conditions they may be quite small. They would, moreover, present considerable variation according to the nature of the substances employed.

No originality is claimed for the ether, water, chloroform experiment, Fig. 1. It occurred to me after a goodly number of the experiments described below had been performed; but in looking over the earlier articles of the rather voluminous literature on the subject of osmosis, I found that in 1854 L'Hermite (Compt. rend. 39,1177) described the experiment in question. His statements concerning its import and bearing are very clear, though, of course, he does not speak of semi-permeable membranes and osmotic pressures, for these concepts were at that time quite unknown. A reference to the article of L'Hermite is also made in the bibliography in Lehmann's Molekularphysik.

However, after having L'Hermite's experiment clearly before me, the principles it illustrates soon became the guide in future experimentation, for they enabled me to foretell whether a membrane would be permeable or impermeable for a certain substance under given conditons; and if permeable for several substances, which of these would go through most readily. I was thus enabled to forecast in which direction the main osmotic current would go. These matters were not only of consequence in direct measurements of osmotic pressures, detailed below, but they were also of importance in dialysis as will appear farther on. Again, by demonstrating the nature of the process, it clearly appeared that in determining the maximum osmotic pressure the contents of the osmotic cell must be continually stirred. For, taking the arrangement as given in Fig. 1 and again imagining the aqueous layer B as quasi solid and firmly fixed in place, it is evident that as ether is drawn into compartment A, the liquid in that compartment becomes enriched with ether just where it touches B, and that consequently the osmotic pressure set up in A is not as great as it would be if the liquid in A were continually stirred so as to rapidly distribute the incoming ether throughout the cell and present a fresh surface to the layer B. When stirring is omitted, this work of keeping the contents of B of uniform concentration has to be performed by diffusion, a process which is very slow, and consequently a lower pressure is obtained. It is clear that for similar reasons the contents of B ought in general also to be stirred in attempting to measure the maximum osmotic pressure, as should also the liquid C. But when B is quite thin, as is the case with some membranes, stirring its contents is of less consequence. Furthermore, when practically very little of A enters C (i. e. when B is a so-called semi-permeable septum) it is obviously not so necessary to stir the contents of C in osmotic pressure measurements. But the liquid in the osmotic cell must *always* be stirred, though it is recognized that in some forms of cells this is far more imperative than in others. Attempts to make direct measurements of osmotic pressures without stirring the contents of the cell are comparable with attempts to make a saturated solution of a salt by placing an excess of it in a dish, pouring the solvent upon it and allowing the whole to stand without agitation. The method of stirring the contents of the osmotic cell will be described below in connection with the quantitative measurements of osmotic pressures.

QUALITATIVE EXPERIMENTS.

The following experiments which are largely of a qualitative nature were performed in the course of the investigation in order to determine the influence which the character of the membrane has upon the permeability of the latter, and also upon the main direction of the osmotic current. Unless otherwise stated, the osmometer used was a very simple one, consisting of an ordinary thistle tube, the mouth of which was closed with the membrane employed. The latter was tied on with thread, in which process great care was used to secure a perfect contact between the glass and the membrane. To accomplish this a thistle tube was selected with a flare of about forty-five degrees at the mouth. On such a tube the membranes used could be perfectly securely tied. Very many evenly applied turns of a stout, moderately fine, white cotton thread were wound on after the membrane had been carefully placed in position over the mouth of the tube. This part of the work required much time, patience and perseverance, as well as skill and practice. After thus securing the membrane over the mouth of the thistle tube, the latter was filled with one of the liquids to a point slightly above the bell of the tube, which was then immersed in a beaker containing the other liquid. The level of the two liquids was, of course, the same to begin with.

For convenience in future reference the experiments performed have been numbered. The liquid in the bell of the osmometer will always be called the inner or *inside* liquid; that in the beaker, the outer or *outside* liquid.

1) The membrane consisted of sheet rubber, vulcanized caoutchouc, such as is used by dentists in making their "rubber dam." This rubber was very elastic, being almost the pure gum. It could readily be stretched over the osmometer and tied on so as to form a perfect joint, for all folds could be avoided. The inside liquid was a 20 per cent aqueous cane sugar solution; the outside liquid was pure water. The experiment ran 48 hours. No change took place. The liquids remained at the same level and no sugar passed through the membrane into the water on the outside.

2) A rubber membrane was used as before. The inside liquid consisted of a 20 per cent aqueous NaCl solution, the outside liquid of pure water. No change took place, the liquids remaining on the same level and not a trace of salt appearing in the water without. When the outside water was replaced with toluene, the latter caused the rubber to swell, but no salt or water passed into the toluene, neither did the toluene go into the brine. On afterward exposing the membrane to the air, it lost its toluene content and shrank to its former size, remaining throughout quite elastic.

Experiments 1 and 2 show that it is possible to get the joint between rubber and glass perfectly tight. It is perfectly clear that no change took place when water and the aqueous solution touched the rubber, for the reason that there is no affinity between these liquids and rubber. Thus the first condition necessary for osmosis to take place was lacking, namely, that the membrane must be able to dissolve (i. e. imbibe or take up) one of the liquids that bathe it. When in the second part of experiment 2 the water was replaced by toluene, the latter was taken up by the membrane; but osmosis did not take place because the liquid on the other side, the brine, having no affinity for toluene to speak of was unable to extract toluene from the rubber.

3) The inside liquid was water, the outside liquid 99.5 per cent alcohol, the membrane rubber. The liquid rose in the osmometer showing the main current to be from the alcohol through the rubber to the water. This is what one would expect for the alcohol is taken up by the rubber and then the water by virtue of its affinity for alcohol extracts the latter from the rubber.

4) This experiment was like No. 3 except that a 20 per cent aqueous cane sugar solution was used as the inner liquid. Again the liquid rose in the osmometer, alcohol passing through the rubber into the sugar solution. No sugar, however, was found in the alcohol on the outside. The rise in the osmometer tube was slow, being about five centimeters in five days. The inside diameter of the stem of the thistle tube was about 3.5mm.

The main direction of the current was, of course, such as was to have been expected from what was said in connection with the preceding experiment. No sugar was found in the alcohol outside for sugar has so little affinity for alcohol, for rubber and for rubber soaked with alcohol that it is not taken up by any of these.

5) The inner liquid was pyridine, the outer liquid water and the membrane parchment. The liquid rose in the osmometer showing the main current to be from the water through the parchment to the pyridine. Some pyridine was also found in the water.

6) The experiment was like No. 5, except that a rubber membrane was used instead of parchment and the water was the inner liquid and the pyridine the outer. The liquid again rose

in the osmometer showing that the main current was in the opposite direction as in the preceding experiment, namely from the pyridine through the rubber to the water. Some water also passed into the pyridine, showing an appreciable minor current.

7) The inside liquid was pyridine, the outside liquid toluene and the membrane parchment. No change took place.

8) This experiment was like No. 7 except that rubber was substituted for parchment. The liquid at once rose in the osmometer, showing the main current to be from the toluene through the rubber to the pyridine. Some pyridine, however, also passed into the toluene.

Rubber readily takes up pyridine, and imbibes toluene still more readily; on the other hand parchment does not, showing that it has but little affinity for these liquids. It is consequently easy to see why no change occurred in No. 7. When it is further remembered that pyridine and water are consolute liquids, as are also toluene and pyridine the observations in Nos. 5, 6, and 8 are easily explained. In No. 5 the parchment imbibes water which is then extracted by the pyridine; but since pyridine is soluble in water soaked parchment, some pyridine also passes into the water outside. In No. 6 the rubber imbibes the pyridine which is then extracted by the water; but as water is somewhat soluble in pyridine soaked rubber, some of it makes its way into the pyridine without. In No. 8 the fact that toluene is imbibed more readily by rubber than is pyridine again determines the direction of the main current, though in this case, owing to the fact that pyridine has considerable affinity for rubber as well as for toluene, the minor current is of considerable consequence. The cases just considered well illustrate how the nature of the septum and of the liquids that bathe it determines what will actually take place.

In 1898, G. Flusin[1] used carbon disulphide, chloroform, toluene, ether, benzene, xylene, petroleum ether, benzyl chloride, turpentine, petroleum, nitrobenzene, methyl alcohol, ethyl alcohol, and acetic acid, taking these liquids in all possible com-

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[1] Compt. rend. 126, 1497 (1898); ibid. 131, 1308 (1900).

binations in pairs and separating them from each other by means of a membrane of vulcanized caoutchouc. He found the main current to be from the liquid which is the more readily imbibed by the rubber, through the septem to the less readily imbibed liquid. Again in 1900[1] he used water, methyl alcohol, amyl alcohol, amyl acetate, chloroform, benzene, ether, and ethyl alcohol. He employed hog's bladder as a septem placing one side of it in contact with ethyl alcohol (the liquid which, of those named, is according to him imbibed least readily) and bathing the other side with each of the other liquids successively. He always found the main current to be in the direction toward the ethyl alcohol and the rate of flow to vary with the amounts of liquid imbibed by the membrane during the first five minutes, which amounts were, of course, determined by independent experiments. I have confirmed all of the results of Flusin where he used rubber membranes. He says nothing, however, about the minor current, which I found to be present in all of these cases to a greater or lesser extent. In other words, the rubber was traversed by both liquids of each pair, though the main direction of the current was quite correctly determined. Flusin shows that the affinity between membrane and liquid is to be measured by the rate with which the latter is imbibed by the former, and not by the total amount of liquid taken up by a given quantity of membrane at the end of a long time, as Tammann[2] contends. Raoult[3] separated methyl alcohol and ether from each other by means of rubber. He always found the direction of the main current to be from the ether through the rubber to the methyl alcohol; and the direction of the main current remained the same, even when the ether was considerably diluted with methyl alcohol. When he substituted a membrane of hog's bladder for the rubber, the direction of the main current was reversed, it being from the methyl alcohol through the septum to the ether. In his article, Raoult has omitted to say anything about the fact that in his experiments there is also a minor current in a di-

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[1] Compt. rend. 126, (1497) 1898; ibid. 131, 1308 (1900).

[2] Zeit. Phys. Chem. 22, 491 (1897).

[3] Compt. Rend. 21, 187 (1895). Zeit. Phys. Chem. 17, 737 (1895).

rection opposite to that of the major; in other words, that when caoutchouc is the membrane and ether is going into the alcohol there is also some alcohol passing into the ether; and when the bladder is employed, there is some ether passing into the alcohol, though the main current is from the alcohol to the ether. Raoult was, however, perfectly clear in his own mind with regard to this matter as will appear from a quotation from a letter written by him printed below.

It is hardly necessary to add that in the light of what has already been said above, the results obtained by Raoult and Flusin are exactly such as might have been foreseen.[1]

9) The inner liquid was glacial acetic acid, the outer liquid distilled water and the membrane rubber. Within five minutes blue litmus paper placed in the water turned red, showing that the acid was passing through the rubber. This was the direction of the main current, which might have been expected, since glacial acetic acid is more readily absorbed by rubber than is water.

10) The inner liquid was a 10 per cent solution of acetic acid in water, the outer liquid water, and the membrane rubber. After 1.5 hours, the blue litmus in the water began to turn red, indicating that in this case, too, the acid was passing through the rubber into the water. The direction of the main current then was not altered by diluting the acetic acid considerably with water, which result is in line with what Raoult found in the ether—methyl alcohol experiment referred to above.

11) The inner liquid was a strong solution of trichloracetic acid in water, the outer liquid water and the septum rubber. But very little acid passed through the rubber into the water. Hardly any change took place in the 20 hours during which the experiment was continued.

12) The inside liquid was a solution of trichloracetic acid in benzene, the outside liquid water and the membrane rubber. In this case acid went into the water in very considerable quantity. This was the direction of the main current. The experiment was run for 20 hours.

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[1] Compare also the remarks by Tammann, Zeit. Phys. Chem. 22, 490 (1897).

13) The inner liquid was a solution of trichloracetic acid in water to which considerable benzene had been added, the outer liquid was water and the septum rubber. Very notable quantities of acid passed through the membrane into the water without. The experiment was continued for 20 hours.

The large increase in the amount of trichloracetic acid which passed through the rubber in 12 as compared with 11 is easily explained by the fact that benzene has considerable affinity for trichloracetic acid and also for rubber. In imbibing the benzene, therefore, large amounts of trichloracetic acid are also absorbed with that hydrocarbon by the rubber. When we dissolve trichloracetic acid in benzene we really unite the acid with the benzene.[1] In this homogeneous liquid, the solution, we have then the acid tied to the benzene; and because of the great affinity of the latter for rubber, the benzene solution of trichloracetic acid, the combination of the acid and the benzene, also has considerable affinity for rubber; and so the acid is also drawn into the rubber because it is united with benzene. To be sure, benzene and acid are not taken up by the rubber in the same proportions in which they occur in the solution, benzene being taken up in relatively larger amounts. This means that the solution is to a certain extent decomposed, i. e. altered in concentration, by the rubber. When the latter soaked with benzene and the acid is in contact with water, the great affinity between water and the acid again comes into play and by virtue of it acid passes into the water, leaving the benzene behind in the rubber almost completely. Similar considerations hold in No. 13 where a solution of trichloracetic acid, water and benzene is separated from water by a rubber septum, except that here the acid passes through in lesser quantity, and minor amounts of water are undoubtedly also absorbed by the rubber, since in the solution the water is tied on to the benzene and the acid.

14) The inner liquid consisted of pure water, the outer of 0.1 normal solution of $AgNO_3$ in pyridine, and the membrane of rubber. The liquid rose rapidly in the osmometer, reach-

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[1] Compare, Kahlenberg, Über das Problem des Lösungen, Chem. Zeitung 29, No. 81, (1905).

ing a height of 25 cm. in 4 hours. The experiment was then stopped and the contents of the cell examined. It was found that pyridine had passed into the water, but no appreciable quantity of nitrate of silver. The entire content of the osmotic cell was evaporated to dryness, the residue extracted with a little water and a drop of nitric acid, and the filtered solution tested with HCl for silver, but none was found.

15) The inner liquid was a 0.1 normal $AgNO_3$ solution in pyridine, the outer liquid pure water, and the membrane parchment. The main current was from the water, through the parchment to the $AgNO_3$ solution in pyridine, for the level of the liquid in the osmometer rose to a height of about 18 cm. in 20 hours. Water then goes into the cell, forming the main current, but both $AgNO_3$ and pyridine were also found in the water outside, showing a considerable counter current toward the outer liquid.

16) The inside liquid consisted of 0.1 normal $AgNO_3$ solution in water, the outside liquid of 0.1 normal $AgNO_3$ solution in pyridine, the membrane being rubber. The main current was from the outer liquid to the inner one, the level rising in the stem of the osmometer. During the night the liquid filled the tube (which was about 32 cm. long) entirely and ran over.

17) The outside liquid consisted of toluene, the inside liquid of 0.1 normal solution of $AgNO_3$ in pyridine, and the membrane of rubber. The liquid rose 22 cm. in the osmometer in 17 hours. The main current consists of the passage of toluene through the septum to the pyridine solution of the $AgNO_3$; however, pyridine was also found in the toluene outside as were mere traces of silver nitrate.

18) This experiment was identical with No. 17 except that a parchment septum was employed. It was continued for 4 days and no change was observed. It seemed as though a slight lowering of the level of the inner liquid might have taken place, so the contents of the cell were evaporated to dryness, the residue taken up with water and a drop of nitric acid and tested with HCl for silver, after having been filtered. Only the faintest indication of the presence of silver was thus obtained.

19) The inside liquid was 0.1 normal solution of $AgNO_3$ in pyridine, the outside liquid pyridine and the septum rubber. The liquid rose slowly in the osmometer, indicating the direction of the main current to be from the pyridine through the rubber to the solution. An examination of the outer liquid showed the presence of only a very small amount of nitrate of silver. The experiment was repeated using a much heavier piece or ordinary gray sheet india rubber as a septum, with the same result. And again, it was repeated using the rubber dam as a membrane once more, but supporting it by tying over the outside of it a piece of muslin. With this arrangement the liquid rose in the stem of the thistle tube to a height of 28.5 cm. in 18 days, remaining there constant for two days, and then receding slightly. The temperature was very nearly 17 degrees throughout the test. The outer liquid was found to contain appreciable amounts of $AgNO_3$, but hardly an estimable quantity. This shows that vulcanized caoutchouc is practically impermeable for $AgNO_3$ under the conditions described; in other words that it is a ''semi-permeable'' membrane.

20) The inner liquid was a 0.05 normal solution of $AgNO_3$ in pyridine, the outer pyridine, and the septum rubber. At 17° C. no change whatever was observed after 6 days. After 19 days a rise of about 0.5 cm. of the liquid in the stem of the osmometer was observed. The outer liquid was then analyzed for silver nitrate. Only traces were found, not an estimable quantity. This shows that the membrane did not leak and that the so-called osmotic pressure of a $AgNO_3$ solution in pyridine which is 0.05 normal is practically nil at 17° C. when vulcanized caoutchouc is used as the semi-permeable membrane.

21) The inner liquid was a normal solution of $AgNO_3$ in pyridine, the outer liquid pyridine, and the membrane rubber. The apparatus was kept at a temperature which varied gradually between —16° and —15° C. At the end of the second day the liquid in the osmometer had risen to a height of 7.2 cm., the temperature being —16.° At the end of the third day the height of the column was 15.6 cm., the temperature being —15.° The membrane was intact and but traces of silver were present in the outer liquids.

22) The inner liquid was normal solution of $AgNO_3$ in pyridine, the outer liquid pyridine, and the membrane rubber. In this case a piece of common vulcanized rubber about 1 mm. thick such as is used on footpower laboratory bellows was employed. It was supported on each side by pieces of muslin and perforated steel discs, and the whole was then securely screwed to the lower end of an osmotic cell made of steel. The maximum pressure which was read on a closed manometer, using mercury between the air space and the inner liquid was 14.95 atmospheres at 20° C. The membrane did not "leak," which was evident from the fact that only mere traces of $AgNO_3$ were found in the outer liquid, though the experiment was run for two weeks, the pressure remaining practically constant for five days. This steel osmotic cell consumed considerable time in its construction, and many difficulties had to be overcome in perfecting it and attaching the manometer to it. However, it is unnecessary to enter into a detailed description of the cell, since no special significance will be attached to the single result recorded here, it being given simply to show that a very considerable pressure may be produced by a normal solution of $AgNO_3$ in pyridine when it is separated from pure pyridine by vulcanized caoutchouc at 20°, whereas at —16° (No. 21) the pressure formed is practically insignificant.

Silver nitrate is insoluble in hydrocarbons, which shows that the affinity between that salt and hydrocarbons is slight. Now as caoutchouc is a hydrocarbon substance, the affinity between it and silver nitrate would be slight. Pyridine has considerable affinity for silver nitrate. It dissolves the salt readily with evolution of a considerable amount of heat. Pyridine is soluble in all proportions in hydrocarbons—is consequently readily imbibed by rubber. When a silver nitrate solution in pyridine is placed in contact with rubber, the latter soaks up pyridine, but also some silver nitrate with it, since the pyridine and the salt are bound to each other by mutual attraction. This accounts for the fact that traces of silver nitrate pass into the pyridine when it is separated from a silver nitrate solution in pyridine by means of a rubber septum as in experiments 19, 20, 21 and 22, though the main current is that of the passage of pyridine through the membrane to the solution. Thus the reason why caoutchouc is a "semipermeable" membrane in these cases is given; and we should expect in all cases in which the solute employed is insoluble in hydrocarbons, like kerosene, benzene, etc., yet is soluble in pyridine, that vulcanized rubber will act as a "semipermeable" membrane when it is employed in separating pyridine from the pyridine solutions of such solutes. This has been confirmed in the case of cane sugar and lithium chloride which are soluble in pyridine yet insoluble in hydrocarbons. The experiments are given below. Conversely, when a substance is soluble in hydrocarbons as well as in pyridine, that substance will always pass through vulcanized caoutchouc in notable quantities when its solution in pyridine is separated from pure pyridine by means of the caoutchouc septum. Examples of such cases will also be found below. Though experiments 19 to 22 are only quasi quantitative in character they are already quite sufficient to show that here the osmotic pressure does not follow the gas laws at all. The change of the pressure with temperature is very much greater than proportional to the absolute temperature; and again the pressure varies much more rapidly with change of concentration of the solute than is required by Boyle's law. Experiment 20 reveals the fact that at room temperature the osmotic pressure of the 0.05 normal solution of $AgNO_3$ in pyridine is practically nil under the conditions described, while on the basis of the vant' Hoff theory the osmotic pressure of this solution ought to be over an atmosphere. We have in No. 20 the case where the solution has been diluted to such a point that its affinity for additional pyridine is practically equal to the affinity between pyridine and the rubber, so that the latter can not be robbed of its pyridine content by the solution, and consequently the liquid in the osmotic cell does not increase in bulk.

The observations made in Nos. 14 and 16 are such as might have been foreseen considering the fact that water has practically no affinity for rubber; that the latter has considerable affinity for pyridine; that water and pyridine are consolute liquids; and that silver nitrate, though soluble in water and pyridine, is yet not soluble in hydrocarbons and consequently has practically no affinity for rubber. Further, recalling that water is readily taken up by parchment, and pyridine and toluene not, and considering these facts in connection with those already mentioned, the data obtained in Nos. 15, 17 and 18 are readily explained.

23) The inside liquid was a 7.06 per cent cane sugar solution in pyridine, the outer liquid pyridine and the membrane rubber, supported by muslin tied over it. The liquid rose in the osmometer to a height of 22.7 cm. in 4 days, the height after the second day being 21.8 cm. Sugar had not passed into the outer liquid except in mere traces.

24) This experiment was like No. 23 except that xylene was used as the outer liquid instead of pyridine. The liquid rose to a height of 16.4 cm. in 4 days. Sugar was not present in the outer liquid in appreciable quantities, but pyridine was.

25) The inside liquid was a 1.2 per cent solution of sugar in pyridine, the outer liquid pyridine and the membrane rubber supported by muslin. The temperature was kept at 22.5° C. A rise of the liquid in the osmometer tube was noted after five minutes. After 3 hrs. the column measured 4 cm.; after 12 hrs. about 5 cm. Sugar did not pass through the septum in appreciable amounts. The experiment shows that the solution used is able to produce but a very feeble osmotic pressure.

26) The inner liquid was a 0.125 normal solution of sugar in pyridine, the outer liquid pyridine and the septum rubber supported by muslin. The whole was kept at 0° C. After 3 days the liquid had risen only 0.5 cm. in the osmometer tube; after 5 days, the rise was but 1.9 cm. The membrane was intact, and practically no sugar had passed into the pyridine without.

27) This experiment was like No. 26 except that a 0.25 normal solution of sugar was used as the inner liquid. The temperature was kept at very nearly —16° C. During the first day the liquid rose to 9.5 cm. in the osmometer; on the second day the column measured 13.5 cm.; and on the fourth day 17.5 cm. The membrane was intact and only very slight amounts of sugar were present in the outer liquid. The same experi-

ment performed at room temperatures showed a very rapid rise of liquid in the osmometer tube,—see pressures measured in the quantitative measurements described below.

28) The inner liquid was a saturated solution of Li Cl in pyridine, the outer liquid pyridine and the septum rubber. The liquid rose in the osmometer and Li Cl did not pass through the septum into the outer liquid in appreciable quantity.

Experiments 23 to 27 show that vulcanized caoutchouc is a "semipermeable" membrane when it separates sugar solution in pyridine from pure pyridine. The data lead one further to the conclusion that the gas laws do not govern the phenomena, which the measurements to be detailed later confirm. No. 28 shows that when Li Cl is used as solute in an otherwise similar experiment, the rubber again acts as a semipermeable septum. The reasons for this behavior have already been discussed in connection with the $AgNO_3$ solutions.

29) The inside liquid was 0.1 normal $AgNO_3$ in pyridine, the outside liquid a saturated solution of cane sugar in pyridine, the membrane being rubber. The level in the osmometer fell, showing the current to be from the $AgNO_3$ solution to that of the sugar. On further examination it was found that $AgNO_3$ had also passed into the sugar solution but in small amount.

30) This experiment was identical with No. 29 except that parchment was used as the septum. The test was continued for 3 days. No change was observed.

In the light of what has already been said, it is clear that the results in Nos. 29 and 30 are such as might have been anticipated.

31) The inner liquid was a solution of $FeCl_3$ in toluene, the outer toluene, the membrane rubber. The main direction of flow is toward the solution, but $FeCl_3$ also passes through the membrane in considerable quantity, which was to have been expected since this salt is soluble in hydrocarbons. The $FeCl_3$ gradually disintegrates the septum.

32) This experiment was identical with that of No. 31 except that iodine was used as the solute instead of $FeCl_3$.

The iodine also passed through the rubber, as was to have been expected; and it disintegrated the septum more rapidly than did the $FeCl_3$. The liquid, however, first rose in the osmometer.

33) The inner liquid was a solution of copper oleate in benzene, the outer benzene and the septum rubber. The liquid in the osmometer rose to a height of 20 cm. showing the main current to be toward the solution; however, large amounts of copper oleate passed through the rubber into the outer benzene. This was to have been expected, since copper oleate is soluble in hydrocarbons.

34) This experiment was identical with No. 33 except that parchment was employed as the septum in place of rubber. No change whatever took place, which was to have been anticipated since none of the ingredients touching the membrane have sufficient affinity for it.

35) This experiment was like No. 33, except that the copper oleate was dissolved in pyridine, and pyridine was used as the outer liquid. Again the main current was toward the solution, but copper oleate passed into the outer pyridine in considerable quantity, which is quite in harmony with the theory advanced.

When we think of a large molecule like that of copper oleate readily travelling through vulcanized caoutchouc as in No. 35, and that under like conditions cane sugar, $AgNO_3$ and LiCl do not pass through that septum, it certainly must convince us that the membrane does not act as a sieve. Again, No. 34 shows that parchment is not a ''porous'' material as is so commonly assumed from osmotic experiments with aqueous solutions in which it is employed as septum.

36) The inner liquid was a strong solution of sodium oleate in water, the outer liquid water, and the septum rubber. No change occurred.

37) The experiment was like 36 except that parchment was employed as the septum. In this case sodium oleate was found in the outer water, though the inner liquid showed slight increase in bulk.

Sodium oleate though soluble in water is insoluble in hydrocarbons; bearing this fact in mind, the results in Nos. 36 and 37 are readily explained.

38) The inner liquid consisted of a normal solution of naphthalene in pyridine, the outer liquid was pyridine and the membrane rubber. Practically no change in level occurred, but large quantities of naphthalene passed into the pyridine without.

39) The inner liquid was a normal solution of camphor in pyridine, the outer pyridine and the septum rubber. Practically no change in level occurred, but considerable amounts of camphor appeared in the outer liquid.

40) The inner liquid was a saturated solution of camphor in 99.5 per cent alcohol, the outer liquid 99.5 per cent alcohol and the septum rubber. After half an hour a slight lowering of the level of the liquid in the osmometer was noticed; it continued to go down for 3 days, when the experiment was stopped. Much camphor had passed into the alcohol without.

41) The inner liquid was a saturated solution of camphor in toluene, the outer liquid toluene and the membrane rubber. The liquid at once rose rapidly in the osmometer. In 9 hours it reached a height of 32 cm. It continued to rise for three days, the duration of the experiment. Large quantities of camphor had passed through the septum into the outer toluene. Camphor is very soluble in toluene.

That naphthalene and camphor should pass through rubber was to have been expected, Nos. 38 to 41, since these substances are very soluble in hydrocarbons. In No. 40 the main current is from the solution of camphor in alcohol to the alcohol; while in 41 the main current is from the toluene to the camphor solution in toluene. This occasions no surprise when it is borne in mind that toluene is imbibed much more rapidly and more copiously by rubber than is alcohol, and that an alcoholic solution of camphor is imbibed by rubber more rapidly than is alcohol. Again, remembering that it is the relative rate of inflow and outflow which determines whether the bulk of the liquid in the osmotic cell will change or not under given conditions, the results in 38 and 39 are readily explained.

42) In the osmometer was placed a solid block of camphor in form of a cube which weighed two grams; the outer liquid was 99.5 per cent alcohol, and the membrane rubber. The os-

mometer was immersed in the liquid so that the membrane was slightly below (about 0.5 cm.) the surface of the liquid. The arrangement is shown in Fig. 5. After five minutes there seemed the least evidence that the camphor was being attacked, but even after 4 hours there was but little further change. Since alcohol is not very readily imbibed by rubber this occasioned no surprise.

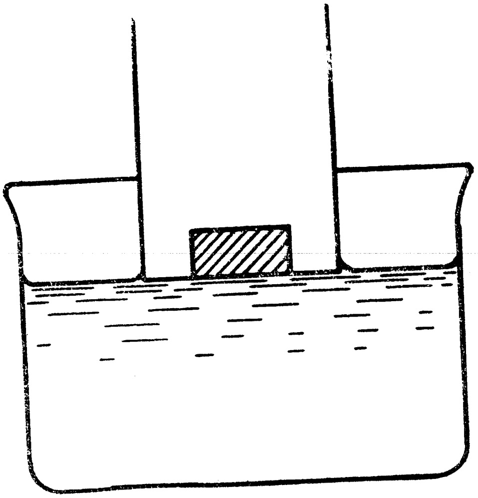

Fig. 5.

43) This experiment was identical with No. 42, except that toluene was used instead of alcohol. Plain evidence that the camphor was dissolving appeared in three minutes. In forty minutes the solid camphor had all disappeared and was found in very large quantities in the outer liquid. The membrane became much distended, bulging downward. The level of the liquid was alike in and outside after four hours, it having risen slightly on the inside.

44) This experiment was like No. 42 except that xylene was employed instead of alcohol. The observations were practically identical with those of No. 43 where toluene was employed, which might have been expected.

45) This experiment was also like No. 42 except that pyridine was employed instead of alcohol. After four minutes it was evident that the camphor was being attacked. A very thin

layer of liquid, less than 1 mm. thick, appeared on the upper side of the rubber. In four hours all the camphor had gone through the rubber into the pyridine, leaving a depth of solution of only about 1 mm. on the upper side of the membrane. During the process the membrane became somewhat distended and bulged upward toward the camphor.

46) In this case the arrangement was again like in No. 42, except that CCl_4 was employed instead of alcohol. After about four minutes it was evident that the block of camphor was beginning to disappear. In forty-three minutes the whole block was gone, having passed through the rubber into the CCl_4, leaving on the upper side of the membrane a camphor solution less than a millimeter deep. The membrane became distended and bulged upward. After four hours, the liquid on the upper side of the membrane was from 1 to 2 mm. deep.

47) This experiment was also like No. 42 except that CS_2 was used as the liquid instead of alcohol. After four minutes it was clearly evident that the block of camphor was being attacked. After 13 minutes a thin layer of liquid was visible on the upper side of the rubber. After 45 minutes all solid camphor had disappeared, having passed through the rubber into the CS_2, leaving on the upper side of the membrane a solution about 3 to 4 mm. deep. After four hours the membrane appeared very much distended, bulging downward, the liquids in and outside being on a level.

48) In this case a cube of paraffine of rather high melting point (70°) was separated from 99.5 per cent alcohol by means of a rubber septum, the arrangement being as described in No. 42. After 24 hours the lower corners and edges of the paraffine cube appeared slightly rounded. No liquid was visible on the upper side of the rubber, however. After three days the large bulk of the paraffine was still intact, though it was distinctly evident that the substance was slowly passing through the rubber septum into the alcohol beneath, without any liquid layer appearing on the upper side of the membrane.

49) This experiment was identical with the preceding one, No. 48, except that toluene was used instead of alcohol. In this case solution gradually accumulated on the upper side of

the rubber, though paraffine passed through into the toluene on the outside. After 24 hours only about one-eighth of the paraffine was left undissolved and the liquid on the inside was practically on a level with that on the outside. Much paraffine was found in the outer liquid.

50) In this case the cube of paraffine was separated from pyridine by means of rubber, the arrangement being as described in connection with No. 42. The observations were practically the same as when alcohol was employed, No. 48.

51) The experiment was identical with the preceding one, No. 50, except that CCl_4 was used instead of pyridine. After 24 hours about three-fourths of the paraffine was still left in the solid state, it floated on the layer of liquid which had formed on the upper side of the rubber. Considerable paraffine had gone through the septum into the CCl_4 below. The membrane was much enlarged and bulged downward. After two days, all the paraffine had dissolved and the liquids in and outside were nearly on a level.

52) About two grams of dry powdered $AgNO_3$ was placed on the upper side of a rubber membrane the lower side being bathed by pyridine. The arrangement was as pictured in Fig. 5, the powdered $AgNO_3$, which was spread out over the surface of the membrane, taking the place of the block represented in the figure. The experiment then was the same as No. 45 except that the $AgNO_3$ was used in place of camphor. After 7 hours no liquid was visible on the upper side of the membrane. Pyridine had, however, passed into the silver nitrate and formed a solid addition product with it. No appreciable amount of $AgNO_3$ had passed into the pyridine without. After 24 hours all the $AgNO_3$ had dissolved and the liquid in the osmometer had risen to a height of about 1.5 cm. and continued to rise. After four days the experiment was stopped and the outer liquid examined for $AgNO_3$. It was, however, found to be present only in mere traces. This shows that vulcanized caoutchouc is indeed a good "semi-permeable" membrane in this case.

Considering experiments Nos. 42 to 52 in which solids were separated from liquids by means of rubber septa, it is evident that whether the solid will pass through the membrane or not depends on the nature of the solid, the membrane and the liquid employed. Furthermore, if the substance composing the solid does make its way through the septum, the fact as to whether the action is accompanied with an accumulation of liquid on that side of the septum occupied by the solid or not is clearly determined by the rate with which the solution formed is absorbed by the membrane (which is determined by the mutual attraction or affinity of the saturated solution and the membrane for each other) and also by the rate with which the pure solvent is imbibed from the other side.

Now it is well known that camphor dissolves very readily in hydrocarbons and consequently has considerable affinity for rubber, and we should, therefore, expect it to pass through the latter when it is employed as an osmotic membrane. An alcoholic solution of camphor is more readily imbibed by rubber than is pure alcohol, and so it occasions no surprise when in No. 42 we find the block of camphor slowly making its way through the rubber septum into the alcohol, and without the appearance of liquid on the side occupied by the camphor. The action is slow because alcohol is not imbibed rapidly or copiously and because the septum holds on to the strong camphor solution very tenaciously, so that only a small portion of the camphor thus saturating the rubber is washed out of the latter by the alcohol on the other side.

When the same experiment is performed using a hydrocarbon as the liquid instead of alcohol, Nos. 43 and 44, the action goes on very much more rapidly on account of the great affinity between rubber and the hydrocarbon or a camphor solution in a hydrocarbon. The strong camphor solution is so greedily absorbed by the rubber that but little liquid appears on the upper side of the membrane while the solid camphor lasts, and it is as though the solid camphor were passing through the rubber septum by mere contact with it. The process reminds one strikingly of the manner in which solid food placed in the alimentary canal is digested and absorbed. Here the presence of the food in contact with the walls of the tract excites the flow of the digestive juices toward the solids, the latter are acted upon and the resulting liquid is absorbed by the walls of the canal. In our experiments the camphor in contact with the rubber saturated with hydrocarbon excites a flow of hydrocarbon toward it. The hydrocarbon acts on the camphor "dissolving" it, and the resulting solution is then so rapidly absorbed by the septum as to leave no liquid on the side occupied by the camphor.

When as in No. 45 pyridine is used instead of a hydrocarbon, the camphor is still absorbed, but not as rapidly; for pyridine is not taken up by rubber as readily or as copiously as are hydrocarbons, neither has camphor so great an affinity for pyridine as for hydrocarbons. On the other hand, camphor very rapidly disappears when CCl_4 or CS_2 (Nos. 46 and 47) are employed, for these substances have great affinity for rubber and also for camphor.

When paraffine instead of camphor is separated from alcohol, toluene, pyridine or carbon tetrachloride by means of rubber, Nos. 48 to 51, the action is in all cases slower, which fact is readily comprehended when it is borne in mind that camphor dissolves more readily in the liquids named than does paraffine.

Finally in No. 52, where solid silver nitrate is separated from pyridine by rubber, we have an illustration of a typical case in which the liquid accumulates very greatly on the upper side of the membrane. Here the solute has very little affinity for the rubber, and so the solution of the salt practically does not get into the septum on account of the fact that pure pyridine is more readily imbibed and silver nitrate is difficultly soluble in rubber soaked with pyridine, that is to say, in a hydrocarbon diluted with pyridine. If a block of cane sugar or one of chloride of lithium were separated from pyridine by means of a rubber septum the action would be similar to that observed in the case of silver nitrate, No. 52. The same would be true if any solid which is soluble in pyridine yet not soluble in hydrocarbons were separated from pure pyridine by means of a rubber membrane. It would be interesting to test this in the case of more substances which are insoluble in hydrocarbons and yet are soluble in pyridine, but the number of such substances is rather limited, for it must be remembered that pyridine is itself nearly a hydrocarbon in character and is consolute with hydrocarbons.

QUALITATIVE TESTS WITH ANIMAL AND VEGETABLE MEMBRANES.

A number of qualitative tests were made with membranes of organic origin. It was thought best to place the results of these experiments on record here, though it is contemplated to take up the whole matter of the action of organic membranes separately at some later time.

Various animal membranes were tested as to their permeability for sodium chloride, urea, and cane sugar. The membranes used were stretched over square wooden frames quite loosely so as to form bags or pockets into which one liquid was placed; these bags were then suspended in the outer liquids, in such a manner that only the lower sagging part, and not the upper edges, came into contact with the outer liquids. As membranes to be tested were selected the pericardium, diaphragm, small intestine, large intestine, stomach, aorta, urinary bladder and gall cyst of a young ox. The animal was perfectly sound and normal and about three years old. The membranes were used soon after the animal had been killed. On the upper side of each membrane was placed an aqueous solution containing 23.4 grams of sodium chloride, 40 grams of urea and 342 grams of cane sugar in 2000 cc.; while the lower side of the membrane was immersed in pure water, the arrangement being as already described. It was found that in case of all of the membranes mentioned the sodium chloride passed through more rapidly than urea and sugar, which fact was established by examining the outer liquid from time to time during the first four hours. After twenty-four hours besides much salt very considerable quantities of urea and cane sugar had gone through all of the membranes. A special test made by separating water from an aqueous sodium sulphate solution by means of the stomach membrane, showed that the latter is permeated but slightly by sodium sulphate; for the amounts of the latter salt in the outer liquid were but small.

The vegetable septa employed consisted of the rinds of California oranges and grape fruit and Florida grape fruit. The grape fruit, also called shaddock, is the fruit of *Citrus decumana.* In each case the fruit was carefully cut in two transversely with a sharp knife, so that the halves after careful removal of the pulp formed two cups. Each half rind was filled to about half of its capacity with the liquid to be tested and then suspended in pure water so that the latter was about on a level with the liquid in the rind. The suspension of the rind was accomplished by means of fine aluminum wire run through small holes pricked through the upper edges of the rind. Each experiment was continued for twenty-four hours, unless otherwise stated. It was found to be impracticable to continue the experiments much longer, for the rinds after being immersed in water for a considerable time undergo alteration, becoming soft and loosing their waxy outer coating.

With the arrangement as just described and employing water as the outer liquid and an aqueous solution as the inner liquid, it was found that sodium chloride readily passes through orange skins, but sodium sulphate very much less readily. Indeed, only traces of the latter salt were found in the outer liquid even when strong solutions were employed. Again, urea readily goes through the orange rinds, but sugar passes through only in very small amounts. When an aqueous solution of potassium alum was used it was found that a slight amount of potassium sulphate appeared in the outer water, but no aluminum sulphate, showing that the latter ingredient is left behind during the time of the experiment at least.

In the upper half of the rind of a Florida grape fruit was placed 100 cc. of a solution containing 10 grams sodium chloride plus 10 grams of sodium sulphate. The rind so charged was suspended in distilled water as above described. The outer water had a volume of 500 cc. Tests of the outer liquid showed that the NaCl was passing through much faster than the Na_2SO_4. This experiment was run for seven days; the rind did not seem to be altered much.

The lower half of the rind of a California grape fruit containing 70 cc. of an aqueous solution, which contained 10 grams of NaCl plus 10 grams of sugar in 100cc., was suspended in a dish in 300 cc. of water. It was found that the sugar passed through the rind much more slowly than the salt. The experiment was continued for seven days.

A similar experiment in which 80 cc. of an aqueous solution containing 10 grams sugar plus 10 grams urea in 100 cc. was placed in the upper half of the rind of a California grape fruit suspended in a dish containing 400 cc. water, yielded the result that both urea and sugar pass through the rind, but the latter more slowly. After twenty-four hours sugar may be detected in the outer liquid with Fehling's solution. The urea acts on the rind thickening and hardening it. Much urea is thus retained in the rind, also considerable amounts of sugar. These facts were determined by an examination made after the experiment had run for seven days.

To 70 cc. of a saturated solution of boric acid in water 10 grams of sugar were added. This solution was placed in the upper half of the rind of a California grape fruit, the outside being bathed by water. The experiment was continued for five days, the rind remaining intact and practically unaltered during this time. Only traces of boric acid passed through the rind. Sugar passed through slowly; but at the end of five days it was found in the outer liquid in considerable quantity.

It was found further that in dilute aqueous solutions H_2SO_4, HCl, and HNO_3 readily pass through the skins of grape fruit; but the rinds are much altered by the acids, appearing shrunken and darkened in color. Citric acid passes through less rapidly than the mineral acids mentioned. It is apparently retained to a considerable extent in the rind.

The experiments made with the rinds of grape fruit were also repeated with the skins of California oranges, with practically the same results.

QUANTITATIVE MEASUREMENTS OF OSMOTIC PRESSURES.

The quantitative measurements of osmotic pressures were made with so-called semi-permeable membranes, that is to say with membranes through which the solvent passes so much more readily than the solute, that the amount of the latter which goes through the septum is practically a negligible quantity. From what has been said above, it appears that the latter quantity is never absolutely nil, and that consequently there is really no such a thing as a semi-permeable membrane, strictly speaking.

Now as has been intimated, the qualitative experiments above detailed enable one to foretell when a membrane will permit the solvent to go through so much more readily than the solute that the amount of the latter which traverses the membrane is so slight that the septum may be called semi-permeable.

From the ether, water, chloroform experiment, already described as a typical case of osmosis, it appears that the ether makes its way through the water into the chloroform because (1) ether is soluble in water and (2) chloroform has much more affinity for ether than has water, so that ether is extracted from the water layer by the chloroform; on the other hand, the water does not permit chloroform to pass into it and into the ether beyond to an appreciable extent, because chloroform is so very slightly soluble in water even when the latter is impregnated with ether.

Holding these things in mind let us look for the proper solvent and solute to employ with rubber as the membrane so that the latter shall be semipermeable. It must first be remembered that rubber (vulcanized caoutchouc) is practically a hydrocarbon. The rubber employed was of excellent quality and was almost the pure gum. On analysis it was found to contain 0.38 per cent ash, and a Carius determination yielded 0.30 per cent chlorine and 0.95 per cent sulphur. Before the analysis was made the sample was wiped superficially, washed with distilled water, dried with filter paper and finally left in a desiccator over strong sulphuric acid for twenty-four hours. It is evident that in order to pass through a rubber membrane a substance must be taken up by the rubber, the rubber must imbibe the substance, in other words the substance must be soluble in rubber.[1] Again the liquid bathing the other side of the rubber must be capable of extracting the imbibed substance from the

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[1] The act of such solution or imbibition is really mutual; i. e. the rubber attracts the substance in question, and the latter in turn attracts the rubber.

rubber, thus completing the transference. On the other hand, those substances which are not soluble in rubber, i. e. are not taken up by the rubber, will obviously not pass through the latter. And so what is required is a solvent which will readily be taken up by rubber, without, however, disintegrating the same, and a solution of such a character that the solute shall not be soluble in rubber. The less affinity the solute has for rubber the better; for then when the solution is brought into contact with the rubber the latter will imbibe practically only solvent to the exclusion of solute, thus leaving the solution slightly more concentrated, that is to say making a partial separation of solvent and solute.

In casting about for a suitable solvent very many substances were tried. It was of course desirable to secure a solvent that was not too volatile at ordinary temperatures or too obnoxious. In the course of this work it soon became evident that water, alcohols and in general compounds containing considerable hydroxyl, relatively speaking, are not suitable for they are not taken up readily enough by rubber nor in sufficient quantity. On the other hand many compounds, like hydrocarbons, their halogen substitution products, carbon disulphide, ether, etc., though taken up readily by rubber and in considerable quantity gradually act upon the latter to such an extent as to form with it a very soft, sticky mass or even a liquid, a combination or solution then of such mechanical properties as to be entirely unsuitable for quantitative osmotic experiments. After trying a large number of liquids, pyridine was finally taken as being the most suitable for the purpose in hand. From its very nature pyridine is a substance which would perhaps be expected to fulfill the requirements. Its high carbon and hydrogen content make pyridine almost a hydrocarbon, indeed, it dissolves in hydrocarbons in all proportions. Since rubber is a hydrocarbon, we should expect it to imbibe pyridine readily. It was found that pyridine is imbibed by rubber, increasing the bulk of the latter somewhat to be sure, but without otherwise materially altering the mechanical properties of the rubber. Indeed, I found that vulcanized caoutchouc may even be boiled in pyridine for hours, in which case there is a slight amount of material extracted from the rubber, giving the liquid a brownish color; but the rubber is not disintegrated or affected materially otherwise. The rubber used as membranes in the actual quantitative measurements was in fact thus extracted with boiling hot pyridine. After such treatment rubber when dried has practically all of its original properties; it is perhaps a little easier to rupture it by stretching it hard. It might at first be somewhat surprising that pyridine does not disintegrate rubber more. However, while pyridine is closely akin to hydrocarbons and consolute with them, it must be borne in mind that water, which has but little affinity for hydrocarbons, also is consolute with pyridine. Pyridine is then a rather unique substance, and it is hardly surprising that it should be imbibed by rubber sufficiently for the purpose in hand without unduly disintegrating it. Direct experiment showed that at room temperature (about 20° C.) 100 grams of the vulcanized caoutchouc used imbibed 144.42 grams of pyridine in 24 hours, while the amount imbibed in 17 days was 145.17 grams.

A suitable solute would be one that is soluble in pyridine yet insoluble in hydrocarbons. For instance, a substance soluble in petroleum or benzene would in general also be soluble in pyridine, but it would also be soluble in rubber (a hydrocarbon) and hence would pass through the latter. Thus oleic acid, the oleates of the heavy metals, ferric chloride, naphthalene, camphor are soluble in hydrocarbons, are consequently readily taken up by rubber, and when dissolved in pyridine pass through the rubber when the latter in an osmotic experiment separates the solution from pure pyridine. Because of the peculiar nature of pyridine already alluded to above, this liquid dissolves a goodly number of substances which are insoluble in hydrocarbons. The solubility of such substances in pyridine is to be sure rather limited as to quantity as a rule. So cane sugar, silver nitrate, lithium chloride are insoluble in hydrocarbons and yet reasonably soluble in pyridine. One would consequently expect that when solutions of either of these substances in pyridine be separated from pure pyridine by means of a rubber septum, practically none of the solute would pass through the rubber; in other words, the latter would be practically impermeable for sugar, silver nitrate and lithium chloride. Now this is actually what was found in the qualitative tests described above. These substances pass through rubber in extremely slight quantities which are quite comparable with the amounts of cane sugar that pass through the much studied tests described above. These substances pass through rubber an aqueous sugar solution. However, on account of the fact that sugar has a high carbon and hydrogen content, one would expect it to have more affinity for a hydrocarbon than either silver nitrate or lithium chloride, and that consequently it would pass through rubber a little more readily than these salts. Experiment has also shown that this is actually the case; though as stated above, the amount of sugar which passes through the rubber membrane is quite small.

The quantitative measurements of osmotic pressures were then made by using solutions of cane sugar, lithium chloride, and silver nitrate in pyridine, these solutions being separated in each case from pure pyridine by means of a membrane of vulcanized caoutchouc previously treated with boiling hot pyridine so as to extract any soluble ingredients. It was not the purpose of the quantitative measurements of osmotic pressures to produce and measure enormous pressures; though as was shown above in No. 22 a pressure of approximately fifteen atmospheres was actually measured in the case of a normal solution of silver nitrate in pyridine. The efforts were rather directed toward determining with a sufficient degree of accuracy moderate pressures, using different concentrations of the solutions employed at several different temperatures.

The osmotic cells were made entirely of glass, excepting of course the surface actually closed by the membrane itself. The different parts of the cells were fused together so as to form one piece, thus avoiding cemented joints of any kind. Figure 6 shows how these cells were made. To a stout, carefully made thistle tube having a flare of about 45 degrees at E, a T was attached, the tube being provided with a bulb and bent as shown in the figure, C. To C was fused a manometer tube D having a bore of about 0.5 mm.; this tube was made as long as the experiment required. The small bulb and bent part of the tube

C contained mercury. At B the tube was somewhat contracted as shown, and after putting the required amount of pure clean mercury into the apparatus and filling the rest of it with the solution to be tested through the orifice F by means of a capillary funnel tube, the apparatus was carefully heated at B by means of a small flame and finally drawn off leaving the whole securely sealed. With practice this part of the operation may be so performed that practically no air bubble remains in the apparatus after the tube has been drawn off at B.

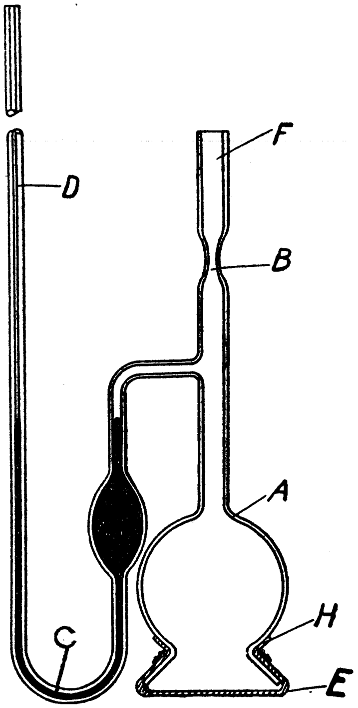

Fig. 6.

Before placing the mercury and the solution into the cell, however, the membrane must be securely put into place. It is first of all essential that the opening of the bell of the thistle tube E be as nearly circular as possible, and that the points on the outer edge of the orifice lie in very nearly the same plane. The rubber membrane consisted of a high quality of sheet rubber as is used by dentists. Its ash, sulphur and chlorine content have already been given above, and it has also been stated that the material was extracted with hot pyridine before using it. The rubber so prepared was carefully tied over the orifice at E. In this process it was stretched only moderately so as not to thin it unduly.[1] In tying the membrane on, the rubber was always stretched to such an extent, however, that no folds whatever remained where it touched the glass. With proper care it is possible to get a smooth surface of rubber to rest snugly against the surface of the glass. The rubber was finally securely fastened into place by carefully winding the whole surface from H to E smoothly and closely with moderately fine thread. When these precautions are observed one hardly ever fails to get a perfectly tight joint between the glass and the rubber, which is so all essential.

The next step consisted of supporting the membrane so that it would withstand pressure. To accomplish this there was first tied over the membrane a piece of smooth, soft yet strong muslin. This cloth was drawn tightly over the membrane, and securely tied on by winding moderately fine yet strong thread over it smoothly and evenly from H to E. It is of course impossible to get rid of folds in the cloth where it is tied on the surface H to E; but it is quite possible to distribute these folds fairly evenly around the circumference, in such a manner that the cloth where it actually touches the rubber on the mouth of the thistle tube and just at the edge at E, lies perfectly smoothly and snugly against the surface of the membrane giving it proper support. After this a circular perforated disc of stout sheet steel made of proper size so as to just cover the lower end of the thistle tube was placed on the muslin covering the rubber; and this disc was then securely held in place by tying over it very firmly another piece of strong muslin by means of stout thread evenly and tightly wound on as in the previous cases. Thus there was the rubber tied on the glass, then the muslin snugly covering and supporting the rubber, then the steel disc pressing against and supporting the muslin, and fi-

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[1] When finally in place, the thickness of the membrane was only a small fraction of a millimeter.

nally the outer layer of muslin firmly holding the disc in the proper position. The perforations in the disc were about 1 mm. in diameter and the disc itself was about 0.5 mm. thick. By observing closely the precautions here laid down, cells were practically always obtained without fail which were perfectly tight, rigid and capable of withstanding pressures up to the point of bursting the glass bell of the thistle tube. Furthermore, on taking such an apparatus apart even after experiments that had continued for weeks, the membrane was found to be intact and in perfect condition. Experiments demonstrating that such membranes do not leak have already been given above, Nos. 1 and 2. We have here then a simple, direct and certain method of preparing so-called semipermeable membranes for osmotic pressure measurements, which are yet not precipitated membranes; and furthermore we know *why* the membranes are semipermeable for the solutions that come into question.

After the membrane has been put into place as described the apparatus is charged with the required amount of mercury and then with the solution to be tested, the tube being drawn off at B before the small flame of a blowpipe so as to leave no air in the apparatus. The latter is then placed in a large vessel containing the pure solvent, and the rise of the liquid in the manometer tube is observed from time to time, a pair of sensitive thermometers graduated to 0.1 degree being placed in the solvent near the osmometer to indicate the temperature. The apparatus was set up in a basement room, whose temperature changed but slightly and only very gradually during the course of the experiments. It is highly essential that the temperature be kept as constant as possible during the experiments and that sudden fluctuations be avoided. From what has been said it is evident that the experiments were finally set up in much the same way in which Pfeffer performed his tests, only in this case additional care was taken to cover up the dish so as to minimize evaporation and to protect the surface of the pyridine from the moisture of the air. All measurements were made with the open manometer and the height of the mercury column was estimated to within 1 mm. in each case. It was found to be quite needless to measure more closely than this, for the results of separate duplicate experiments showed as a rule greater variation.

I will first give the results of the experiments performed by using the method just described, which method, though it is the usual one, had to be modified later in one important particular in order to secure reliable results. The individual experiments are numbered consecutively with those preceding so as to facilitate future reference to them.

53) The liquid in the osmometer was a solution of cane sugar in pyridine containing one-fourth of a gram-molecule per liter of the solution. The outer liquid was pure pyridine. The experiment was run in triplicate, that is three separate individual tests were made with the same solution. The temperature in each case was 17.5° C. a) In the first of these experiments the pressure came up slowly, remaining nearly constant after the second day. The experiment was nevertheless allowed to go on for nine days when the pressure was finally measured and found to be 186.2 cm. at 17.5° C. Before discontinuing the experiment, the whole of the outer vessel was packed in melting ice for ten hours. The mercury column became constant at 125 cm. after three hours and remained there for the remaining seven hours. The temperature was then permitted to rise slowly and after three days the mercury column was 159.5 cm. high, the tempreature being 16.4°. When the whole had finally reached the temperature 17.5°, the mercury column was again 186.0 cm. high. This then would seem to be pretty good evidence that the maximum pressure had indeed been reached. During the operation care was taken not to disturb the apparatus by jarring it in any way. The outer liquid was found to contain traces of sugar, but the exact amount was not determined. b) In the second independent yet perfectly similar experiment, the pressure also rose gradually, changing but slightly after the second day. The temperature was kept at 17.5° C., and at the end of eleven days the column of mercury measured 155.6 cm. This experiment was then allowed to run for four days longer. At 8 in the morning of the fourteenth day the mercury column measured 166.4 cm. at 16.4°

C.; while at 5 in the afternoon of the fifteenth day it measured 166.7 cm. at 16.0° C. As in the previous case, care was taken not to jar the apparatus during the experiment. In this case, too, traces of sugar were found in the outer liquid. c) In a third similar experiment the pressure also increased but little after the second day. The experiment was stopped at the end of four days in this case, when the mercury column was 107.4 cm. high, the temperature being 17.5° C. Only traces of sugar were found by testing the outer liquid.

It will be noted that the highest pressure was observed in (a). In this experiment the pressure came up more rapidly than in the other two cases, accomplishing 115 cm. in the first twenty-four hours. The discussion of the causes of the discrepancies in the results of (a), (b) and (c) will be left until a little later.

54) This experiment was performed in duplicate. The arrangement was exactly like that in No. 53 except that the liquid in the osmometer consisted of a solution of cane sugar in pyridine containing one-eighth of a gram-molecule per liter of the solution. a) The pressure rose slowly increasing but slightly after forty-eight hours. On the sixth day the mercury column measured 62.4 cm., the temperature being 17.5° C. The whole was then surrounded with melting ice, and after two hours the mercury column measured 0.2 cm.; at the end of ten hours the pressure was 0.6 cm. The temperature then gradually rose during the night as the ice disappeared. At 14.5° C. the column measured 48.8 cm. and at 17.5° C. it again came up to 62.0 cm., nearly where it was before the chilling process. In this case the experiment was left set up for five days longer, when the column measured but 43.6 cm. at 18° C. Sugar was found to be present in the outer liquid in small amount. b) This was a duplicate of (a). The observations made were practically the same as in (a) except that the pressures were different. The chilling process with ice was omitted in this case. After two days the pressure increased but slightly. On the third day it was measured carefully and found to be 52.8 cm. at 17.5° C. During the next two days the temperature fell very gradually to 14.5° C. and the column then measured 42.5

cm. The temperature then rose gradually to 18° C. during the succeeding three days, when the pressure was 46.3 cm.

55) In this experiment the inner liquid was a solution of cane sugar in pyridine containing 0.25 gram-molecule per liter of solution, while the outer liquid was a solution of cane sugar in pyridine containng 0.125 gram-molecule per liter of solution. The pressure rose slowly, the mercury column reaching a height of 137.5 cm. at 16.8° C. By far the most of this was accomplished during the first two days. The final measurement just given was taken after thirty days. The experiment was then left undisturbed for twenty-six days longer. The pressure began to diminish gradually and finally measured only 89 cm., when the experiment was discontinued. The membrane when examined at the end of this time was to all appearances intact and but slightly changed, if at all.

56) Two experiments were made with solutions of cane sugar containing 0.25 gram-molecule of cane sugar per liter of solution in pyridine as the inner liquid, and pure pyridine as the outer liquid, employing in this case, however, a common thick sheet rubber—such as is used on the ordinary foot-power laboratory bellows— in place of the finer vulcanized caoutchouc used in the other experiments. The experiments were conducted side by side as duplicates of each other. The pressure rose gradually, the mercury column reaching a height of 43.0 cm. in one case and 39.5 cm. in the other at 22° C. The rubber was found to be much softened when examined at the end of ten days, the duration of the experiments. The maximum pressure was practically reached after forty-eight hours. The pressure diminished gradually after seven days. Small amounts of sugar were found in the outer liquid, though the exact amount was not determined.

From the results just detailed it is evident that the methods employed are not capable of yielding concordant values. Though only attempts to measure osmotic pressures of sugar solutions in pyridine have been described, similar experiments were made using lithium chloride and silver nitrate solutions in pyridine. In these cases the results were no more concordant than in those in which sugar was solute. At first it was thought that the difficulty lay in the membrane itself, the initial experiments having been performed by using the sheet rubber without further treatment; but actual tests showed that whether the rubber was previously extracted with boiling hot pyridine or not made no perceptible difference in the results. It will be noted that one set of experiments was made using a common thick sheet rubber (No. 56). In this set the results were very different from those obtained with the thin rubber of high grade (No. 53), and yet the duplicates did not differ from each other more than when thin rubber was used. It was also determined by several trials that the non-concordance of the results of duplicate experiments could not be laid to the fact that in some cases the rubber was stretched rather more than in others. It was not to be expected, of course, that the maximum pressure would be reached in the same time in two experiments that were similar, for the areas of the surfaces of the membranes and their thickness were not exactly the same. It might further be possible that the differences in pressure observed in the experiments that were duplicates of each other occurred because slightly different amounts of sugar passed through the different membranes; in other words, that there was more leakage of solute in one case than in the other. Such leakage or lack of semi-permeability would operate to diminish the osmotic pressure in two ways, (1) by directly letting material out of the cell as the pressure rises, and (2) by increasing the amount of solute in the outer liquid and so weakening the cause which creates the pressure. It is quite true that in all cases small quantities of sugar passed through the membranes, and that these were, perhaps, not always exactly the same. The qualitative tests made, however, always showed that the quantity which had passed through was very small and far below the amount required to produce a noticeable osmotic pressure with the apparatus employed during the time of the experiments. It will be recalled that 1.2 per cent solution of sugar in pyridine yields practically no osmotic pressure (No. 25) and that the same is true of a 0.05 normal solution of silver nitrate in pyridine at room temperature (No. 20). Furthermore, when two similarly charged osmometers were set in one and the same outer vessel filled with pyridine, they nevertheless in general failed to indicate identical pressures. All this led me to the conclusion that the non-concordance of the pressures measured could not be due to leaks, defects or various degrees of "semi-permeability" of the membranes.[1]

I confess that I was about at my wits' end to understand why such duplicate experiments made as nearly as possible the same in every way should yet yield results that were not more concordant. In all, some seventy independent trials had been made, in which great care had been used to get the experiments alike, but to no avail. Yet the membranes were not defective. One day after measuring carefully the height of the mercury column in one of the experiments which had been running for two weeks and in which the pressure had changed inappreciably for several days, I happened to brush against the apparatus in such a way as to thoroughly jar it without, however, upsetting it or otherwise modifying it in any way. Some minutes later when I chanced to look at the apparatus, I noticed that the level of the mercury, which had been practically constant, had risen over two centimeters higher. The apparatus was then shaken repeatedly from time to time, seizing it with an insulating cloth so as not to alter the temperature, with the result that each time the pressure rose some-

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[1] Measurements of the electrical resistance of the membranes were also made which further establish this fact. Placing on each side of the membrane of an osmotic cell, such as was employed in the pressure measurements, a circular platinum electrode about 2.5 cm in diameter, so that the planes of the electrodes were parallel to that of the membrane, and that the metallic surfaces were close to the membrane without actually touching it, the cell was filled with an aqueous saturated solution of NaCl and the same solution was used as the outer liquid. The electrodes were thus immersed in the saturated NaCl solution and the membrane separated them from each other. The electrical resistance between the plates was measured by means of the Kohlrausch method; but it was found to be so great that it was not possible to estimate it accurately, being upwards of 70,000 ohms. Various rubber membranes tested in this manner gave results of the same order of magnitude. When the same experiment was performed using a parchment membrane the edges of which protruded so that they did not dip into the brine, the resistance measured was less than an ohm. Using the same arrangement with a rubber membrane and a normal solution of silver nitrate in pyridine as the liquid on each side, the resistance was over 90,000 ohms, and remained the same for four days, when the experiment was discontinued. A small hole pricked through the membrane with a needle in the latter case caused the resistance to drop to 450 ohms.

what higher. The explanation of this was, of course, perfectly obvious at once. The pressure is produced by the entrance of solvent into the omsotic cell, consequently right on the inner side of the membrane the solution becomes more dilute than at other points in the interior of the cell. If the cell is left undisturbed the very slow processes of diffusion seek to equalize the strength of the solution in the cell, but clearly shaking the apparatus, or better yet, stirring the interior content of the cell would at once accomplish what it would take diffusion processes a very long time to do, even though they be aided somewhat by gravity owing to the form of the osmotic cell. And it was, moreover, perfectly evident, too, that to shake the osmometer filled as it was with considerable pressure on, would not thoroughly mix the contents of the osmotic cell. It is clear that with a more dilute solution in immediate contact with the inner side of the membrane than is in the rest of the osmotic cell the maximum osmotic pressure can not be attained. Furthermore, as in cells slightly different in form when left perfectly at rest the dilute layer in contact with the inner side of the membrane would in general not disappear at the same rate by diffusion and disturbances due to gravity and difference of density of the layers, concordant results could not be expected in duplicate experiments, however carefully performed, without stirring the contents of the osmotic cell. *In all direct measurements of osmotic pressures which have for their aim the determination of the maximum pressure attainable in a given case, it is consequently necessary to continually stir the interior contents of the osmotic cell while the measurement is being made, in order that the concentration of the contents of the cell may remain uniform and a layer of maximum concentration be kept in immediate contact with the inner side of the septum.* Again, since there really is no such thing as a semi-permeable membrane in the strict sense of the word—which fact has already been emphasized above—it is evident that some of the inner content of the cell, be it ever so slight, is making its way through the membrane into the outer liquid. Thus right next to the membrane on the side bathed by the outer liquid, the septum is really not in contact with the pure solvent—the outer liquid—but rather with a *solution* more or less dilute. When the apparatus is at rest, the slow diffusion processes, aided by gravity perchance, tend to keep the outer liquid of uniform concentration; but these agencies clearly can not be relied upon to keep pure solvent in contact with the outer surface of the membrane in osmotic pressure measurements. Indeed, when the surface of the membrane is not smooth and presents considerable area to the outer liquid, a film of liquid is apt to adhere tenaciously to the outer surface of the septum in spite of the effects of diffusion. In the usual osmotic experiments using aqueous sugar solutions in a cell made by precipitating copper ferrocyanide on the inner side of an unglazed cup, the slight amount of sugar that passes through very likely lingers very tenaciously in the pores of the cup just outside of the actual membrane, forming there a film of solution of such strength that its effect upon the osmotic pressure is not a negligible quantity. At least its effect can not be assumed to be negligible without further experimental work. Nor would stirring the outer liquid in such a case as this be apt to remove the difficulty. In the osmotic cells described above where thin rubber membranes supported by cloth and perforated steel discs were used, the effect in question is no doubt less than in the walls of a porous cup, but it is by no means negligible. Here the cloth and the disc hinder diffusion, and it is very necessary to stir the outer liquid thoroughly and continuously in making the osmotic pressure measurements.

I would like to emphasize here once more then that it is very essential to stir the contents of the osmotic cell and also the outer liquid continuously in any attempt to measure directly the maximum osmotic pressure that may be produced in a given case; and that since in all past experiments this has been entirely neglected, the results of such experiments can not be considered as final and conclusive. In reality, as has been pointed out above in connection with the ether, water, chloroform experiment of L'Hermite, the membrane itself ought also to be stirred during the process. This is of course less necessary when the septum is quite thin than when it is thick. It should be borne in mind, however, that even stirring the contents of the cell and the outer liquid simultaneously can never entirely keep the concentration of the liquid layers in immediate contact with the two sides of the septum exactly the same as the concentration of the liquids further away from the membrane; yet in many cases this may be accomplished with a fair degree of approximation. Moreover, by using the stirring process, the osmotic pressures may be measured fairly approximately even when the amount of material passing out of the cell is not negligible; in other words, when the membrane is not semipermeable.

The apparatus devised for stirring the contents of the cell and also the outer liquid simultaneously during the measurement of osmotic pressures is pictured in Fig. 7 in diagramatic form. In the figure, the beaker B of a capacity of 1000 cc. or more, contains the outer liquid. The latter is stirred by means of the stirrer F, which is moved up and down by the motion of the crank C. In the experiments performed it was admissible to make this stirrer of iron, for this is not attacked by pyridine. F consisted then of a bright, stout iron wire bent in ring form. Just above the cork closing the beaker, this stirrer F was jointed so that it would not need so large an opening in the cork in which to move up and down. A thermometer is placed in the liquid to register the temperature. The whole apparatus is set in a constant temperature room, or the beaker B is immersed in a bath of constant temperature, not shown in the figure. The osmotic cell S is made exactly as heretofore described, except that before tying on the membrane, the stirrer is inserted into the apparatus through the opening in the bell of the thistle tube. This stirrer consists of a perforated disc of light sheet iron fastened by riveting and a drop of solder, to a sufficiently stout, yet flexible, iron wire the upper end of which carries a lug of soft iron soldered on, as shown in the figure. In order that the perforated disc at the lower end of this stirrer might not pound on the delicate membrane and injure it, prongs of wire were soldered on the main vertical wire of the stirrer; and after the latter had been introduced into the osmotic cell, these prongs were bent outward in such a way that they would strike the side of the bell of the thistle tube (see

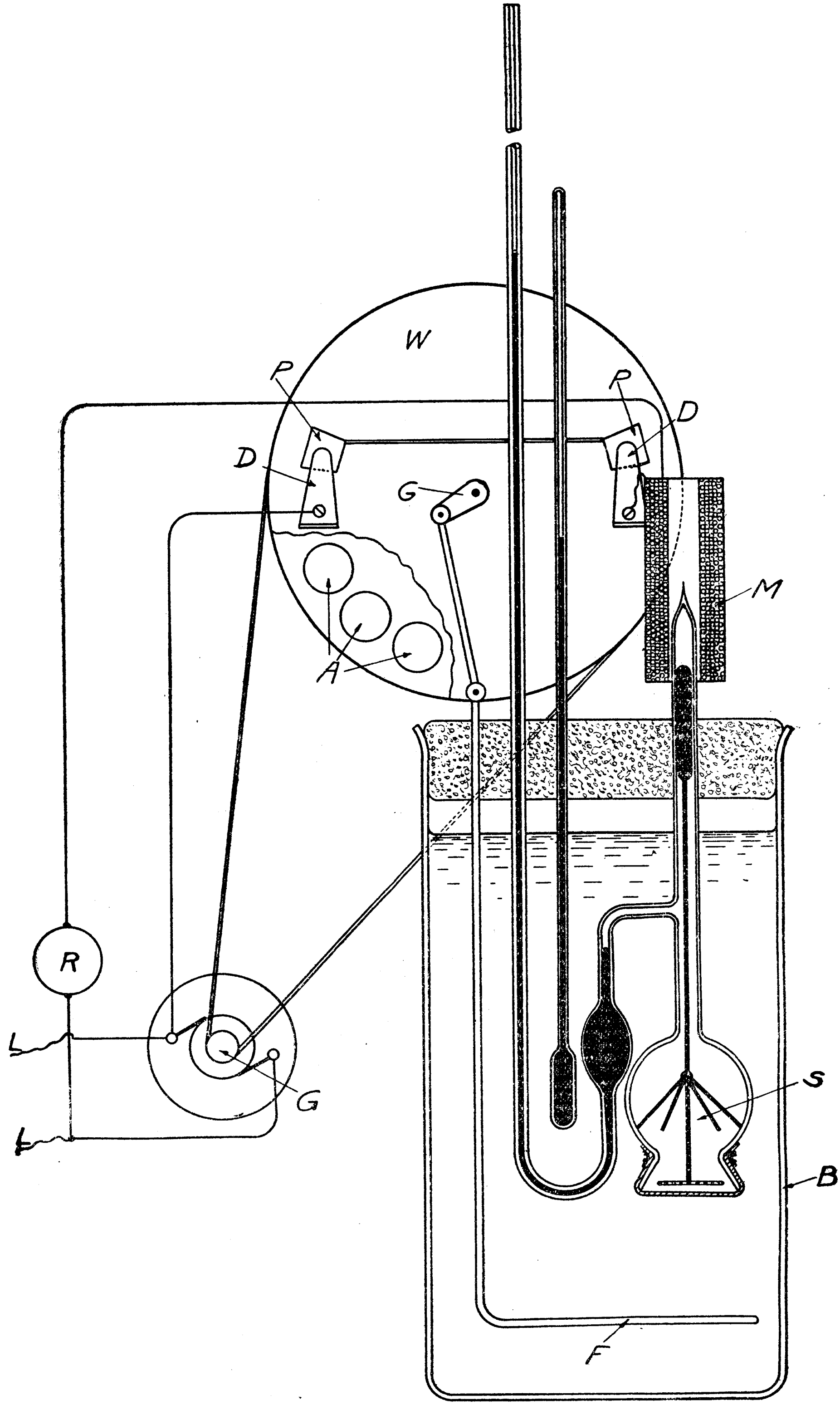

Fig. 7.

figure) as the stirrer moved downward, thus allowing the perforated disc to come close to the membrane without actually touching it. These prongs of course do not interfere with the upward movement of the stirrer. The soft iron lug at the upper end of the stirrer had a longitudinal groove (not shown in the figure) filed into it to facilitate the introduction of the liquid into the cell. After the glass part of the osmotic cell had been blown in one piece, the iron stirrer was introduced and the prongs bent into the required position; the membrane was then tied on and supported with cloth and steel disc as heretofore described. The proper quantity of mercury was then introduced and finally the cell was filled with the solution. A fine long funnel tube was employed in introducing the mercury and the solution into the osmotic cell. The upper end of the osmotic cell, through which the cell had been filled, was then drawn off before a blowpipe flame, as already described, so that practically no air remained in the cell after it was sealed. The osmotic cell was mounted in the beaker as indicated in Fig. 7, the cork closing the cell securely, leaving only a little play for the stirrer F.

Over the sealed end of the osmotic cell is placed the solenoid M; and by making and breaking the current in M, the stirrer in the osmotic cell is moved up and down. By means of the wires LL the small electric motor G is connected with a number of storage batteries; the latter are not shown in the figure. The motor G turns the wheel W, through the agency of a belt, and thus operates the crank C which moves the stirrer F. Now the wheel W is made of hard rubber and on it are fastened two small brass plates PP which are connected with each other by a wire. Mounted on separate supports, independent of the wheel W, are the two brass brushes DD, which make contact with PP. These brushes DD are connected with the source of current and the solenoid M by means of wires as shown in the figure. As the wheel W turns and the brushes DD come into contact with the brass plates PP on the wheel, the current in the solenoid M is established, the soft iron lug is attracted and the stirrer in the osmotic cell moved upward only to be dropped again as soon as W has turned far enough to destroy the electrical connection.[1] Thus both the outer liquid and that in the cell are stirred continuously. R is a resistance to regulate the current in the solenoid. It was found that in order to get good steady motion the hard rubber wheel W was too light. This defect was remedied by boring holes A through the wheel near its edge all around the circumference, and filling these with lead. On the same axis with W was another wheel (not represented in the figure) of the same size and weight, which served to balance the apparatus and at the same time to operate the stirrers of a second osmotic experiment like that shown in the figure. Thus osmotic experiments could be performed in duplicate, using but one motor. The arrangement for making and breaking the current in the solenoids of course did not require duplication, since the same current could be sent through both coils in series.

With this new apparatus the osmotic pressures of 0.125 normal LiCl solution in pyridine and also of 0.125 solution of cane sugar in pyridine were measured, using vulcanized caoutchouc as the membrane and pure pyridine as the outer liquid. The 0.125 normal solutions were selected rather than 0.25 normal, because the latter yielded a rather higher pressure than could be conveniently estimated with an open manometer. The preliminary results (above recorded) obtained with 0.25 normal sugar solutions without shaking do not indicate this, for the highest pressure observed was below 200 cm.; but it was found that by stirring this pressure could be about doubled.

With the stirring apparatus described, Fig. 7, it, of course, takes much more time to set up each individual experiment for the arrangement is more complicated. The necessity of stirring in osmotic experiments was found out after a long series of preliminary tests made in the old-fashioned way without stirring, and so during the time left only a limited number of tests could be carried out. Working with pyridine, moreover, has a very depressing effect on the nervous system, it being impossible

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[1] The arrangement was such that the slight heating effect produced by the current in passing through the solenoid was negligible, for the readings of the manometer did not change perceptibly after the current had been turned off and sufficient time allowed for any difference in tempearture to become equalized.

to keep the air in the laboratory entirely free from it. The effect appears to be cumulative in character, for one's system seems to become more and more sensitive to the substance. For this reason I felt constrained not to prolong the work with pyridine unduly. It will be remembered that I have carried on experiments with pyridine continuously for about two years, and off and on for a much longer time. During the investigations I have at times found it necessary to counteract the depressing effects of pyridine by taking small doses of strychnine.

The results of the experiments performed with the stirring apparatus will now be given. It need hardly be stated again that the LiCl and the sugar were dry and of a high degree of purity, as was also the pyridine.

57) The inner liquid was 0.125 normal solution of LiCl in pyridine and the outer liquid pure pyridine. It required 46 cc. to fill the cell. The volume of the outer liquid was 600 cc. The temperature was 19.0,° and the experiment was run for three days with constant stirring. The maximum height which the mercury column reached was 51.2 cm. at 19.0° C, corrected. This height was practically attained during the first 24 hours. At the end of the experiment, the whole of the outer liquid was evaporated to dryness and the LiCl in the residue estimated. It was found that the 600 cc. of outer liquid contained 0.0130 grams of LiCl, which shows definitely to what extent the solute has actually passed into the outer liquid.

In the light of what has been said in the preceding pages, it is evident that a determination of the exact amount of solute present in the outer liquid at the time when the maximum pressure is attained is an essential part of all final osmotic pressure measurements, yet such estimations have hitherto always been omitted even in experiments in which great care has been bestowed upon other details.

58) This experiment was exactly like the the preceding one (No. 57) except that the outer liquid consisted of 1200 cc. of pyridine instead of 600 cc. and the temperatures were as indicated below. The experiment was continued for 27 days, at the end of which time an examination showed the membrane

to be perfectly intact. The entire outer liquid was finally evaporated to dryness and the LiCl determined in the residue. It was found that the 1200 cc. of the liquid contained but 0.0267 grams of LiCl. The pressure rose slowly, the mercury column measuring over 50 cm. after the first day. On the sixth day the mercury column measured 51.5 cm., the temperature being 24.0.° On the twelfth day the mercury column measured 53.2 cm., the temperature being 20.95;° on the fourteenth day, 54.9 cm., the temperature being 21.96;° on the fifteenth day, 54.2 cm., the temperature being 19.6.° The beaker containing the outer liquid was then packed in melting ice. The temperature sank to 2.0° and was kept there for 12 hours After two hours of thus cooling, the mercury column was only 9.0 cm. high and remained there without change for the remaining ten hours. The ice was allowed to melt gradually for the next twelve hours. When the temperature had reached 21.4° C. the mercury measured 56.1 cm., this was on the seventeenth day. During the next three days the outer beaker was again kept surrounded with melting ice. It was difficult to keep the temperature of the apparatus perfectly constant during all this time. It gradually sank to 2.2° and finally to 1.6.° When the temperature had reached 1.6° the pressure indicated by the manometer was 2.9 cm. and remained constant for six hours. The temperature was then gradually raised, and at 20.5° the mercury column reached a height of 53.0 cm. where it remained constant for twelve hours. The outer beaker was then surrounded with a bath of a temperature of 36° C., the change to that temperature being made gradually; which, of course, was also done in the preceding cases where the temperature of the bath was radically altered. At 36° the height of the mercury column became constant at 101.0 cm. and remained so for about half a day, when the temperature of the bath was gradually raised to 58.7° and kept there. At this temperature the mercury column rose to a height of 128.3 cm., where it remained practically constant for two hours. The temperature was then gradually permitted to fall, and at 25.5° C. the column in the manometer finally registered 51.9 cm. on the last day of the experiment. It was after all this that the entire outer liquid was evaporated to dryness and the amount of LiCl in the residue determined with the result already recorded. The discussion of the results of this experiment will be deferred until after the next two experiments have been described.

59) The inner liquid was a solution of sugar in pyridine containing 0.125 gram-molecule per liter. The outer liquid was pure pyridine and the septum vulcanized caoutchouc. The experiment was run with constant stirring or three days, the temperature being kept constant at 20° C. The maximum pressure reached was 98.3 cm., nearly the whole of which was attained during the first day. On concluding the experiment the whole of the outer liquid consisting of 400 cc. was evaporated to dryness; the residue was taken up with water, and the sugar determined with Fehling's solution, after inverting with HCl. It was found that the 400 cc. contained 0.1149 grams of sugar.

60) This experiment was as nearly as possible a duplicate of the preceding one (59). On the fifth day the mercury in the manometer reached a height of 91.8 cm., the temperature having gradually gone up to 21° C. In this case, too, the pressure increased but little after the second day. On the morning of the seventh day the pressure was 94.0 cm., the temperature being 21.95° C.; in the evening of the same day 95.5 cm. at 22.15.° On the eighth day the outer beaker was packed in melting ice, the temperature in the beaker being 2.0° C. During this time the mercury in the manometer registered practically no pressure, or at best only a few millimeters. The ice was then permitted to melt gradually, and on the ninth day the pressure registered 92.0 cm. at 19.4° C. The temperature was then slowly raised to 46° C., where it was kept for twelve hours. The pressure became constant after about three hours at 114.2 cm. and remained there for nine hours. The experiment was then discontinued. The whole of the outer liquid consisting of 1200 cc. was then evaporated to dryness, and the sugar was taken up with water, inverted with HCl and determined with Fehling's solution. The result was that in the 1200 cc. 0.2205 grams of cane sugar were found.

Taking into consideration the pressures developed while the apparatus is kept nearly at a constant temperature, we find that according to No. 57 the osmotic pressure of the 0.125 normal solution of LiCl is 51.2 cm. at 19.0,° and according to No. 58 it is 54.2 cm. at 19.6.° Again, according to No. 59 the osmotic pressure of a 0.125 normal sugar solution is 98.3 cm. at 20.0,° while according to No. 60 it is 91.8 cm. at 21.0.° Even in these cases the agreement of the duplicates leaves much to be desired, for the differences in pressure observed can hardly be due to the slight differences in temperature. They are more likely due to individual differences in the membranes used, and to the fact that even with constant stirring, it was not quite possible to keep the liquids in immediate contact with the membrane of perfectly uniform concentration on each side. These difficulties have already been discussed at length above. There can be no doubt, however, that the results obtained are approximately the osmotic pressures of the solutions in question under the conditions of the experiments.

The effect of stirring is clearly shown by a comparison of experiment No. 54 where the highest osmotic pressure of a 0.125 normal sugar solution was found to be 62.4 cm. at 17.5° without stirring, and the result recorded in No. 59, where with stirring the osmotic pressure was found to be 98.3 cm. at 20.0.°

Taking the highest values found, namely 54.2 cm. for 0.125 normal LiCl solution at 19.6° and 98.3 cm. for 0.125 normal sugar solution at 20.0,° we note (1) *that the electrolyte gives by far the lower pressure,* and (2) that neither the LiCl nor the sugar give anywhere near the pressure called for by the gas laws, according to which a 0.125 normal sugar solution ought to give a pressure of approximately three atmospheres (228 cm. Hg) at 20.° Further, it will be recalled that a 1.2 per cent solution of sugar yielded practically no osmotic pressure at 0,° whereas according to the gas laws it ought to have produced about 0.8 of an atmosphere. And again, 0.05 normal solution of $AgNO_3$ yielded no osmotic pressure, whereas according to the gas laws it ought to have produced over an atmosphere. Further, the changes of pressure above recorded as caused by changes of temperature are so very much greater than they would be if they were proportional to the absolute temperature that additional comment seems quite superfluous. The osmotic pressures here investigated, then, do not follow the gas laws at all. Again, the electrolyte LiCl yields a much lower pressure than the non-electrolyte, sugar, which is exactly the opposite of what the theory of electrolytic dissociation requires.

OBSERVATIONS CONCERNING DIALYSIS.

When both cane sugar and copper oleate are dissolved together in pyridine and the solution is separated from pure pyridine by means of a vulcanized caoutchouc membrane, the copper oleate passes through the septum and the sugar remains behind. This is what one would expect from what has been said above. But here we have a case where a crystalline body, the sugar, is separated from a non-crystalline substance, the copper oleate, by dialysis in which process the non-crystalline or colloid body passes through the septum and the crystalloid remains behind. Again, when camphor and sugar are together dissolved in pyridine and the solution is separated from the solvent by means of a rubber septum, the camphor passes through and the sugar remains behind, which might have been anticipated. Here we have a case in which crystalloids are separated from each other by dialysis.

In fact it is clear that in general any substance which is soluble in both hydrocarbons and in pyridine may be separated from sugar by dialysis, when the pyridine solution of it and the sugar is separated from the pure solvent by a rubber septum. In such cases the sugar will always remain behind in the dialyser and the other substance will pass through whether it be crystalline or non-crystalline. Any other substance soluble in pyridine but not soluble in hydrocarbons may, in general, be substituted for sugar. Thus the rôle of the nature of the membrane in the process of dialysis is demonstrated. The current view that crystalloids always pass through membranes more readily than colloids is evidently untenable, for it has been shown that just the opposite may occur, and that even crystalloids may be separated from each other by dialysis when the proper septum is chosen. *Whether substances can be separated by dialysis or not does not depend at all upon their crystalline or non-crystalline nature as is so commonly supposed, but upon their affinity for the septum employed.* After what has been said above, it is evident that stirring the liquids hastens dialysis.[1]

Several experiments in addition to those here described have been performed, but as I hope to continue the experimental work on dialysis their presentation will be reserved for a later communication.

GENERAL REMARKS.

The conclusion reached above that the process of osmosis depends upon selective solubility (in other words, upon the affinities involved) is in agreement with what Overton[2] has found in his physiological studies. The importance of the recognition of the true nature of osmotic processes for progress in physiology can hardly be overestimated.

From the time of Dutrochet all investigators who worked on osmosis experimentally with different membranes and various liquids have been impressed with the important rôle of the nature of the membrane in determining whether osmosis would occur at all or not, and if so, in what direction. The following quotation taken from the article of L'Hermite, cited above, shows how clearly he had grasped the situation. "Je pense avoir demontré par la discussion des expériences de mes devanciers et des miennes, que l'endosmose n'est point le resultat d'une force particuliére, mais de l'affinité elle-même en étendant l'acceptation de ce mot à l'attraction capillaire qui en est le premier degré". I should also like to quote the opinion of Raoult, which he voiced in a letter written January 7, 1897, to Prof. W. D. Bancroft, and which, through the latter's kind-

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[1] Since the above was written it came to my notice that in 1848 Jolly, (Lieb Ann. 68, 6) refers to a case where he shook the dialyzer to hasten the process.

[2] Vierteljahrsschr. d. naturforsch. Ges. in Zürich, 44, 88 (1899). See also the discussion in chap. 5 of Höber's Physik. Chem. d. Zelle u. Gewebe, Leipsig, (1902). Compare also Livingston, The Rôle of Diffusion and Osmotic Pressure in Plants, Chicago (1903).

ness I am able to present here. This quotation, which requires no further comment, gives everything contained in the letter relative to the osmotic process.

"La question de l'osmose, que beaucoup croient résolue par des formules mathématiques fondées sur des hypothèses commence à peine à être posée. Mes expériences auxquelles vous faites allusion, prouvent qu' il y a certainement des diaphragmes *actifs.* Existe-t-il également des diaphragmes *passifs,* comme pour les gaz? Pour le moment tout le mond paraît le croire, sur la foi de van't Hoff; mais pour mon compte, je n'en ai jamais pu trouver un seul.

"Si je n'ai pas continué mes publications sur ce suject, c'est que j'ai rencontré de très grandes difficultés pour mesurer les pressions osmotiques avec des diaphragmes mous. Existe-t-il, d'ailleurs, une veritable pression osmotique? Je n'en suis pas sûr. Il arrive certainement toujours une pression pour laquelle le mouvement osmotique semble s'arrêter; mais j'ai lieu de croire qu'elle correspond au moment où la quantité de liquide qui passe dans un sens par *osmose,* est égale à celle qui passe en sens opposé par *filtration.*

"La question est intéressante et fort délicate. Aussi, verrais-je avec un trés grand plaisir d'autres expérimentateurs s'engager dans cette voie."

Again, very recently Barlow (1. c.) working in the laboratory of J. J. Thomson has reemphasized the fact that the osmotic current is caused by the mutual potential energy of solution of the liquids and that the direction of osmosis is conditioned by the character of the membrane.

Precipitated membranes, like those of copper ferrocyanide, etc., are hydrates, that is they contain more or less water. When such a membrane is used to separate water from an aqueous solution, the latter abstracts water from the septum—the hydrate—which again takes up water from the side bathed by the pure solvent. The affinity of the solution for additional solvent must be sufficient to extract some water from the membrane in order that an osmotic pressure may be produced. If, in addition, the solute has considerable affinity for the septum, copper ferrocyanide for example, as many of the salts of the alkali metals are known to have, for instance, the solute will pass through to a notable extent; if on the other hand, the solute has very little affinity for the septum, as is the case with cane sugar, the membrane will allow but very small quantities to pass through, and hence will be "semipermeable." It is perfectly clear, too, that, in general, different precipitated membranes would lose part of their water with different degrees of readiness, and hence in quantitative measurements of osmotic pressures different results would be obtained when different precipitated membranes are used. This is shown to be true by the experiments of Pfeffer (Osmotische Untersuchungen) who found that when copper ferrocyanide membrane was used a one per cent sugar solution yielded an osmotic pressure of 51.0 cm. at 14.2°; when a Prussian blue septum was employed the pressure was 38.7 cm. at 13.9°; and when a calcium phosphate membrane was used the pressure was 36.1 cm. at 15.2°. In all three cases the sugar which passed through the septa was insignificant according to his determinations. While the results of Pfeffer are not final as determinations of the maximum osmotic pressures, inasmuch as he did not stir the liquids while measuring the pressures, the experiments nevertheless serve to show that the pressures developed with the different septa are by no means the same. If one were to compute the molecular weight of sugar from the osmotic pressures which a one per cent aqueous solution of it develops when copper ferrocyanide, Prussian blue and calcium phosphate are used as membranes respectively, different values would obviously be obtained, that is the conclusion would be reached that the molecular weight of sugar in one and the same solution varies in different cases, which is obviously absurd. The experimental facts are, of course, readily explained by what has been said above.

Further, when precipitated membranes are used and the osmotic process goes on very rapidly, it generally occurs that the solution robs the precipitated hydrate of water faster than the latter is taken up from the pure water bathing the other side of the septum. In such cases the membrane develops ruptures through which the solution oozes out of the cell, hence the necessity of the usual practice of adding the "membrane formers'' to the liquids on each side of the membrane to repair such leaks. It is obvious that in any osmotic experiment the composition of the septum is always in a state of change, though the extent of this may be slight in some cases. Here lies one of the chief difficulties of measuring osmotic pressures (which are equilibrium pressures) with soft diaphragms to which Raoult alludes.

It must be borne in mind that the application of the gas laws, either in simple or modified form, to dilute solutions is based upon the experiments which Pfeffer made with copper ferrocyanide membranes, and which Morse and Frazer have recently sought to verify. But these experiments have all been made without stirring and with but one membrane, and hence are not final. Furthermore, the osmotic pressures of sugar solutions in pyridine, using vulcanized caoutchouc as the semi-permeable membrane, show definitely that the gas laws do not obtain here at all. In the face of the experimental facts which we now have as showing the nature of the osmotic process and the magnitude of the osmotic pressures under different conditions, the general, indiscriminate application of the gas laws in their simple or somewhat modified form to all dilute solutions, and even to some that are not dilute, as now in vogue, can not be too greatly deplored. To speak of the osmotic pressure of any isolated solution without specifying what membrane separates it from what other liquid is nonsense,[1] in the light of the facts here presented. And further, to assume that solutes are polymerized or dissociated in dilute solutions because the osmotic pressures developed by the latter in given cases happen to deviate from values computed from the gas laws is evidently equally unjustifiable practice.

SUMMARY.

In this paper it has been shown that whether osmosis will take place in a given case or not depends upon the specific nature of the septum and the liquids that bathe it; and if

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[1] Compare also views expressed by Van Laar, Chemisch Weekblad, 2, 1—16. (1905.)

osmosis does occur, these factors also determine the direction of the main current and the magnitude of the pressure developed. The motive force in osmotic processes lies in the specific attractions or affinities between the liquids used, and also those between the latter and the septum employed. These attractions or affinities have also at times been termed the potential energy of solution, etc., they are to the mind of the writer essentially the same as what is commonly termed chemical affinity.

It has been emphasized that osmotic pressures are equilibrium pressures, and that in osmotic processes there is always a current in both directions; though the main current may in specific cases be so much stronger than the minor that the latter almost sinks into insignificance. In such cases the septum is termed "semipermeable."

Vulcanized caoutchouc has been found to be a "semipermeable" membrane when it separates pyridine solutions of silver nitrate, lithium chloride, and cane sugar from the pure solvent.

The necessity of stirring the contents of the osmotic cell and also the outer liquid during osmotic pressure measurements has been pointed out, and a new apparatus for measuring osmotic pressures accordingly has been devised.

The results of the osmotic pressure measurements show that the gas laws do not hold; and it has consequently been pointed out that the latter can not serve as a basis for a satisfactory theory of solutions.

The advantage of stirring in processes of dialysis has been indicated; and it has been shown that whether substances pass through membranes or not does not depend upon their colloidal or crystalloidal character, but solely upon their affinity for the membrane employed and for the liquids that bathe it.

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