

BOTANICAL MICRO-CHEMISTRY

POULSEN - TRELEASE

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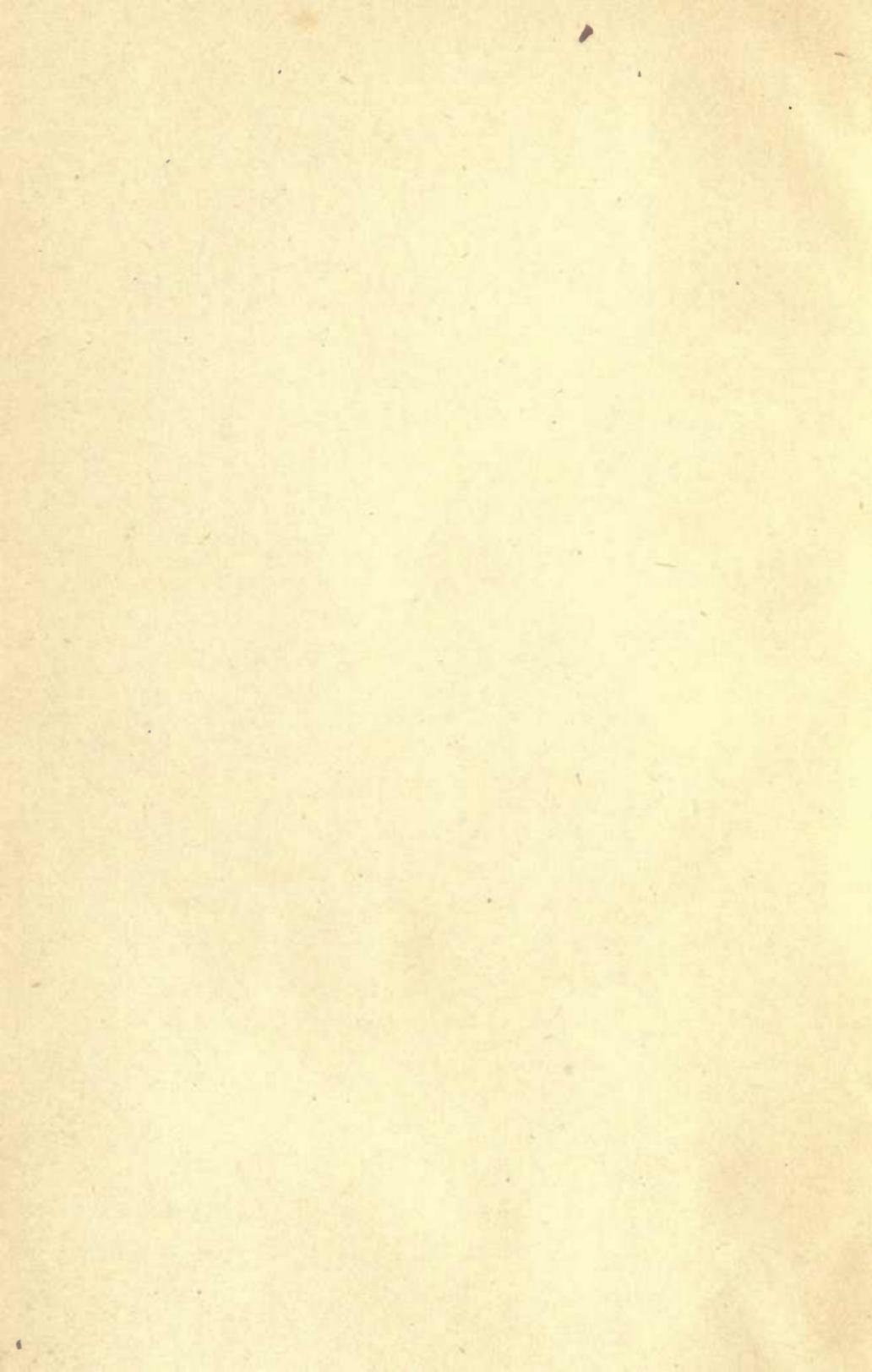
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ANALYTICAL
CHEMISTRY

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BERKELEY, CALIFORNIA



BOTANICAL
MICRO-CHEMISTRY

AN
INTRODUCTION TO THE STUDY OF
VEGETABLE HISTOLOGY

PREPARED FOR THE USE OF STUDENTS

BY
V. A. POULSEN

*TRANSLATED WITH THE ASSISTANCE OF THE AUTHOR
AND CONSIDERABLY ENLARGED*

BY
WILLIAM TRELEASE
PROFESSOR IN THE UNIVERSITY OF WISCONSIN



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ELECTROTYPED.
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TO
My Esteemed Teacher,
DOCENT EUG. WARMING, Ph.D.

AND

My Respected Friend,
PROFESSOR LEOPOLD KNY,

THIS BOOK IS DEDICATED IN THANKFULNESS
BY THE AUTHOR.

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PREFACE
TO
THE AMERICAN EDITION.

THE translation of the Danish original into German, Italian, and French, and now into English, is an honor not dreamed of when the original edition was published.

I wish to cordially thank all of my collaborators, especially my friends Carl Müller of Berlin, and Prof. Aser Poli of Melfi, and my honorable colleagues Dr. Lachmann of Lyon, and Prof. Trelease of Madison, who have not merely enlarged the book, but enriched it with many notes and paragraphs of great value.

Once more I wish to express the hope that, in the new shape in which it appears, my book may be of use to the students of vegetable histology, for whom it was written.

V. A. POULSEN.

ROSENVAENGETS HOVEDVEJ, COPENHAGEN, June 1883.

TRANSLATOR'S PREFACE.

WHILE studying in the laboratory of Dr. Farlow, at Harvard University, in 1881, I first became acquainted with the German translation of Poulsen's little treatise, which made its appearance in this country about that time. It was proved so valuable by every-day use in the laboratory, both there and with my classes in the University of Wisconsin, that I began a translation of the most frequently used parts, having in mind at first only the convenience of my own students. But several requests having been made that I should complete the translation and publish it, I decided to do so, having received an offer of additional notes from the author. Various causes, however, have delayed my work, so that it is but now, above a year since it was begun, that it is completed.

It should be said that the translation is in no sense a literal one. The obvious meaning of the author has been given, but with no effort to preserve the idiom or vocabulary, and the usual English terminology has commonly been employed.

In presenting my translation to American teachers of vegetable histology, I feel confident that it will meet the every-day wants of the laboratory.

WM. TRELEASE.

MADISON, Wis., April 12, 1883.

AUTHOR'S PREFACE.

THIS little attempt at a comprehensive presentation of the more important micro-chemical reagents and methods is the first which has appeared in the Danish language. It has been undertaken on the recommendation of Dr. Eug. Warming, who has often felt the need of a compact guide to micro-chemistry in his work with students of vegetable anatomy at the Botanic Garden. To him I am indebted for many valuable suggestions, which I find it a pleasant duty to acknowledge here.

I am also indebted to Mr. R. Pedersen, Docent in Vegetable Physiology at the Copenhagen University, for many criticisms.

Should those inexperienced in microscopy draw even a little useful information from this treatise I shall have accomplished what was aimed at.

V. A. POULSEN.

ROSENVÆNGET, October 1880.

INTRODUCTION.

RECENTLY microscopic technology has risen to an importance undreamed of in its early days. The perfection of the microscope, by whose help so many beautiful and important discoveries are made, has given us such an insight into the natural history of the cell as to stimulate the study of elementary organs, so far as possible, with all available aids. The spectroscope, the polariscope, and the induction coil, placed in our hands by Bunsen, Huygens, and Faraday, are applicable and must augment the value of the instrument first given us by Hans and Zacharias Janssen; and even photography has of late been employed. Physics has thus striven to bring this instrument to as great a degree of perfection as possible; it remains for chemistry to find means of recognizing and rightly understanding the composition of the objects we investigate. In other words, if we employ a thorough system of chemical analysis with the optical apparatus we shall be able to answer all questions lying within the range of possibility. It is this analysis, applied to objects

under the microscope, that we designate by the word *micro-chemistry*.

I have endeavored to successively make the reader acquainted with the most valuable reagents used in micro-chemistry, *i. e.* with those substances whose action on the bodies to be studied allows their chemical composition and nature, and sometimes their physical structure, to be recognized. In the first section I have considered the chemicals used in the laboratory; in the second, the vegetable substances to be tested for and the reactions by which they are known.

The correctness of the statements which follow rests in part on the long experience of my respected teacher, Dr. Warming, and in part on my own. I have also been able to profit by the practice and teaching of Professor Hanstein, of Bonn, made known to me by Dr. Warming. Finally, the scattered experiments and methods, recorded in a large and scattered literature, have been used so far as possible,—far be it from me, however, to suppose that I have exhausted the literature; nor have I tried to take up all of the chemicals that have been used, my endeavor being to collect only such as are most useful.

At the close of the first section I have introduced a short chapter on media for the preservation of permanent preparations; to which are added a few words on the cements used in mounting.

Pleasant as it would have been to add a historical outline of the development of micro-chemistry, I have thought best not to do so, partly that the book might be kept within proper limits as to size, partly because that treatment of the subject would more properly find place in a theoretical and comprehensive text-book than in a compact guide for purely practical use.

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PART I.
MICRO-CHEMICAL REAGENTS
AND
THEIR APPLICATION.



MICRO-CHEMICAL REAGENTS.

IODINE.

THIS is one of the most valuable and indispensable substances used in microscopic botany. It is employed in solution, either in water,¹ alcohol, or a solution of potassic iodide in glycerine, chloriodide of zinc, or water. The degree of concentration of the solution depends upon the particular case in which it is to be used, the effects of much-diluted solutions being often very evident. For the recognition of starch, iodine is a very convenient reagent, and the only certain one known. A preparation which contains the minimum of free iodine suffices to reveal this substance, though only in case the starch contains water. With an aque-

¹ Nägeli: *Das Mikroskop*, 1877, p. 473. Dippel: *Das Mikroskop*, 1872, p. 273. Sachs: *Jahrbücher für wiss. Bot.*, 1863, III. Mohl: *Vermischte Schriften*, 1845, p. 335. Hofmeister: *Handbuch*, I, *Pflanzenzelle*, 1867, pp. 252, 387. Weiss: *Allgemeine Botanik*, 1878, I, pp. 18, 77, 159. Schleiden: *Wiegmann's Archiv.*, 1838, pp. 39, 59. Raspail. *Développement et Analyse microscopique de la Féculé*.—*Ann. Sci Nat*, 1 Sér., T. VI., p. 388. Meyen: *Untersuchungen über den Inhalt der Pflanzenzellen*, Berlin, 1828, p. 21. Sachs: *Lehrbuch der Botanik*, 1874, pp. 34, 40, 58.

ous solution, this condition is fulfilled ; but in case a solution in absolute alcohol is used, water must be added to the preparation.

The well-known reaction, discovered by Stro-meyer, is as follows :¹ According to the strength of the solution and the duration of its action, the starch grains assume a more or less deep-blue color, which vanishes when the preparation is warmed, but reappears when it again becomes cool, and is entirely destroyed by the action of hyposulphite of sodium. Dry starch slowly turns brown when treated with a solution of iodine in chloroform or absolute alcohol, which renders it a good test for the presence of water in alcohol. According to Nägeli, the starch grains are composed of granulose diffused through a skeleton of starch-cellulose. The former, only, is turned blue by iodine, for after its removal (by digestion with saliva at 45–55° C., or the action of pepsin, diastase, organic acids, or the prolonged action of dilute sulphuric or hydrochloric acid) the grains turn yellow or brown.

Most cellulose membranes color yellow or brownish with iodine, especially when a freshly-made solution is employed. An exception, however, is afforded by the paraphyses and asci,—the hymenium of lichens, which often turn blue, like starch. If, however, the cell-wall has been first

¹ Nägeli: Die Stärkekörner, Zürich, 1858. Wiesner: Technische Mikroskopie, 1857, p. 73.

treated with strong sulphuric or phosphoric acid, or with an aqueous solution of zinc chloride, it colors blue when iodine is added, these reagents converting the cellulose into amyloid,¹ a carbo-hydrate related to starch. Hydriodic acid also acts in the same way, and hence old solutions of iodine sometimes color the cell-wall blue, when this acid has formed through the action of the water or alcohol of the solution. Cellulose membranes that have been allowed to dry after treatment with a solution of iodine, and are again moistened with water, often turn blue, the organic matter having induced the formation of hydriodic acid. The epidermal cell-walls of many seeds and pericarps that swell into mucilaginous masses when moistened, assume a blue color with iodine-water only after the swelling has reached a certain stage. Not unfrequently a membrane lying in an iodine solution passes through several shades of color in the course of a longer or shorter time. Most reagents that cause cellulose to turn blue when used in combination with iodine, also cause it to swell, approximately, in the following ascending order: Potassic iodide, iodide of zinc, nitric acid, phosphoric acid, potassic hydrate, hydriodic acid, and sulphuric acid.

We have thus an excellent test for pure cellulose in iodine and any amyloid-producing reagent.

¹ Not to be confounded with the substance known to animal histologists by the same name.

If, however, a membrane does not color blue when treated with sulphuric acid and iodine, it is permeated by other substances, or is to be regarded as changed chemically; this is true of wood-cells, vessels, cork, and, usually, the root-cap. The substances (lignin, suberin) taken up by the walls in these cases must first be removed by the alternate use of acids and alkalies, and finally by washing in alcohol, ether, or chloroform, before the cellulose reaction will appear; but, as yet, with the single exception of the silicified frustules of diatoms, no cell-wall has been found that is not shown by this treatment to contain cellulose.

In the study of lichens, chemical reagents play an important part.¹ The preparation usually employed, consists of 5 cg. iodine, 20 cg. potassic iodide, and 15 g. distilled water. In thin sections, the hymenium is usually colored blue by this solution, the medullary layer, occasionally, and the gonidial layer, less commonly. A wine color may also appear under this treatment, which shows that these cell-walls have a composition different from those of other plants.

All living protoplasm² is killed by iodine, which is then rapidly imbibed and stains the protoplasm

¹ Deichmann-Branth and Rostrup: *Lichenes Daniæ*, p. 17. De Bary *Morphol. und Physiol. der Pilze, Flechten und Myxomyceten.*—Hofmeister's *Handbuch*, II., 1866, p. 281.

² Sachs: *Lehrbuch*, 1874. Weiss: *Allgem. Bot.*, 1878, I. Tangl: *Protoplasma der Erbse.*—*Sitzungsber. wiener Akad.*, 1877-8, Bd. LXXVI.-LXXVIII.

brown. This is especially true of the nucleus and chlorophyll bodies, as well as of the nitrogenous fundamental part of the protein grains, for the detection of which a rather concentrated solution should be used. Since a very small quantity of iodine is quickly fatal to protoplasm, besides coloring it, this reagent is especially useful in studying bacteria and other ciliated micro-organisms. In the study of protein grains the glycerine solution is best, because of its clearing action.

The starch¹ which occurs in chlorophyll bodies is easily detected by treating very thin sections with alcohol, or potassic hydrate and acetic acid, and afterwards adding a solution of iodine in water which contains potassic iodide, when the swelling starch grains assume the characteristic blue color.

The so-called crystalloids consist of protein substances, and consequently turn yellow when treated with iodine. This is also the case with inuline, where, however, the color does not depend upon a real imbibition of the iodine, but only upon a condensation of the brown fluid in the fine fissures of the sphaero-crystals.

All objects colored with iodine fade in the course of time; and iodine solutions destined for use should be kept in the dark, to prevent the formation of hydriodic acid, which is greatly promoted by the action of light. When sections or

¹ Sachs: *Jahrbücher für wiss. Bot.*, 1863, III., p. 200; *Flora*, 1862, p. 166. Weiss: *Allgem. Bot.*, 1878, I., p. 111.

other preparations are removed from water into a concentrated alcoholic tincture of iodine, small, black, rhombic crystals of iodine often make their appearance.

CHLOR-IODIDE OF ZINC.

In the preceding section mention has been made of a solution of iodine in chloride of zinc.¹ We may now consider this a little more fully.

This preparation is made by dissolving zinc in pure hydrochloric acid, evaporating the solution to the density of sulphuric acid, in contact with metallic zinc, and adding as much potassic iodide as the solution will take up. Finally, it is saturated with metallic iodine.² The color of the reagent should be reddish-brown; it should have the odor of iodine, and small crystals of pure iodine should precipitate with time. As a precaution against the formation of hydriodic acid, it should be kept in the dark, although this is less important than with the other iodine preparations.

Chlor-iodide of zinc is especially useful for the detection of pure cellulose, since the zinc chloride

¹ Nägeli: Verhalten der Zellhaut zum Jod.—Sitzungsber. d. bayr. Akad. der Wiss., 1863, p. 383; Das Mikroskop, 1877, p. 474. Mohl: Blaue Färbung der Vegetab. Zellmembran durch Jod.—Flora, 1840. See also the works cited above.

² The directions of Grönland, Cornu and Rivet (*Des Préparations Microscopiques*, Paris, 1872, p. 75), are incorrect, since the most important element—the iodine—is not present.

converts this substance into amyloid, which is then colored blue or violet by the free iodine. Cell-walls that have suffered degeneration of the cellulose are not colored blue. Wood-cells, vessels, cork-cells, and the cells of the root-cap, as well as the cuticularized layer of the epidermis, the extine of pollen grains and spores, in fine, all lignified or corky membranes, are colored yellow; the true cuticle, however, is uncolored. Starch colors blue, but the grains rapidly swell up and undergo disorganization. The walls of fungus hyphæ, composed of the so-called fungus-cellulose, remain uncolored to a noticeable degree.¹ [They are also usually uncolored by sulphuric acid and iodine.]

For the detection of tannin, a very dilute solution of chlor-iodide of zinc is employed, the contents of cells which contain tannin becoming reddish or violet under this treatment.²

POTASSIC HYDRATE (Caustic Potash).

Next to iodine, caustic potash takes the most important place among micro-chemical reagents.³

¹ Schacht: Die Pflanzenzelle, p. 143, *et seq.* Hofmeister: Handbuch, I., Die Pflanzenzelle, p. 258.

² Sanio: Bot. Zeit., 1863. Dippel. Das Mikroskop, I., p. 375.

³ Nägeli: Das Mikroskop, 1877, pp. 472 and 525. Dippel: Das Mikroskop, I., p. 278. Wiesner: Technische Mikroskopie, 1867. Sachs: Ueber die Stoffe, welche das Material der Zellhäute liefern. — Jahrb. für. wiss. Bot., 1863, III.; Keimung von *Allium Cepa*. — Bot. Zeitung, 1863, Nos. 8-9; Zur Keimungsgeschichte der Gräser, Keimung der Dattel. — Bot. Zeitung, 1862.

The commercial potassic hydrate is used in an aqueous or alcoholic solution, whose concentration depends upon the particular purpose for which it is to be employed. In general, a moderately concentrated solution is preferable, since it may be diluted when this is necessary, and a very strong solution is seldom to be recommended, since, although its action is more rapid, it destroys the tissues too quickly. In making an aqueous solution, care should be taken to add the alkali in small quantities, to avoid undue heating. The alcoholic solution is made as follows:¹ 85–90 per cent. alcohol is mixed with a concentrated aqueous solution of caustic potash until a precipitate is formed. After being repeatedly shaken it is set aside for twenty-four hours, at the end of which time the clear, pale-yellow fluid is decanted, and, after dilution with distilled water, is ready for use. Russow recommends the employment of solutions of two densities, one consisting of one part of distilled water and two parts of the saturated solution; the other, of one part of water to three of the original solution.

Since potash solutions readily take up carbonic acid from the air, and further tend to crystallize out in the neck of the bottle in which they are kept, it is necessary to keep them in bottles with glass stoppers, which must be frequently loosened.

¹ Russow: *Mém. Acad. St. Pétersbourg*, Sér. 7, Tome 19, No. 1, p. 15, note.

[A slight coating of paraffine will prevent the stopper from sticking.]

In microscopic manipulations the value of potash depends upon its solvent and softening actions, those which accompany the absorption of water. It dissolves many of the fine granules in protoplasm, bleaches various coloring matters, forms soluble soaps with fats, and effects the swelling of the starch which often renders tissues opaque. It thus destroys the protoplasmic structure of cells and makes these clearer and more transparent, thus, when dilute, playing a very important part as a clearing medium in the study of otherwise opaque sections; while it permits the examination of thick masses of tissue, or even of entire organs, as embryos, trichomes, sections of the *punctum vegetationis*, or whole stems and leaves, as in the study of the course of fibro-vascular bundles [and laticiferous tissue].¹

This method was first proposed by Hanstein. The sections are treated with a solution of potash, washed, and neutralized with hydrochloric or acetic acid. If this renders them too opaque they may be cleared by washing first in pure water, then in ammonia water. If, on the other hand, they are too transparent, they may be improved by washing

¹ Hanstein: Die Scheitelzellgruppe im Vegetationspunkt der Phanerogamen, 1868; Entwicklung des Keimes, Botan. Abhandl., I., Heft 1, p. 5. Koch: Cuscuta, in Hanstein's Bot. Abhandl., II., Heft 3, p. 25. Oels: Anat. d. Droseraceen., Diss., Breslau, 1879, p. 13.



in alum water. Sometimes the process has to be repeated several times before the desired effect is obtained. After washing in distilled water, preparations made in this way can be kept for a long time in glycerine, which clears them still more.

Russow's alcoholic potash is used for the same purpose as the aqueous solution, and in most cases is preferable, since the presence of alcohol prevents the excessive swelling of the cell-wall, that often occurs when the Hanstein process is employed. With this reagent acetic acid may be used for neutralization, and the preparations keep well in glycerine. Starch swells and is destroyed in potash, which is therefore useful for clearing up tissues, like the albumen of seeds, that contain much starch.

The aqueous solution of potash, causing swelling of the cell-wall, often facilitates the study of its striation and stratification; this is especially the case in collenchyma.

A warm solution of potash, being a solvent for the so-called intercellular substance, is sometimes employed for isolating cells by maceration.

Potash is useful as a test for suberin.¹ When thin sections of corky tissue are well boiled in this reagent the suberin is extracted from the cell-wall, appearing as yellow drops, that soon run together.

¹ Höhnel: Kork u. verkorkte Gewebe. — Sitzber. wien. Akad., 1877, Abth. I., p. 16.

Cell-walls (wood-cells, ducts, etc.), that do not immediately give the cellulose reaction with iodine and sulphuric acid, because of the presence of so-called incrustation matters, give this reaction promptly after treatment with boiling potash¹ — often used in connection with nitric or other acids — since this removes the foreign substances.

Sometimes tannin may be recognized by the use of potash, as cells which contain it — and which color green with salts of iron — assume a yellow color with potash.

Cells containing chrysophanic acid² become purple-red with the same fluid.

Sachs has employed potash as an analytic reagent in various histologo-physiological studies, obtaining good reactions when using it with cupric sulphate in testing for different sugars, protein matters, and carbo-hydrates.³

When protoplasm is first treated with nitric acid, and afterwards with dilute potash or ammonia, it assumes a beautiful yellow color from the formation of potassic or ammoniac xanthoproteate.⁴

The so-called crystalloids swell and change their angles in potash, thus showing their organic nature.

¹ Hofmeister : Handbuch, I., several places.

² Borscow : Bot. Zeit., 1874, p. 20. Weiss : Allg. Bot., 1878, I., p. 288.

³ See cupric sulphate, below. The reader is referred to the preceding papers, and to H. de Vries' Keimung des rothen Klees. — Landwirthsch. Jahrb., 1877, VI., p. 468.

⁴ Dippel : Das Mikroskop, II., pp. 10, 18.

AMMONIA.

A concentrated solution of ammonia in water is often used instead of potash, where the latter would act too powerfully.¹ We have also mentioned its use in clearing tissues by Hanstein's method.

As a test for protein combinations, including crystalloids, it is employed with nitric acid to intensify the color of the xantho-protein reaction. When a thin section of a tissue composed of cells with thickened walls is successively treated with nitric acid and ammonia, the middle lamella (intercellular substance) is colored yellow.²

Finally, it is employed in the preparation of cupro-sulphate and carminate of ammonia.³

Ammonia is also valuable for restoring the form of herbarium specimens of phænogams, algæ, and mosses, as well as spores, pollen grains, etc., that are to be examined microscopically.

CUPRAMMONIA (Cuoxam, Cramer; Cupridiamin).

This important reagent must be freshly made when needed for use, as it deteriorates with age. When it is desirable to keep it for a time it should be set in the dark.

¹ Dippel: Das Mikroskop, I., p. 279. Weiss: Allgem. Bot., 1878, pp. 77, 144.

² Dippel: Das Mikroskop, II., p. 100.

³ See the section on staining agents.

To prepare it an aqueous solution of sodic hydrate is slowly added to a solution of cupric sulphate, until a precipitate of cupric hydrate forms. The precipitate is collected on a filter, transferred to a test tube, washed, and dissolved in strong ammonia. The solution, which is of a beautiful dark-blue color, is at once ready for use.

It may also be prepared by allowing 16 per cent. ammonia to stand upon copper turnings in an open flask. However prepared, it should be used only so long as it has the power of quickly dissolving cotton fibers.

Pure cellulose swells much and is dissolved without conversion into amyloid. Cell-walls incrustated with lignin, suberin, etc., are only dissolved after these substances have been removed by Schultze's maceration.

In general, neither the cuticle nor the so-called middle lamella or intercellular substance dissolves.

According to Kabsch, cuprammonia is a test for pectose. When a tissue containing this substance is treated with the reagent a fine skeleton of cupric pectate is left behind.¹

¹ Schweitzer: Vierteljahrschrift, nat. Ges., Zürich, 1857, II. Kabsch: Jahrb. wiss. Bot., 1863, p. 357. Hofmeister: Handbuch, I., p. 240. Dippel: Das Mikroskop, I., p. 280. Wiesner: Technische Mikroskopie, 1867, p. 38, note 2. Nägeli: Das Mikroskop, 1877, p. 474. Frémy: Mémoires de l'Académie, Paris, 1859; Journ. de Pharm. et Chim., XXXVI. Epstein: De Conjunctione cellulosæ cum cupro oxydato. Dissertatio, Breslau, 1860 (Cf. Bot. Zeit., 1860, p. 234).

MINERAL ACIDS.¹

SULPHURIC ACID (Oil of Vitriol).

Sulphuric acid is used in a concentrated or dilute form according to circumstances. The most useful proportion is obtained by diluting one volume of strong acid with three of water.²

Dilute sulphuric acid causes starch grains to swell, and similarly affects cellulose, especially in collenchyma, at the same time transforming it into one of its isomers, amyloid, which differs from cellulose in assuming a blue color when treated with iodine. Hence, to determine whether a cell-wall consists of pure cellulose, it is only necessary to treat it first with a tincture of iodine and then with sulphuric acid,³ when it turns blue, if unincrustated.

Concentrated sulphuric acid dissolves both cell-wall and starch grains, greatly swelling all parts it

¹ In using any of these acids a large cover-glass should be employed to prevent injury to the objective.

² Dippel: *Das Mikroskop*, I, p. 276. Nägeli: *Das Mikroskop*, 1877, p. 474. Weiss: *Allgem. Bot.*, 1878, I, p. 62, etc. Hofmeister: *Handbuch*, I, p. 252, etc.

³ [M. Vetillart advises the use of the following mixture, in place of pure acid, in the cellulose test. Three volumes of sulphuric acid (spec. grav. 1.84), one of water, and two of glycerine, are slowly mixed to avoid heating. This does not destroy the tissue. — *Christy's Fibres*, p. 17. W. T.]

comes in contact with. Starch is thus converted into dextrin.

The cuticularized parts of the cell-wall (cork, cuticle,¹ extine of pollen, exospore, root-cap, etc.) resist the action of this reagent, as does the previously mentioned middle lamella. Protoplasm is destroyed after a time, while young protoplasmic bodies are often colored rose-red under its action,² the reaction being rendered more certain by the addition of a solution of cane-sugar. Fat-bodies occurring in the protoplasm are not dissolved, but run together, forming small refractive drops. Oil which previously existed, diffused through the protoplasmic mass, manifests itself similarly.³

NITRIC ACID (Aqua fortis).

This is employed as a macerating reagent in combination with potassium chlorate,⁴ *q. v.*

When the contents of a cell are treated with nitric acid alone, or with this reagent followed by ammonia, they assume a bright yellow color when protein matters are present, through the formation of xantho-protein acid.

According to Höhnel, nitric acid, either alone or

¹ DeBary: Hofmeister's Handbuch, III., 1878, pp. 84, 131. Brongniart: Ann. Sci. Nat., 1830, 1 Sér., T. XXI., p. 427.

² Sachs: Bot. Zeitung, 1862, p. 242.

³ Sachs: Bot. Zeitung, 1862, p. 146.

⁴ Dippel: Das Mikroskop, I., p. 275. Nägeli: Das Mikroskop, 1877, p. 474. Sanio: Bot. Zeit., 1863, p. 362, note.

with potassic chlorate, is a good test for suberin.¹ To obtain this so-called ceric reaction, thin sections of the tissue are treated with the reagent. If suberin is present, after the solution of the other parts of the cell-wall, yellow or spherical masses remain, which are at first granular but later become homogeneous. They consist of ceric acid, and are soluble in alcohol, ether, benzol, and chloroform.

Warm nitric acid, followed by ammonia, colors the middle lamella (or intercellular substance) yellow.²

Nitric acid dissolves starch grains after causing them to swell greatly; hence it may often be used in a dilute condition for clearing tissues which contain much starch.

CHROMIC ACID.

This must be free from sulphuric acid. It is employed concentrated or dilute, according to circumstances.³ Since it tends to form crystals in the neck of the bottle in which it is kept, the stopper should be coated with vaseline, or frequently loosened by turning.

The cell-wall swells and finally dissolves in chro-

¹ Höhnel: Sitzber. wien. Akad., 1877, Abth. I.

² Dippel: Das Mikroskop, II., p. 100.

³ Dippel: Struktur der Zellhülle, 1878. Sanio: Bot. Zeitung, 1860-1863. Kabsch: Jahrb. wiss. Bot., III., p. 387. Nägeli: Das Mikroskop, 1877, p. 475.

mic acid, and, as the swelling proceeds slowly, the dilute acid is useful for showing the stratification of the wall. Only silicified and corky¹ layers resist its action. Lignified cells are entirely dissolved. Those containing suberin become very transparent and nearly invisible; but after the acid has been removed by washing they usually reappear, though the prolonged action of the reagent dissolves them.

In general, chromic acid is useful in the study of the stratification of the cell-wall, starch-grains, etc. It is also sometimes applicable to the fixation of protoplasm [*e. g.* the plasmodia of Myxomycetes], but must be used in a very dilute form for this purpose.

PEROSMIC ACID.

This poisonous and ill-smelling reagent,² which is usually kept in the crystalline form in hermetically-sealed tubes, and dissolved in water when needed for use, has of late years been much employed in the investigation of the minute structure

¹ Höhnel: Ueber Kork. — Sitzungsber. wiener Akad., 1871, Abth., I. Höhnel differs here from Pollender: Chromsäure als Lösungsmittel für Pollenin und Cutin. — Bot. Zeitung, 1862, p. 385.

² Dippel: Das Mikroskop, I., p. 375. Strasburger: Befruchtung und Zelltheilung, 1878; Studien über Protoplasma, 1876. Pfitzer: Die Bacillariaceen. — Hanstein's Bot. Abhandl., I., Heft 2, 1871, p. 33. Nägeli: Das Mikroskop, 1877, p. 476. Ranvier: Histologie, 1875, p. 55. Robin: Microscope, 1877, p. 220.

of protoplasm. As its vapor attacks the mucous membrane of the eyes and air passages, the use of the osmiamid has been recommended, instead of the acid, which it replaces well in all reactions. Oil and fats reduce osmic acid, precipitating metallic osmium, which colors the oil drops brown or even black. Tannin is recognizable by the same reaction.

A one per cent. solution is especially valuable for the instantaneous hardening of living protoplasm. The stages in the division of the nucleus of cells, and other structural peculiarities of protoplasmic bodies are thus fixed in a few minutes, and, after washing, may be preserved for future study in a dilute solution of glycerine, which, however, has the disadvantage of rendering the preparations very transparent.

Recently perosmic acid has been used in the study of young meristem tissues.¹ The organs to be studied were laid in a very dilute aqueous solution (.1-1 per cent.) of the acid until they blackened, after which they were treated with alcohol, cleared with clove oil, and imbedded for sectioning in cocoa-butter (*butyrum cacao* of pharmacists). The sections were mounted in Canada balsam.

A mixture of nine parts of .25 per cent. chromic acid and one part 1 per cent. perosmic acid

¹ Parker: Journ. Roy. Microsc. Soc., 1879, II., p. 382. I have not tested the method.

color. Hydrochloric acid is further valuable for showing the nucleus of diatoms, etc.¹

Crystals based on carbonic acid emit bubbles of this gas, when treated with hydrochloric acid, the carbonates being converted into chlorides. On the other hand, crystals based on oxalic acid dissolve without effervescence.

Recently, Pringsheim² has employed hydrochloric acid as a reagent for hypochlorin, one of the components of chlorophyll bodies, *q. v.* Newly-cut sections are allowed to lie in the acid for several hours, when the hypochlorin separates as small semi-fluid exudation-masses, brownish or red in color, at first nearly spherical, but afterward forming needle-shaped crystals.

ORGANIC ACIDS.

ACETIC ACID.

This acid³ is used, in the form in which it is kept by pharmacists, in various micro-chemical investigations; *e. g.* it may replace hydrochloric acid in Hanstein's method for clearing opaque meristem (see the section on potassic hydrate, *Cf.* p. 11). The sections, first rinsed in water, are placed in a drop

¹ Pfitzer: Bacillariaceen. — Hanstein's Bot. Abhandl., I., Heft 2, p. 31.

² Monatsber. berlin. Akad., Nov. 1879.

³ Dippel: Das Mikroskop, I., p. 277; II., p. 10. Nägeli: Das Mikroskop, 1877, p. 476. Hanstein: Scheitelzellgruppe, 1868; Entwicklung des Keimes. — Bot. Abh., Bd. I., Heft 1, p. 5. Strasburger: Studien über Protoplasma, 1876, p. 5.

of acetic acid on the slide. With the neutralization of the alkali they often become somewhat opaque, but may be re-cleared, often to great transparency, by laying them in glycerine.

In the study of crystals, those composed of an oxalate may be distinguished by being insoluble in acetic acid, but soluble in hydrochloric acid, (p. 22); while salts of carbonic acid, occurring as crystals or as incrusting components of the cell-wall, are soluble with effervescence in either.

Acetic acid sharply differentiates the nucleus, and is often a valuable medium in the study of the intimate structure of protoplasm. Strasburger¹ employs a one per cent. aqueous solution for fixing the nucleus when staining the latter with methyl-green; at the same time it often clears up protoplasmic structures, and swells the condensed ectoplasm.

Acetic acid has also found application in cochineal solution, and in glycerine in which preparations stained with carmine are to be preserved.

OXALIC ACID.

The aqueous solution² is employed with certain coloring matters in staining tissues. The alcoholic

¹ Zellbild. u. Zelltheil., 1880, 3 ed.

² Dippel: Das Mikroskop, I., p. 285. Frey: Das Mikroskop, p. 77. Bachmann: Dauerpräparate, p. 27. Wiesner: Technische Mikroskopie, pp. 247, 258.

solution is useful for removing some of the color from too deeply stained sections. A concentrated aqueous solution is used as a test for pectose, which it dissolves after previous treatment of the section with potash.

CARBOLIC ACID (Phenol).

This has a very limited application in micro-chemistry.¹ Cell-walls that, after treatment with carbolic acid, assume a greenish-yellow or bluish-green color, when moistened with hydrochloric acid, are considered to be lignified, and phenol thus becomes a reagent for lignin (?).

Leitgeb² has employed a solution of carbolic acid in alcohol as a clearing medium in studying the histogeny of mosses.

Phenol should be added in small quantity to glycerine-jelly to prevent molding, and when dilute it may be used as a preservative for bacteria (Warming), which remain sharply defined, but become clear and homogeneous internally.

¹ Bot. Zeitung, 1877, p. 786.

² Nägeli: Das Mikroskop, 1877, p. 476.

ALCOHOLS.

ALCOHOL (Ethyl Alcohol).

The known disinfecting value of spirits of wine, or alcohol,¹ depends upon its fatal action on all protoplasm; hence its application as a preservative for animal and vegetable preparations. Absolute alcohol has the property, in common with perosmic acid, of rendering protoplasm rigid, and it is thus applicable in studying the more intimate structure of protoplasmic bodies, the division of the nucleus, etc. Its avidity for water causes the protoplasm to contract from the cell-wall, so that the ectoplasm becomes visible; and the same peculiarity is taken advantage of when we employ alcohol for hardening tissues that are too soft for section-cutting when fresh. It may also be used advantageously for removing the air from intercellular spaces, etc., in preparations, since it penetrates into capillary cavities much more readily than water does. In difficult cases warming the sections often helps this action, alcohol being added from time to time to replace that lost by

¹ Dippel: *Das Mikroskop*, I., p. 282. DeBary: *Vergl. Anat.*, p. 86. Nägeli: *Das Mikroskop*, 1877, p. 476. Sachs: *Bot. Zeit.*, 1864, Nos. 12-13. Weiss: *Allg. Botanik*, 1878, pp. 182, 185. Tangl: *Protoplasma der Erbse*.-Sitzber. wien. Akad., Abth. 1, 1877-8. Strasburger: *Zellbildung und Zelltheilung*, 1875, p. 2; *Befruchtung und Zelltheilung*, 1878, p. 38.

evaporation. When this expedient does not produce the desired effect, recourse should be had to the air-pump.

Alcohol is a solvent for volatile oils and resins, while fatty oils and vegetable wax are insoluble in cold alcohol, though it causes the oil globules to become confluent.

In tissues, *e. g.* nectaries, which contain much cane sugar, this may be forced by the use of absolute alcohol to separate in small stellate crystals soluble in water.¹

In all tissues containing inulin the prolonged action of alcohol effects its precipitation within the cell in the form of sphaero-crystals; *e. g.* in *Inula*, *Helianthus*, *Dahlia*, etc. Other sphaero-crystal-forming substances, such as hesperidin,² crystallize under the same treatment. (See Inulin and Hesperidin.)

Asparagin may also be detected by the use of absolute alcohol, the sections that are tested being alternately moistened with the reagent and allowed to dry, when the asparagin³ crystallizes out—often with other substances. It is recognized by its in-

¹ Bonnier: Les Nectaires. — *Ann. Sc. Nat.*, 1879, T. 8, pl. 8, figs. 124, 126.

² Prantl: *Das Inulin*, 1870. Rosenvinge: *Sfærøkrystaller hos Mesembrianthemum*. — *Nat. Foren. vidsk. Meddelelser*, 1877-78, p. 305, with table (with literature of the subject). Pfeffer: *Sachs' Lehrbuch*, 1874, p. 65. Russow: *Leitbündelkryptogamen*, 1872, p. 110.

³ Hartig: *Entwicklung des Pflanzenkeimes*, 1858. Pfeffer: *Ann. Sci. Nat., Bot.*, 5 Sér., T. XIX., p. 391. Sachs: *Lehrbuch der Botanik*, 1874, p. 689. Borodin: *Bot. Zeitung*, 1878, p. 803. Detmer: *Vergl. Physiol. d. Keimungsprocesses*, 1880, p. 171.

solubility in a warm solution of asparagin. The same treatment may, perhaps, be used with effect in testing for other substances; it has already been found satisfactory for tyrosin.

Alcohol is also employed as a solvent for a part of the reagents used in micro-chemical tests; *e. g.* anilin dyes, corrosive sublimate, phloroglucin, iodine, etc., as well as in the capacity of an anhydrating medium for preparations that are to be mounted in volatile oils or Canada balsam.

GLYCERINE.

This fluid,¹ especially useful as a preservative for permanent preparations, for which use I prefer the nearly anhydrous form known as glycerinum Wilsoni, is also employed for many other purposes. According to circumstances it is diluted with alcohol or water, or with both. Preparatory to final mounting it is often well to place preparations temporarily in a mixture of equal volumes of glycerine, distilled water, and absolute alcohol.

It is used like alcohol as an anhydrating medium in the study of protoplasm. It can be employed very successfully as a clearing medium in many cases; *e. g.* in studying the histology of the fibrovascular bundles, and as a preservative or final

¹ Kraus: Bot. Zeitung, 1877, p. 329. Sachs: Bot. Zeitung, 1864, Nos. 12, 13. Nägeli: Das Mikroskop, 1877, p. 475. Hegelmaier: Entwicklung dicotyledoner Keime, p. 11.

clearing fluid in the Hanstein and Russow methods of clearing tissues. A mixture of dilute potash and glycerine has been employed by Hegelmaier in the study of the embryo.¹

Kraus has employed glycerine as a reagent for sugar and inulin. When sections that contain these substances in solution are placed in glycerine, strongly refractive rounded drops appear in the cells. If inulin is present these drops change to the characteristic spaero-crystals, and remain; but if only sugar is present in the tissue they rapidly dissolve again. The sections should not be laid in water, but must be placed directly in the glycerine. The reliability of this reaction is certainly worthy of further tests. With larger masses of tissue, glycerine can also be used for the separation of inulin, and it is a good preservative medium for inulin preparations.

Iodin-glycerine is useful in the study of protein grains. It is prepared by dissolving a little iodine in glycerine, to which a small quantity of iodide of potassium has previously been added. More specific directions as to the relative quantities are unnecessary.

Warm glycerine is also used for the same purpose. In it the protein grains, which under natural conditions are uniformly refractive, become differ-

¹ [For the use of glycerine in the cellulose test, see sulphuric acid, p. 16. For its employment instead of potassium tartrate in Fehling's sugar test, see cupric sulphate, p. 36. w. T.]

entiated so that globoids and crystalloids are distinguishable.

Since the evaporation of glycerine is almost imperceptible, it is one of the best preservatives for permanent preparations; but as it absorbs the moisture of the air when concentrated, the cover-glass should be sealed with some air-tight cement.

ETHERS, ETC.

ETHER, BENZOL, CHLOROFORM, BISULPHIDE OF CARBON, ETHEREAL-OILS.¹

As reagents for determining to what extent a substance is a fat, a volatile oil, a resin, etc., substances have been employed which, from a chemical standpoint, are often quite different. They allow us to recognize these substances because of their solvent powers. For this purpose ether, chloroform, alcohol, benzol, oil of turpentine, and carbon bisulphide are used.

¹ Weiss: Allgemeine Bot., 1878, p. 178. DeBary: Vergl. Anatomie, p. 86. H. de Vries: Keimung des rothen Klees.—Landwirthschaftliche Jahrb., 1877, Bd. VI., p. 468. Nägeli: Das Mikroskop, 1877, p. 476. Dippel: Das Mikroskop, I., p. 374. Wiesner: Technische Mikroskopie, p. 81. For the use of carbon bisulphide as a reagent for sulphur, see this substance in Part II.

Resins are soluble in ether, cold absolute alcohol, carbon bisulphide, and oil of turpentine.

Fatty oils are soluble in carbon bisulphide, ethereal oils, hot alcohol, and ether. When treated with concentrated potassic hydrate they form soaps, which are soluble in water.

Ethereal oils are easily soluble in oil of turpentine and cold absolute alcohol. Most of them are also dissolved by ether and carbon bisulphide.¹

It will be seen that their behavior, when treated with alcohol, is distinctive for fatty and volatile oils; but for resins other reactions must be relied upon, which will be spoken of later.

The substances which dissolve fatty oils may also be used as reagents for wax.

With respect to the application of these reagents, we can only say that since carbon bisulphide, ether, oil of turpentine, and benzol are insoluble in water, the sections should be placed immediately in them to secure the best results. They cannot be prepared in water which is replaced by allowing the reagent to penetrate under the cover-glass, as with so many other reagents. The most convenient plan is to treat the sections in a watch-glass, with a considerable quantity of the reagent.

¹ Dippel (Mikr., I., p. 374) states that they are insoluble in ether, which I do not understand.

ETHEREAL OILS.

Several volatile oils beside turpentine, which has already been mentioned, find use in microscopy. Oil of cloves and lemon oil are especially useful as clearing fluids in the study of pollen. They are also good preservative media for objects which cannot be studied in water, but require a fluid of some other refractive index. Since they decrease the refraction they are very useful in the study of many strongly refractive substances, by the aid of polarized light. Preparations which have been in oil must be washed with ether or chloroform and afterward with alcohol before they can be placed in water or glycerine.

INORGANIC SALTS.

CHLORIDE OF SODIUM (Table Salt).

A dilute aqueous solution¹ is used as a morphological reagent for the contraction of protoplasmic bodies, a phenomenon which is to be attributed to its avidity for water. Many other salt-solutions have this property. A dilute solution of table salt

¹ Dippel: Das Mikroskop, I., p. 279.

has the power of dissolving crystalloids, at least in the embryo of *Bertholletia*.¹

CHLORIDE OF CALCIUM.

In an aqueous solution (two to three parts of water to one of the salt) this substance is sometimes used as a mounting medium for permanent preparations excepting those which contain amyllum, although for this purpose glycerine has largely replaced it. Recently it has found application for clearing tissues. The section which is to be treated is placed in a few drops of water and sprinkled with the dry pulverized salt. It is then warmed over a gentle flame until nearly dry, and again moistened with a few drops of water, after which it is laid in glycerine, where, in the course of a few hours, it acquires a very satisfactory degree of transparency. (Treub's method.)²

CHLORIDE OF MERCURY (Corrosive Sublimate).

A very dilute aqueous solution (1:100) is used to make the finest protoplasmic currents evident.³

¹ Weyl: *Zeitschr. für phys. Chemie*, Bd. I., p. 90.

² Treub: *Méristème primitif de la racine*. — *Musée de Leyde*, 1876, Tome II., p. 9. Eriksson: *Meristemet i dicotyla växters rötter*, p. 10. Flahault: *Accroissement terminale de la racine*. — *Ann. des Sc. Nat., Bot.*, 1878, p. 24.

³ Dippel: *Das Mikroskop I.*, p. 281.

Pfeffer¹ has used it in a two per cent. alcoholic tincture in the study of protein grains. It unites with these albuminoids, forming a compound insoluble in water; but to secure satisfactory results the preparation must lie at least twelve hours in the fluid.

CHLORIDE OF IRON.

An aqueous solution may be employed as reagent for tannin² when this is not present in too small quantity. The cells to be examined, when placed immediately in the reagent without the previous contact of water, which easily removes the tannin, assume a dark green or bluish-black color, according to the nature of the tannin compound. The green cells color yellow if potash is added.

The solution should not be too concentrated, as the tannate of iron which is formed is soluble in an excess of this compound, and its prompt solution renders the test less evident. For this reason acetate and sulphate of iron³ have largely replaced the chloride. Being more certain in their action, they are to be preferred.

¹ Pfeffer: *Jahrb. für wiss. Bot.*, 1872, VIII., p. 491. Weiss: *Allg. Bot.*, p. 140, note. Sachs: *Lehrb.*, 1874, p. 55. Duchartre: *Eléments de Bot.*, 1877, p. 102.

² Karsten: *Gesammelte Beitr. zur Anat. u. Phys. d. Pflanzen*, I., 1865, p. 253. Dippel: *Das Mikroskop*, I., p. 375. Wiesner: *Technische Mikroskopie*, p. 83. Weiss: *Allg. Botanik.*, I., p. 181; *Die Pflanzenhaare*, Karstens *Bot. Unters.*, I. Nägeli: *Das Mikroskop*, 1877, p. 475.

³ Cf. Link: *Grundlehrend. Anat.*, 1807, p. 80, where, to my knowledge, it is mentioned for the first time as a micro-chemical reagent.

CHLORATE OF POTASSIUM.

This reagent is used with nitric acid, either in a concentrated aqueous solution, or, better, in the crystalline form, for the destruction of the "middle lamella" and the consequent isolation of cells, especially in investigations of wood.¹ The mixture, the so-called Schultze maceration-medium, is boiled for a few minutes in contact with pieces of the tissue to be studied. After careful washing in alcohol, these macerated preparations may be preserved in glycerine. This process must be carried on at a distance from microscopes and other apparatus that can be injured by the gases developed.

The Schultze mixture has also been found useful as a reagent for suberin. Thin sections are boiled for a long time in it; all parts of the cell-wall soon become clear, but those which contain suberin possess dark and sharp contours and resist the action longer than the others, though finally they become distorted, abruptly swell, and melt to form rounded drops of ceric acid, which are soluble in ether, benzol, chloroform, caustic potash, and boiling alcohol. In the process a part of the suberin is dissolved by the reagent, only a part being changed into ceric acid. Membranes which are

¹ Sanio: Bot. Zeitung, 1863, p. 362, note; Anatomie der Kiefer.—
Jahrb. für wiss. Bot., IX. Höhnel: Ueber Kork.—Berichte der wiener
Akad., 1877, Abth. I. Schacht: Das Mikroskop, 1855, p. 27; Anatom. u.
Physiol. der Gew., I., p. 14. Dippel: Das Mikroskop II., p. 101. Nägeli:
Das Mikroskop, 1877, p. 474.

only slightly suberiferous will, therefore, hardly show this reaction; and to detect their suberin, the sections are placed in the cold fluid a few moments, and then removed to a solution of potash. The walls, which after the first treatment stand out sharply, assume an ochre-yellow color under the action of the potash—in all cases after being slightly warmed.

CUPRIC SULPHATE (Blue Vitriol).

This substance has a very extensive application in micro-chemistry.¹ It is always used in a tolerably concentrated aqueous solution, and must be chemically pure.

It is used in the following (Trommer's) test for sugar. A moderately thick section of the tissue which is to be studied is allowed to lie from two to ten minutes in a concentrated solution of the salt. The surface is then rapidly rinsed with distilled water, and the section transferred to a boiling mixture of equal parts by weight of water and potassic hydrate. Cells which contain cane sugar (*saccharose*) assume a bright blue color, while those

¹ Nägeli: Das Mikroskop, 1877, pp. 475, 525. Dippel: Das Mikroskop, I., p. 372; II., p. 20. Weiss: Allgem. Botanik, 1878, I., pp. 77, 171, 174. Sachs: Flora, 1862, p. 289; Jahrbücher für wiss. Bot., III., p. 187. De Vries: Keimung des rothen Klees.—Landwirths. Jahrb., 1877, VI., p. 468. Wiesner: Technische Mikroskopie, p. 79. Fresenius: Quantitative chemische Analyse, Braunschweig, 1853, p. 496. Fehling: Ann. der Chemie und Pharmacie, Bd. LXXII., p. 106.

which contain grape sugar (*glucose*) become clouded by the deposition of a finely granular or flocculent orange precipitate of reduced oxide of copper.

By this test we are thus able to determine which kind of sugar was present in the tissue. If the blue solution of cane sugar is boiled with dilute sulphuric or nitric acid it is changed into grape sugar, which then gives the red reaction.

Trommer's test also serves for the detection of *dextrine*. The contents of cells containing this sugar assume a vermilion color, while small granules in the precipitate exhibit the Brownian movement. If the dextrine is mixed with protein compounds, however, the precipitate is yellowish.

If in manipulation too much of the cupric sulphate has penetrated the cells the reaction is often masked by a precipitate of cupric hydrate. To avoid this difficulty Fehling's fluid, which gives the same reaction, may be used. It is prepared in the following manner. Dissolve 4 gm. of cupric sulphate in 16 gm. distilled water; and 16 gm. of potassium tartrate in the minimum of water. The two solutions are to be mixed. The reagent should be kept in the dark and needs frequent renewal.

[Prof. W. S. Haines has found in glycerine a very desirable substitute for the tartrate in Fehling's test. The proportions employed by him for qualitative examinations are: Cupric sulphate, 2

gm. ; potassic hydrate, 6 gm. ; pure glycerine, 7.5 cc. ; distilled water, 178 cc.¹ — W. T.]

Arabin (arabate of potassium) *Cerasin* (metagummate of potassium) and *Bassorin* do not reduce the Trommer reagent ; a dark blue precipitate only is formed, the flocks of which unite into balls when heated.

Protein compounds are sometimes recognized by the same tests, the contents of young cells assuming a beautiful violet color, though older cells fail to show the reaction.

Cell-walls which are not lignified are colored faintly blue by long soaking in an aqueous solution of cupric sulphate.

POTASH ALUM (Alum).²

An aqueous solution³ is employed as a mordant, in various staining processes, *e. g.* in Frey's haematoxylin and Grenacher's alum-carmine ; as an anhydrating medium ; or, finally, as an aid in the Hanstein method of clearing tissues, *q. v.*

POTASSIC NITRATE (Niter, Saltpeter).

This has been used in a one-fourth per cent. aqueous solution as a culture-fluid for the living

¹ Wheeler : Organic Chemistry, p. 187.

² The ordinary commercial alum, which is ammonium-alum, is quite as useful.

³ Frey : Das Mikroskop, p. 93. Bachmann : Dauerpräparate, p. 28. Hanstein : Scheitelzellgruppe, 1868.



tissues of higher plants during observations on the division of the nucleus.¹

MERCURIC NITRATE (Millon's reagent).²

This is made by dissolving mercury in its weight of concentrated nitric acid, and diluting with an equal volume of distilled water. It should be freshly prepared when required for use.

It causes the cell-wall to swell, and so renders its striation more evident, but its most important use is for the detection of protein compounds. These, after lying in it for some time, and especially after slight warming, assume a very red color. The surface membrane (Hautschicht) of protoplasm is slightly colored if at all. It should, however, be added that the reagent is not very sensitive, and the reaction is not always obtained (Nägeli).

CHLORIDE OF GOLD.

A one-half per cent. solution has recently been used in America for coloring the tissues of fungi. It requires from one to six hours to produce the

¹ Treub: Quelques recherches sur le rôle du noyau dans la division des cellules végétales, 1878, p. 9. — Naturk. Verh. d. koninkl. Akad., Vol. XIX., Amsterdam.

² Dippel: Das Mikroskop, I., p. 281; II., p. 18. Nägeli: Das Mikroskop, 1877, pp. 475, 527. Weiss: Allgem. Bot., I., pp. 77, 144. Millon: Ann. de Chim. et de Phys., 3 Sér., Tome 29, p. 507. Radlkofer: Ueber krystal. proteinart. Körper, Leipzig, 1859.

proper effect. Preparations stained with it may be mounted in dilute glycerine.¹

NITRO-PRUSSATE OF SODIUM.

This is useful in its aqueous solution for the detection of free sulphur.² It should be prepared when required for use. The crystals need to be kept from the air, from which they very readily take up water. The preparation to be tested is heated with potassic hydrate, after which treatment the granules of sulphur unite to form larger yellow masses, which are colored violet by the nitro-prussiate.

POTASSIC FERRO-CYANIDE (Yellow Prussiate).

An aqueous solution precipitates ferric salts, thus causing a blue color. This reaction has been taken advantage of for the detection of ferric hydrate in the cell-wall, *e. g.* in *Crenothrix*.³ Cells, the brown color of whose walls causes a suspicion of an encrusting compound of iron, are treated with a mixture of hydrochloric acid and the ferro-cyanide. If the beautiful color of Prussian blue appears the suspicion caused by the brown color is confirmed.

¹ W. Hassloch: *New York Med. Journ.*, Nov. 1878,—also *Journal Royal Microscop. Soc.*, 1879, Vol. II., p. 170. I have not tested this myself.

² Cohn: *Untersuchungen über Bacterien*, II.—*Beitr. zur Biol. d. Pfl.*, Bd. I., Heft 3, p. 175.

³ Cohn: *Ueber den Brunnenfaden*.—*Beitr. zur Biol. d. Pfl.*, Bd. II., Heft 1, p. 119.

SULPHO-CYANATE OF POTASSIUM.

An alcoholic solution is employed, sometimes in combination with hydrochloric acid, for the detection of iron in the cell-wall. (See the second section, Iron.)

POTASSIUM BICHROMATE.

An aqueous solution is used for the detection of tannic acid.¹ Masses of tissue of considerable size are left in the reagent for some time. Cells which contain tannin assume a reddish-brown color. The iron-reactions, however, are preferable.

It is also used for hardening resin masses.

NITRATE OF SILVER.

A very dilute alkaline solution in water has been employed for testing the living condition of protoplasmic bodies which, in this condition, contain aldehyde. According to Loew and Bokorny,² the discoverers of this reaction, the reagent is prepared as follows: 1. A one per cent. solution of nitrate of silver is made in distilled water. 2. A mixture of 13 cc. potassic hydrate solution (s. g. 1.333), 10 cc. ammonia (s. g. .964), and 77 cc. dis-

¹ Dippel: *Das Mikroskop*, I., p. 280. Sanio: *Bot. Zeitung*, 1863, p. 17. Hanstein: *Organe der Harz und Schleimabsonderung*. — *Bot. Zeitung*, 1868, p. 702. Nägeli: *Das Mikroskop*, 1877, p. 475. Weiss: *Allgem. Bot.*, I., p. 187, note.

² *Pflügers Archiv.*, 1881, XXV.; *Jahrb. wiss. Bot.*, 1882.

tilled water is made. Before using these solutions they are mixed, 1 cc. of each being taken, and the mixture diluted so as to make a liter. The mixture can be made only at the moment when it is to be employed; otherwise metallic silver is precipitated by the light.

This reagent colors living protoplasm black, while dead protoplasm remains uncolored. The reaction may be obtained with a solution diluted very greatly (even 1 : 1,000,000).¹

Tannic acid also gives a reaction, but this is effected only with a less dilute solution (1 : 10,000).

Glucose gives a reaction with a solution of 1 : 100,000, the cells coloring brown, and not black. In this proportion, therefore, nitrate of silver is an excellent reagent for this sugar.

¹ These very dilute solutions cannot be employed in as small quantity as micro-chemical reagents usually are. To obtain good results it is necessary, according to Bokorny, to immerse a few cells (*e. g.* of *Spirogyra*) in a large quantity (.5—1 liter) of the reagent for six to twelve hours.—*French Translator.*

ORGANIC SALTS.

ACETATE OF IRON

Is used in an aqueous solution, as has been described for the chloride.

ACETATE OF COPPER (Verdigris)

Is used as a means of recognizing resins. Masses of tissue are soaked five or six days in an aqueous solution of the salt, after which treatment the resinous masses assume an emerald green color.¹

SULPHATE OF ANILIN.

An aqueous solution of this substance, the so-called Wiesner anilin reagent, is used for the detection of lignin, the constituent of wood.² The sections are first placed in a dilute solution of the sulphate until they are well saturated with it; even with no other treatment, lignified membranes often assume a faint yellow color, which, however, is

¹ Franchimont: Origine et constitution chimique des résines de terpènes. — Cf. Archives Néerlandaises, 1871, T. VI., p. 427, with Pl. 8.

² Wiesner: Technische Mikroskopie, 1867, p. 64. Karsten: Bot. Untersuchungen, I., p. 120, note. Burgerstein: Sitzungsber. der wiener Akad., 1874, Bd. LXX., Abth. I., p. 338. Höhnel: Ueber Kork, etc.—Sitzungsber. der wiener Akad., 1877, Bd. LXXVI., Abth. I., p. 21.

intensified by transferring the section to dilute sulphuric acid. If it is desired, the reagents may be mixed before using.

Since this anilin salt is found in the market only in a very impure and barely soluble form, it is better to replace it by the next.

CHLORIDE OF ANILIN

Is used in aqueous solution for the same purposes as sulphate of anilin, and in the same manner, except that the acid used must be hydrochloric.¹ An alcoholic tincture of either anilin salt may replace the aqueous solution, and the colors produced are then more intense.

CHLORAL.

This has been recently introduced into micro-chemistry by Meyer.² It is employed in aqueous solution: five parts of chloral to two parts of water; and should be used at a temperature of 15° C., as crystals are precipitated at lower temperatures. Its effect upon fats and volatile oils is similar to that of alcohol. It dissolves the same saccharine and amylaceous matters (Kohlenhydrate) as water, and causes the swelling of starch grains. It swells or dissolves protein matters, and is, therefore, frequently useful in clearing tissues.

¹ Höhnel: Ueber Kork, etc., p. 21.

² Arthur Meyer: Das Chlorophyllkorn, Leipzig, 1883.

OTHER ORGANIC COMPOUNDS.

SOLUTION OF CANE SUGAR (Syrup).

Vegetable cells containing much sugar often assume a beautiful red color on the addition of sulphuric acid. This fact has been utilized in obtaining a reaction for protoplasmic substances. The tissues to be tested are first saturated with a solution of cane sugar and water. On the addition of sulphuric acid the red color manifests itself. This reaction (Raspail's)¹ is, however, often difficult to obtain, and not very good. The sections have to be left in the acid for some time before the color appears.

Syrups of different degrees of concentration also find application as anhydrating media.² A three per cent. solution had been used as a fluid of preparation in the study of transparent ovules, *e. g.* embryo sac of *Monotropa* and *Orchis*; and a five per cent. solution has been recommended for pollen-cultures under the microscope.

¹ Raspail: *Chimie organique*, 1839, II., p. 139. M. Schultze: *Ann. der Chem. u. Pharm.*, Bd. LXXI., p. 270. (Second discovery of the reaction.) Schacht: *Mikroskop*, p. 27; *Pflanzenzelle*, pp. 27, 28; *Anat. u. Physiol. der Gewächse*, I., pp. 46, 61. Dippel: *Mikroskop*, I., p. 283. Nägeli: *Mikroskop*, 1877, pp. 476, 526. Frey: *Mikroskop*, p. 73. Hofmeister: *Pflanzenzelle*. — *Handb. der phys. Bot.*, I., p. 2. Weiss: *Allg. Bot.*, I., p. 77. Duchartre: *Éléments de Bot.*, 2 ed., p. 25.

² Strasburger: *Befruchtung u. Zelltheilung*, pp. 16, 29, 52, etc.

ASPARAGIN.

Borodin¹ has recommended a tepid concentrated aqueous solution for the detection of asparagin, which has been precipitated in the tissue by alcohol (p. 27), on the principle that a crystallized substance is insoluble in a saturated solution of the same substance.

Asparagin is prepared by evaporating the well-boiled and filtered sap of young seedlings of leguminosæ (especially lupines) which have germinated in the dark, or by the evaporation of the dialyzed aqueous decoction of althæa root; when it separates as crystals.

DIPHENYLAMIN.

This officinal substance has of late been used as a reagent for nitrates and nitrites.² The sections to be tested are allowed to dry on the slide and afterwards moistened with a solution of .01-.1 gm. of diphenylamin in 10 cc. pure sulphuric acid. Even very small quantities of compounds containing nitric acid are indicated by the appearance of a dark blue color in the cells; the reaction is very distinct.

¹ Borodin: Bot. Zeitung, 1878, p. 804, *et seq.* Detmer: Physiol. u. Keimung d. Samen, 1880, p. 171, *et seq.*

² Molisch: Ueber den Mikrochemischen Nachweis von Nitraten und Nitriten etc.—Ber. d. deutschen bot. Ges., 1883, I., p. 150.

BRUCIN

Has been introduced into micro-chemistry for the same purpose as diphenylamin, but it does not serve so well for the detection of small quantities of nitrates and nitrites. Molisch¹ recommends a solution of .2 gm. brucin in 10 cc. pure sulphuric acid. A dry section of a tissue which contains $N_2 O_5$ assumes a bright red or reddish-yellow tint when placed in a drop of the solution.

INDOL.

This substance has been employed quite recently by Niggel as a reagent for lignin, or lignified membranes.² The discovery of this reaction is due to Professor Baeyer, of Munich.³ Several crystals of indol are dissolved in a sufficient quantity of warm distilled water. The sections to be tested are placed in a drop of the reagent for several minutes, after which they are washed in dilute sulphuric acid (1 pt. : 4 pts. water). Lignified cell-walls assume a very intense red color.

PHLOROGLUCIN.

One of the prettiest and best reactions of micro-chemistry has recently been discovered by Wiesner ;

¹ Molisch : Ber. deutsch. bot. Ges., 1883, I., p. 150.

² Niggel : Flora, 1881, No. 35, p. 545.

³ Ann. d. Chem. u. Pharm., Bd. CXL.

viz. the phloroglucin test, for lignin. An aqueous solution or, better, an alcoholic tincture of the reagent is employed, and even in excessively small quantity induces the reaction.¹ The section to be studied is treated with hydrochloric acid, and then placed in a drop of phloroglucin on the slide. The parts containing lignin assume a beautiful and intense rose-red color with a rapidity depending upon the concentration of the solution. The preparations may be kept for a considerable time. If difficulty is experienced in getting the reagent—and at present it is quite expensive and hard to obtain—an extract of cherry wood diluted with water may replace it. This contains the substance in question among others, but gives a more violet reaction than the pure reagent.²

ROSOLIC ACID

Is recommended by Janczewski³ as “the best of the reagents which color the callosities of sieve tubes.” A little ammonia or sodic carbonate should be added to it.

¹ Wiesner: Sitzungsber. der wiener Akad., Bd. LXXVII., Abth. I., Januarheft.

² This has been called the xylofilin reaction by its discoverer, Höhnel.—Sitzungsanzeiger d. wiener Akad., 1877, No. 23, pp. 228, 229.

³ Études comparées sur les tubes cribreux.—Mém. de la Soc. des Sc. nat. de Cherbourg, 1881, XXIII., p. 350. The discovery is due to Szyszyloviez.

COLORING AGENTS.

Lately various dyestuffs have been employed for the differentiation of the tissue systems, as well as for the recognition of some of the cell contents; of these only the most important will be mentioned.

TINCTURE OF ALCANNA.

A red dye, extracted by alcohol from the root of *Alcanna tinctoria*, is employed for coloring resins, which have a special avidity for this substance. Protoplasm is also colored pale red by it.¹ Preparations colored by alcanna do not endure drying.

COCHINEAL.

An aqueous extract of cochineal is employed with acetic acid or alum, for staining the prosenchyma (bast cells) of the phloem in fibro-vascular bundles. After lying in the dye for some time, these cells assume an intense red color, while the other elements are not affected, or but slightly colored. Yet there are certain kinds of wood which imbibe the cochineal extract, but after-treat-

¹ N. J. C. Müller: Untersuchungen über die Vertheilung der Harze. — Jahrbücher für wiss. Bot., V., p. 387. Hanstein: Organe der Harz- und Schleimabsonderung. — Bot. Zeitung, 1869, pp. 707, 708.

ment with hydrochloric or sulphuric acid removes the color from everything except the bast cells, in which it becomes more intense.

As a coloring agent in the investigation of protein bodies, this substance has also found use.¹

CARMINE.

A solution of carmine in dilute potash, such as is sold by dealers, is used for staining the nuclei of cells.² The solution, which should contain very little undissolved carmine, is filtered and then mixed with alcohol or glycerine in various proportions. The object requires to lie in the solution for some time. Only the nucleus (and protein grains) imbibe the color.

The carminate of ammonium (ammonia carmine) is, however, more commonly used.³ It is prepared in the following manner, suggested by Hartig. Carmine powder is dissolved in a strong solution of ammonia until this is saturated; the solution is evaporated to dryness over a water bath, and the carminate thus formed is prepared for use by solution in water.

¹ Wigand: Bot. Zeitung, 1862, pp. 129, 139. Maschke: Bot. Zeitung, 1859, p. 22. Vogl: Anat. u. Histol. der unterirdischen Theile von *Convolvulus arvensis*. — Sitzungsber. d. wiener Akad., 1863, XIII.

² Hartig: Der Füllkern, etc. — Karsten's botan. Untersuchungen, I., p. 282, note.

³ Dippel: Das Mikroskop, I., p. 184. Frey: Mikroskop, 1873, pp. 87, 88, 90. Bachmann: Dauerpräparate, p. 26. Tangl: Protoplasma der Erbse. — Sitzungsber. der wiener Akad., Bd. XXVI.-XXVIII.

Thiersch gives the following formula for its preparation. One part of carmine is dissolved in one part (by weight) of concentrated ammonia and three parts of distilled water. This solution is mixed with eight times its volume of dilute oxalic acid (1:22). Twelve volumes of absolute alcohol are then added, and the whole is filtered. The filtrate may be rendered more orange in color by the addition of oxalic acid, or more violet by the addition of ammonia. If oxalate of ammonium is precipitated, it can be removed by filtration or redissolved by the addition of a few drops of ammonia.

Grenacher's alum-carmine, recently introduced into vegetable histology by Tangl,¹ is prepared by him in the following manner. A saturated aqueous solution of alum is made. The desired quantity of carmine is dissolved in it, the solution is boiled for about ten minutes, allowed to cool, and filtered. Walls consisting of cellulose are colored bright-red by this preparation, while those containing suberin or lignin remain unstained. Protoplasmic bodies and the nuclei of cells are also stained with difficulty, and but slightly. It is recommended that parts of plants from which sections are to be cut, shall be hardened in absolute alcohol, as this increases the power of the membranes to take up the coloring matter.

¹ Tangl. Ueber offene Communication zwischen den Zellen des Endosperms. — *Jahrb. für wiss. Bot.*, 1880, XII., p. 170. Grenacher: *Archiv. f. mikr. Anatomie*, 1879, p. 465; *Zeitschrift f. Mikroskopie*, 1879, Jahrg. II., p. 55.

If the preparation should be too deeply stained by this carmine fluid, it can be bleached in an alcoholic solution of oxalic acid.

Carmine stains all protoplasm if allowed to act a sufficient length of time. The nuclei of cells are most deeply colored. Living protoplasm does not imbibe the coloring matter. This occurs only after it has been killed by the addition of the reagent. In general it may be said that it colors most vegetable albuminoids, while starch and cellulose take it up in much smaller quantity or not at all.

[The double-staining of clear sections, usually bleached by the action of Labarraque's solution, or some similar fluid, is capable of yielding very good results where convenience of demonstration in the class-room is concerned. As good directions as any are those of Dr. Rothrock,¹ who uses Woodward's ammonia carmine and the aniline color known as iodine-green.

The section is placed in alcohol faintly colored by the addition of a few drops of a concentrated tincture of the green, where it is allowed to remain from twelve to twenty-four hours, according to circumstances. It is then successively passed through a series of fluids in the following order: one, water; two, carmine; three to five, alcohol; six, absolute alcohol; seven, oil of cloves: being merely

¹ Rothrock: Staining and double-staining of vegetable tissues.—*Bot. Gazette*, Sept. 1879, Vol. IV., pp. 201-6.

dipped into the water, remaining from twenty seconds to a minute in the carmine, being well rinsed in the first watch-glass of alcohol, and staying ten to twenty minutes in each of the others. It is left in the oil of cloves until cleared up, when it is ready for mounting in balsam. The general experience of teachers seems to be, however, that more time is consumed in making the few successful preparations than they are worth. — W. T.]

Beale's carmine, which is especially useful in differentiating the nucleus, is prepared by dissolving .6 gm. carmine in 2 gm. boiling ammonia water. The solution is set aside for an hour to allow some of the ammonia to escape. Sixty gm. distilled water, 60 gm. glycerine, and 15 gm. absolute alcohol are then added. After standing for some time, the fluid is filtered and ready for use.¹

Strasburger, in the study of the embryo sac, stains the protoplasm with a boracic solution of carmine, prepared as follows: Four parts borax are dissolved in fifty-six parts distilled water. To this one part of carmine is added. One volume of this solution is diluted with two volumes absolute alcohol and filtered. By the use of this dye the study of the forms of the nucleus is greatly facilitated. The preparations may be preserved in glycerine or glycerine jelly.²

¹ Frey: Mikroskop, p. 90.

² Strasburger: Zellbild. u. Zelltheilg., 1880, p. 9.

Czokor recommends the following coloring fluid: 7 gm. cochineal are pulverized with an equal quantity of calcined alum. This is dissolved in 700 gm. distilled water, and the whole is boiled down to about 400 gm. After it is cool a drop of carbolic acid is added, and the fluid is filtered. It should last at least six months without deterioration. At the end of this time it should be re-filtered after the addition of another drop of carbolic acid.¹

PICROCARMINATE OF AMMONIUM (Picrocarmine).

This staining agent, which is much used by students of animal histology, is employed in botanical micro-chemistry, chiefly for differentiating the nucleus.² It is prepared by adding a strong solution of ammonium-carminate to a concentrated aqueous solution of picric acid, until this is neutralized. After evaporating it to four-fifths its original volume, it is set aside for a time, and then filtered, when the dark orange fluid is ready for use.³ Another method has been recommended by Gage. Equal parts by weight of picric acid and

¹ Czokor: Die Cochenille Carminlösung. — Arch. f. mikr. Anat., 1880, XVIII., p. 412 *et seq.* — See also Bot. Centralblatt, 1880, p. 1280.

² Treub: Actes du congrès international à Amsterdam, 1877, Leyden, 1879, p. 146.

³ Frey: Mikroskop, p. 91. Bachmann: Dauerpräparate, p. 27. Treub: Rôle du noyau dans la division des cell., 1878, p. 23. Pelletan: Le Microscope, 1870, p. 207. Gage: American Monthly Micr. Journ., 1880, p. 22; Journal of the Royal Micr. Soc., 1880, Vol. III., p. 501.



carmines are dissolved; the former in one hundred parts of water, the latter in fifty parts of concentrated ammonia. The solutions are then mixed and filtered, evaporated to dryness, and the residue is redissolved in a hundred times its weight of water.

Protoplasm is colored a yellowish-red by it. The nucleus quickly assumes a deeper color, especially after very short action of the coloring matter. The best degree of concentration is a one per cent. solution. Maupas recommends the use of alcohol, picro-carmines, and glacial acetic acid for staining the nucleus.¹

[Mayer's picro-carmines² is prepared as follows:—
“To a mixture of powdered carmines (2 g.) with water (25 cc.), while heating over a water-bath, add sufficient ammonia to dissolve the carmines. The solution may then be left open for a few weeks in order that the ammonia may evaporate; or the evaporation may be accelerated by heating (Hoyer). So long as any ammonia remains large bubbles will form while boiling, but as soon as the free ammonia has been expelled the bubbles will be small, and the color of the fluid begin to be a little lighter. It is then allowed to cool, and filtered. To the filtered solution is added a concentrated aqueous solution of picric acid (about four

¹ Maupas: *Comptes rendus*, July 1879, No. 4, p. 250.

² Mayer: *M. T. Zool. Stat. Neapel*, 1880, II., pp. 1-27. — *Journ. Roy. Mic. Soc.*, Dec. 1882, Ser. 2, Vol. II., pp. 876-7.

volumes of the acid to one of the carmine solution). The addition of the acid should cease before a precipitate begins to form. In order to protect this fluid against changes attributed to bacteria by Hoyer,¹ Dr. Mayer places a small crystal of thymol in the containing bottle; Hoyer uses chloral-hydrate (1 per cent. or more) for the same purpose."

Weigert² prepares the reagent by the following process: "Over 2 gm. of carmine are poured 4 gm. common ammonia, and the whole left twenty-four hours in a place protected against evaporation; 200 gm. of a concentrated picric acid solution are then poured in; the mixture is left twenty-four hours, until all soluble matters are dissolved. Very small quantities of acetic acid are then added, until a slight precipitate comes down even after stirring; a rather copious precipitate is usually thrown down in the course of the next twenty-four hours; it should be removed by filtration. A picro-carmine which does not stain readily may be improved by the addition of acetic acid."—W. T.]

HÆMATOXYLIN.³

This substance is the active principle in the extract of logwood, but is not found in great quantity in the *tinctura ligni campeschiani*. It may be

¹ Hoyer: Beitr. z. histol. Technik. — Biol. Centralblatt, 1882, II., pp. 17-19.

² Arch. pathol. Anat. (Virchow), 1881, Vol. 84, pp. 275-294. — Journ. Roy. Micr. Soc., Feb. 1883, Ser. 2, Vol. III., p. 139.

³ Frey: Mikroskop, p. 91. Pelletan: Le Microscope, p. 209. Ranvier:

obtained in the market ready prepared. The staining fluid is made by dissolving .35 gm. hæmatoxylin in 10 gm. water. To this are added a few drops of an alum solution (which acts as a mordant in fixing the color), made by dissolving 3 gm. alum in 30 gm. water. This makes a beautiful violet fluid, which colors the nucleus deep blue. It is the best staining fluid that is now known for the nucleus.¹ Preparations need to lie in it for some time. They may be preserved in glycerine.

I have used with success the method of staining bacteria first published by Koch. The dried preparation is treated with a concentrated extract of Campeachy-wood in water. After removing the superfluous dye with distilled water, the color is fixed by the use of dilute chromic-acid. The preparation can be preserved, after drying, in glycerine or Canada balsam. Cilia and the bodies of the cells are sharply differentiated by this method.² Koch has later recommended coloring with hæmatoxylin. Staff-shaped bacteria, however, do not color by this substance, according to him.³ I have however used the hæmatoxylin tincture with success even on certain staff-shaped bacteria. After rinsing, my preparations are preserved dry.

Histologie, p. 103. Robin: *Microscope*, 1877, p. 250. Bachmann: *Dauerpräparate*, p. 28. Schmitz: *Niederrhein. Gesellsch.*, Nov. 1879.

¹ Johow: *Zellkerne d. höheren Monocotylen.*, Diss., Bonn, 1880, p. 9, note.

² Koch: *Conserviren und Photographiren der Bacterien.*—Cohn's *Beitr. z. Biol. d. Pfl.*, II., p. 421.

³ *Wundinfektionskrankheiten*, 1878, p. 30.

NIGROSIN.

[Errera recommends¹ this tar derivative for staining the nucleus. It is soluble in water, but insoluble in alcohol and ether. After remaining a short time in a solution of nigrosin, the section is transferred to water, where it remains until no more of the color is removed, when it may be mounted in glycerine or glycerine jelly, or transferred to alcohol, and afterward cleared in oil of cloves and mounted in balsam. The latter medium serves best for specimens intended to show the chromatin; while the former are preferable for those intended to show the achromatin of Flemming. — W. T.]

EOSIN.

This beautiful rose-colored derivative of phthalic acid has a pronounced green fluorescence. It is employed in aqueous [or alcoholic] solution. Even a very small quantity has great coloring capacity.² It has been used for staining *Sarcina* and *Sarcinoglobulus*. It does not appear adapted for use on bacteria (*Bacillus*, *Bacterium*, etc.). [I have succeeded in obtaining fair preparations of *Bacillus subtilis* by staining in alcohol-eosin, and

¹ Procès-verbal de la séance mensuelle du 25 Juin, 1881, Soc. belge de Microscopie, p. CXXXIV.

² Poulsen: Om nogle mikroskopiske Planteorganismer. — Nat. Foren. vidsk. Meddel., 1879-80, p. 235. Preparations stained with eosin may be preserved in glycerine.

mounting in balsam. — W. T.] In the tissues of higher plants, where its effect has not yet been fully studied, it stains dead protoplasm a beautiful rose color. Eosin is especially useful for coloring the plasma of sieve tubes and the nuclei of cells. It has also been used as the first dye in double-staining preparations, which are afterwards treated with Nicholson's blue, fixed with absolute alcohol and mounted in dammar.¹

ANILINE COLORS.²

Within a few years the beautiful aniline colors have been generally applied in many histological researches. In none, however, are they more useful than in the preparation of bacteria, not only in sections of animal tissues, but also in films obtained by drying (and hardening through a flame) a thin layer of fluid containing these organisms on the cover-glass. The discovery, by Weigert, in 1871, of the power of carmine to color bacteria³ led him to try the effect of the aniline salts, in which a very energetic coloring matter — almost a reagent for bacteria — was found. The names

¹ Journ. of Roy. Micr. Soc., 1880, III., p. 693.

² Wigand: Bot. Zeitung, 1862, p. 17. Hanstein: Organe der Harz- und Schleimabsonderung in den Laubknospen. — Bot. Zeitung, 1868, p. 708.

³ The "sulphate of rosanilin" has been strongly recommended by Salomonsen for staining bacteria in putrid blood. It is used in a concentrated aqueous solution prepared by heating, and filtered after it has become cold. — Cf. Studier over Blodets Forraadnelse, Copenhagen, 1877, p. 15.

of Ehrlich and Koch are also well-known in connection with the further improvement of this method of research.

In the following paragraphs only some of the most useful aniline dyes will be mentioned; their number is daily increasing. In general it may be stated, that all preparations stained by aniline colors should be carefully washed before being mounted; they must also be kept in the dark, as most of these colors soon fade in the light.

FUCHSIN,

In an alcoholic tincture, colors especially well thickened cell-walls; the different layers often with different intensity. Sections which are to be stained should be free from potash, which destroys the color, and they must be treated in alcohol, the addition of water precipitating the dye. Stained preparations remain unchanged only for a limited time.

Fuchsin has recently been applied by Ehrlich¹ similarly to vesuvin, with methylene blue, for the staining of the *Bacillus* of tuberculosis.

HANSTEIN'S ANILINE VIOLET

Is prepared by mixing about equal parts of methyl violet and fuchsin, and dissolving them in alcohol. Its action depends upon the different avidity of

¹ Ehrlich: *Zeitschr. f. klin. Medicin*, 1882, II.

the various substances which are found in the tissues or cells for the mixed colors.

Protoplasm is stained violet-blue. Amyloid substances, the nucleus and gums assume various shades of red; the membrane of the nucleus stains bluish; resins, pure blue (the cuticle also colors blue in many colleters). Tannin assumes a foxy color; the cell-wall stains pale violet, deeper if it contains lignin, reddish if it is more gelatinous. Bast cells are stained a deep red; sieve tubes and the soft bast do not assume any intense color, which is of advantage in the study of the fibrovascular bundles of endogens.

METHYL VIOLET

Has been recommended as a staining agent for bacteria by Koch,¹ whose methods we give.

A few drops of a concentrated tincture of methyl violet are added to 15 to 20 gm. of distilled water, so that this is deeply colored. With a small pipette a couple of drops of this are placed upon the film of bacteria to be colored, where the fluid is allowed to flow back and forth until it is thought that the specimen is sufficiently stained. After a little practice it is easy to determine the proper concentration for the fluid, and the length of time it requires to act. If it is too weak the bacterian film loosens from the glass, while if it

¹ Koch: Cohn's Beitr. z. Biol. der Pfl., II., p. 406.

contains too much of the coloring matter the mass in which the bacteria lie stains too deeply. After the process of staining is finished, the preparation is rinsed with distilled water, or with a ten per cent. solution of potassic acetate.

After lying for half an hour exposed to the air, the slide is ready for mounting in balsam. Glycerine cannot be used, as it removes the color. Preparations which are to be photographed should be mounted in a fifty per cent. solution of potassic acetate and sealed air-tight.

The coloring matter is so quickly taken up by the bacteria that we have in it a useful reagent for these organisms, which might be easily confounded with small oil globules or other very minute rounded bodies.

ANILINE BLUE.

Wilhelm¹ and Russow² have recommended an aqueous solution of this dye for staining the callous-plates of sieve-tubes. After the sections have been submitted to the action of the dye they are rinsed in water. The protoplasm colors violet-blue; the nuclei, deep indigo. Cellulose membranes assume a blue color, while the callous-plates become azure. Preparations mounted in glycerine change in a few days, so that only the

¹ Wilhelm: Siebröhren, 1880, p. 36.

² Russow: Sitzber. d. naturf. Ges., Dorpat, 1881, p. 63.

nuclei and callous-plates remain colored. The latter are very evident, because of their considerable refractive power. Such preparations remain unchanged for several months.

I can recommend this blue for giving a very intense color to bacteria. It is used precisely like methyl violet. The preparations should be mounted in pure Canada balsam, as the chloroform solution removes the coloring matter.

[More recently methyl blue has been used for detecting the *Bacillus* of consumption. The tuberculous matter is dried in a thin film on a cover-glass in the usual way, and then floated for twenty-four hours on a fluid composed of 1 part saturated methylene blue in alcohol, 2 parts 10 per cent. potash, 200 parts distilled water. After rinsing it is submitted to the short action of a few drops of vesuvin. Only the bacteria retain the blue color. — W. T.]

ANILINE BROWN

Is used in the same way as methyl violet.¹ It is better than the latter for specimens which are to be photographed; but the preparations must be mounted in glycerine, since the color is removed by potassic acetate. A concentrated solution in equal parts of glycerine and distilled water is recommended as a staining fluid, the superfluous color being washed away with glycerine.

¹ Koch: Cohn's Beitr., II., p. 406.

ANILINE GREEN (Methyl Green)

Has been recommended by Hanstein for staining chlorophyll grains, to which it imparts a deep green color, different from their natural tint.

Treub¹ recommends it for staining the nucleus. Nuclei which are not in process of division are colored pale green. In those which are dividing the so-called nuclear-plates assume an evident green color. Strasburger² has used it for the same purpose in combination with 1 per cent. acetic acid.

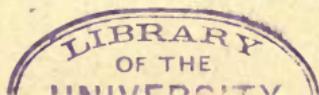
[For the use of iodine-green in double staining cell-walls for convenience in class demonstrations, see Ammonia Carmine.]

MAGENTA.

[Dr. Gibbs recommends magenta and chrysoidin for staining consumptive sputum. Three fluids are required: A — Magenta crystals, 2 gm.; pure anilin, 3 gm.; alcohol (s.g. 830), 20 cc.; distilled water, 20 cc.; B — A saturated solution of chrysoidin in distilled water, to which a crystal of thymol has been added to prevent deterioration; C — Dilute nitric acid (1:2). The sputum, after being thoroughly dried on the cover-glass, is floated on A for 15–20 minutes, then washed in

¹ Treub: Sur des cellules végétales à plusieurs noyaux.—Archives Néerlandaises, 1880, T. XV.; pp. 7, 17 of the Separate.

² Strasburger: Zellbild. und Zelltheil., 3d ed., 1880.



C till the color disappears, rinsed in pure water, which restores a little of the color, floated on B a few moments, transferred to absolute alcohol, and finally dried and mounted in Canada balsam. The Bacillus of consumption is stained by the magenta, which does not color putrefactive bacteria, and so differentiated in the brown sputum.¹
— W. T.]

The above coloring matters are those which are most commonly employed in micro-chemistry, and which give the best results. There are some others which are used for various purposes, but they may be omitted here.

¹ The Lancet, Aug. 5, 1882.

APPENDIX TO PART I.

MOUNTING MEDIA.

Before this section is closed the most important substances used in mounting botanical specimens should be noticed. In many cases the student must learn by trial the best medium for a preparation. Still there are a number of substances which are known to be so well adapted for the preservation of very different objects that they should be tried first.

GLYCERINE is excellent for nearly all botanical preparations. The Florideae and Diatoms, however, usually require preservation in other media; the former since their cell-walls often swell greatly in this fluid, especially if they have not been previously anhydrated with absolute alcohol; the latter because their structure does not appear in it with the required degree of distinctness. Bacteria, also, become so transparent in glycerine, and in particular if they have not been stained, as to be almost indistinguishable.

[FARRANT'S SOLUTION, which is employed somewhat in animal histology, is a good substitute for glycerine in mounting many vegetable tissues. Frey's formula is: Equal parts of gum arabic, glycerine, and a saturated solution of arsenious acid. If the slide is allowed to lie a day or two

before being sealed, the gum hardens at the edge of the cover, and so aids in fastening it. This medium does not render sections so transparent as pure glycerine.

GLYCERINE AND ACETIC ACID in equal parts make a convenient preservative for many fungi and other preparations. The fluid should be boiled and filtered to remove mold spores and other impurities.

Like other fluid media, this requires the employment of cells of some sort,—the usual ring of asphalt or the wax cell, if firmly fixed, answering very well. In covering such a cell it is best to lower the cover gradually from one side, so that any superfluous fluid which is forced out shall penetrate between the cell and cover-glass only at one side, where it must be carefully wiped off with blotting-paper before the cell is sealed.

A neat and useful cell may be made by placing three small balls of white wax on the slide, at equal distances apart, just within the line where the edge of the cover is to come, and flattening them to the requisite thickness by pressing the slide against any flat body covered by a piece of muslin or paper. A drop of the mounting fluid is put in the centre, and the object arranged in it as it is to remain, after which the cover-glass is slightly warmed, placed in position, and gently pressed against the wax supports by the handle of a dissecting needle or other convenient object. Any

fluid which has escaped beyond its edge is neatly removed with bits of bibulous paper, and a moderately-heavy ring of benzole-balsam is painted round the cover. When this is dry a coating of asphalt may be added to toughen the cell if desired. With a little practice one learns to estimate the quantity of fluid needed in a given case, so that very little is forced out, and, the slide being perfectly dry and clean, except at the one point of escape, the cell which is built up adheres well. — W. T.]

GLYCERINE JELLY. — Nordstedt's¹ directions for the preparation of this substance (for mounting algæ) are as follows: One part of pure gelatine is dissolved in three parts of hot distilled water and four parts of glycerine. To prevent moulding, a small piece of camphor or a drop of carbolic acid is added. The mass hardens on cooling, and must be slightly warmed when needed for use.

Kaiser² gives the following recipe: One part by weight of the best French gelatine [Cox's gelatine is equally good] is soaked in six parts of distilled water for two hours, seven parts of chemically pure glycerine are added, and 1 gm. of carbolic acid is added to 100 gms. of the mixture. The whole is then heated ten to fifteen minutes, mean-

¹ Om anvændandet af gelatinygerin vid undersökning og preparering af Desmidieer. — Botaniska Notiser, 1876, No. 2.

² Botanisches Centralblatt, 1880, No. 1, p. 25. — Cf. also Glycerin-Gelatine for Mounting. — Journ. of Royal Micr. Soc., 1880, Vol. III., p. 502.

time being constantly stirred, until the fluid has become clear, after which it is filtered through glass wool.

This glycerine jelly, which, in a thin layer, is completely transparent and as clear as water, is useful for preparations that are to lie immovably under the cover-glass, but which are too small to be held by the pressure of the latter. Pollen grains, starch, yeast-cells, spores, and especially unicellular algæ, *e. g.* Desmidiacæ, should be mounted in this medium. If it is desired to preserve the structure of the protoplasm and the arrangement of the chlorophyll bodies as far as possible, the plants must be previously hardened by immersion in an aqueous solution of perosmic acid (1 : 800) or in absolute alcohol. After being hardened the preparations are placed in dilute glycerine before being mounted in glycerine jelly. When this is cold the cover-glass is sealed.

[Taking advantage of the insolubility of gelatine which has been acted upon by potassium bichromate and exposed to the light, Dr. Goodale has recommended lightly painting the edge of the cover-glass with a solution of this salt in place of the usual ring of varnish. — W. T.]

CHLORIDE OF CALCIUM is often used in aqueous solution for many kinds of preparations, — starch, however, excepted. One part of chloride of calcium to three of distilled water, with a trace of hydrochloric acid (to prevent crystallization under

the cover-glass), has been recommended. There are, however, so many unpleasant features about the use of this preservative that I prefer not to use it.

ACETATE OF POTASSIUM. — A concentrated aqueous solution is used for preparing algæ for anatomical preparations as well as for the preservation of bacteria stained in methyl-violet. Slides should lie twenty-four hours before the covers are sealed down.

[MONOBROMO-NAPHTHALIN is employed by Möller in mounting diatoms for test objects, because of its great refractive power. The markings of the frustules come out nearly as well as in dry mounts, while the specimens present a much better appearance.¹

PHOSPHORUS is also used for the same class of objects, giving, it is said, even better results. — W. T.]

CANADA BALSAM, which liquefies when slightly warmed, is much used in mounting diatoms, the fine structure of their frustules being brought out very clearly by it. Instead of the pure balsam, a concentrated solution in ether or chloroform, which is far cleaner, may be used.

[For most purposes benzole-balsam is, by some, preferred to either of these solutions. It is prepared by exposing commercial "balsam of firs" to gentle heat in a shallow dish, loosely covered to

¹ Cf. Journ. Roy. Micr. Soc., 1880, III., p. 1043.

exclude dust, until all water is evaporated, and the balsam becomes perfectly hard on cooling. It is then dissolved in enough pure benzole to make a thin solution, and filtered. The usual directions advise subsequent evaporation to the consistency of cream, but it may be satisfactorily employed for most purposes in a much more fluid form. Glass stoppers, which are always advisable for reagent bottles, are indispensable for bottles intended to hold this or Dammar. Sections may be mounted in this solution directly from benzole, or from either of the commonly used clearing fluids — clove oil or turpentine. — W. T.]

Only such things as contain little water should be mounted in balsam. If the objects are delicate and contain much water, they must be anhydrated in absolute alcohol, or dried in the air, and when it is necessary they should be cleared in clove oil before mounting. Although balsam preparations cannot dry up, or move about after the medium has once hardened, they need to be sealed, as a jar may at any time loosen the cover. Such a partial or complete loosening is easily detected by the appearance of Newton's rings.¹

¹ Literature of Mounting Media. — Dippel: *Das Mikroskop*, I., p. 470, *et seq.* Schacht: *Mikroskop*, 1855, p. 28. Sanio: *Bot. Zeitung*, 1863, p. 359. Koch: *Cohn's Beitr. z. Biol. d. Pfl.*, Bd. II., p. 407. Bachmann: *Dauerpräparate*, 1879. Frey: *Mikroskop*, pp. 122, 125. Harting: *Mikroskop*, III., p. 409. Pelletan: *Le Microscope*, 1876, p. 178, *et seq.*

CEMENTS.

A large number of compositions have been proposed for use as cements for mounting preparations. As only a few of these appear to me deserving of attention, I do not hesitate to add a few words on this subject to the digression already made in behalf of mounting media.

Cements should adhere to glass strongly, so as to be both air and water-tight. They should be unaffected by the mounting fluid, and should not crack with age.

Those which I would recommend, from my own experience, are asphalt varnish (Brunswick black), which can be bought ready for use, and is made by dissolving asphalt in linseed oil or turpentine; prepared gold-size (a sort of Copal varnish); and an alcoholic solution of Holmblad's best sealing wax. All these substances should have a rather ropy consistency, and must not harden too quickly. They are applied with a small camel's-hair pencil. The safest sealing is effected by applying a layer of asphalt first, and covering this with the sealing-wax solution when it is half-hard.

In my opinion the following new composition is to be recommended. We have tried it in the Copenhagen laboratory, and so far are pleased with it. 50 gm. Canada balsam; 50 gm. shellac; 50 gm. absolute alcohol, and 100 gm. ether are mixed,

filtered, and evaporated over the water-bath to the consistency of thick syrup. I call this the "Gram-Rützou" varnish, after its discoverers.

I have not personally tested the mastic cement (Maskenlack, No. 3), recommended by German investigators, nor Ziegler's and other foreign compositions. I can, however, recommend the so-called Japan varnish, which seems to me very useful for the first coat, when applied similarly to asphalt.

In the laboratory at Rome a cement is used, prepared by dissolving with the aid of heat 100 parts gum dammar in 100 parts benzole, and adding 60 parts ivory black ground with oil.

An alcoholic solution of shellac can be used for fastening the label to the slide, as gums and other adhesive substances do not adhere well to the glass. [Fish glue and the liquid glue made with acetic or nitric acid are useful for this purpose, though rather disagreeable to use. — W. T.]

PART II.

VEGETABLE SUBSTANCES

AND THE

METHODS OF RECOGNIZING THEM.

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[The reader is requested to consult the principal literature in the first section, under the different reagents.]

CELLULOSE.

Pure cellulose is colored violet by chlor-iodide of zinc, blue by iodine and sulphuric acid, and brown, or yellowish brown, or even yellow, by an aqueous or alcoholic solution of iodine. On the addition of water to membranes which have been dried after treatment with iodine, they often become clear blue. Young cellulose frequently colors blue with iodine and sulphuric acid, only after treatment with hydrochloric acid, or after being compressed strongly under the cover-glass.¹

The cellulose of the seeds of *Paeonia* colors blue in iodine dissolved in iodide of potassium.

¹ Richter: Sitzb. wien. Akad., 1881, Bd. LXXXIII.

Cellulose swells in an aqueous solution of potassic hydrate, as well as in the so-called mineral acids, and the stratification of the cell-wall is frequently rendered much sharper and more distinct as a result. It is dissolved by concentrated sulphuric acid with the formation of amyloid, and in cuprammonia without change. From this solution it can be precipitated by the use of absolute alcohol.

Cellulose is colored with unequal intensity by the various aniline colors. Alcannin and carmine, on the other hand, are inactive, or nearly so. After heating with concentrated potassic hydrate, young cellulose is not colored by cupric sulphate; but when somewhat older it is colored pale blue by this reagent. Grenacher's alum carmine colors it red (p. ~~49~~).

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LIGNIN.

This substance probably consists of, 1, vanillin; 2, coniferin(?); 3, some gum; 4, a substance which is colored yellow by hydrochloric acid; and 5, the wood-gum of Thomsen. It is thus seen to be a very complex substance.¹

Lignified cell-walls are colored yellow by chloride of zinc. Concentrated sulphuric and chromic acids dissolve them, but they are insoluble in cuprammonia and the Schultze maceration fluid. After treatment with an aqueous solution of cupric

¹ Max Singer: Anzeiger des kais. Akad., Wien, 1882, No. 11.

sulphate, the addition of warm concentrated potassium hydrate often colors them brown. An aqueous solution of sulphate of aniline (naphthalidin or toluidin), followed by saturation with dilute sulphuric acid, colors them a beautiful yellow (Wiener's reaction). Frequently this reaction is obtained without the use of any acid. All membranes which contain lignin assume an intense and very beautiful rose color, due to the presence of vanillin, when treated with hydrochloric acid and phloroglucin. The same reaction is given by this acid in combination with an extract of cherrywood (Xyloflin), but the color is more violet. On the other hand, a bluish-green color appears after the successive action of hydrochloric acid and phenol.

All aniline colors are taken up with avidity by lignified membranes. Indol gives them an intense red color. Grenacher's alum carmine does not color them. A weak aqueous solution of eosin produces no color after short action, though the same is true of pure cellulose. Lignified membranes lose the so-called incrusting substances by being heated with alkalis, concentrated nitric acid, or the Schultze maceration fluid, after which the cellulose reaction can be produced by the proper reagents.

INTERCELLULAR SUBSTANCE ("Middle Lamella").

Insoluble in concentrated sulphuric acid, cuprammonia, and dilute chromic acid; soluble with

difficulty in concentrated chromic acid; soluble in the Schultze maceration fluid (which is specially important in the investigation of wood), and occasionally in hot potash, sometimes even in boiling water.

The intercellular substance is easily and strongly colored by the aniline dyes. With chlor-iodide of zinc it assumes a yellow color. Hot nitric acid and ammonia give it a beautiful yellow color.¹

SUBERIN,

The constituent of cork, is insoluble in concentrated sulphuric acid and cuprammonia, and very resistant to chromic acid. Treated with boiling potash, corky membranes secrete peculiar ochre-yellow granular masses, and when heated with nitric acid and chlorate of potassium they form masses of ceric acid, which are soluble in alcohol, ether, benzole and chloroform.

The walls of cork cells are colored yellow by chlor-iodide of zinc. Grenacher's alum carmine does not stain them.

Olivier indicates the following method of coloring corky membranes. Sections of the tissue to be studied are laid in a solution of fuchsin in equal parts of alcohol and water, which is taken

¹ Solla: Beitr. zur Kenntniss d. chem. und phys. Beschaffenheit der Intercellularsubstanz. — Oesterr. botan. Zeitschr., Nov. 1879. Höhnel: Mittellamelle der Holzelemente u. d. Hoftüpfelschliessmembran. — Bot. Zeitung, 1880, No. 26.

up by the entire preparation. By after-treatment with absolute alcohol, the coloring matter is removed from the cellulose portions, remaining only in those which are corky.¹

FUNGUS-CELLULOSE.

In 1866 De Bary gave this name to the substance composing the cell-walls of fungi.² Previously (1852) Schacht had shown³ that these walls were very resistant to micro-chemical reagents, and especially that the reaction characteristic of pure cellulose seldom succeeds. Since then, the names fungine and metacellulose have been given to this doubtful substance. Recently Richter has succeeded in showing that the walls of hyphae are, in reality, formed of cellulose as a fundamental substance,⁴ its detection being rendered difficult by the presence of infiltrated matters, — possibly of a protein nature.

After fungus tissues have been treated with concentrated potash, frequently renewed, for several weeks, and especially after they have been finally boiled in this fluid, they assume a blue color with

¹ Olivier: Note sur le système tégumentaire des racines chez les Phanérogames. — Bull. Soc. bot. de France, 1880, T. XXVII., pp. 234-235.

² De Bary: Morphol. u. Physiol. d. Pilzen, Flechten u. Myxomyceten, p. 7, *et seq.*

³ Schacht: Pflanzenzelle, p. 9.

⁴ Richter: Beitr. z. genaueren Kenntniss d. chem. Beschaffenheit d. Zellmemb. bei den Pilzen. — Sitzber. wiener Akad., 1881, Bd. LXXXII., Abth. 1.

the reagents used for detecting pure cellulose.¹ There is, therefore, no reason for considering "metacellulose" as a distinct substance. It should be looked upon simply as a modification similar to that of wood and bark.

The paraphyses and asci of lichens are, as a rule, colored blue by iodine, as are the hyphae of the medullary layer in some cases. This is to be attributed to the presence of lichenin,—a substance characteristic of these plants.

PROTEIN SUBSTANCES

Are easily characterized by the brown color which is imparted to them by iodine, the rosy red which they assume when acted upon by Millon's reagent, especially after gentle warming, and the yellow which is produced by nitric acid² either alone or in combination with ammonia. After lying for about twelve hours in corrosive sublimate in alcohol, they form a compound, insoluble in water, which is especially interesting in preparations of aleuron grains. Protein compounds become violet when treated with Trommer's reagent; sugar and sulphuric acid (Raspail's reagent, 1833) color them red. The imbibition and condensation of different coloring matters, *e. g.* cochineal, carmine, a solu-

¹ Van Tieghem: *Traité de botanique*, 1882, p. 569.

² Discovered in 1686 [?] by Glauber (*Explicatio miraculi mundi*). Mulder has given the yellow compound the name "Xanthoproteic acid."

tion of aniline blue in water, etc., is characteristic of protein substances.¹

For the preparation of aleuron-grains the following method is employed in the Copenhagen laboratory: The sections are treated for several days with a five per cent. alcoholic solution of corrosive sublimate, according to Pfeffer's plan. They are then colored by an aqueous solution of eosin, and mounted for study in acetate of potassium and water in equal parts. The crystalloids are thus rendered very distinct; and, if the eosin solution has not been too concentrated, they commonly assume a red shade, different from that of the ground mass.

PROTOPLASM.

Protoplasm is the living portion of the cell. The chemical cause of this life — if, indeed, such chemical cause exist — has not yet been discovered; but it appears as if some steps have been taken toward the solution of this interesting problem. Quite recently a reaction for living protoplasm has been discovered. In this state it contains free aldehyde, which does not exist in dead protoplasm. This substance precipitates metallic silver from even an extremely dilute alkaline solu-

¹ Cf. Vines: Chemical composition of aleuron-grains. — Royal Society of London, May 13, 1880. — Nature, 1880, vol. 22, No. 552, p. 91. — Journ. of the Royal Micr. Soc., 1880, Vol. III., p. 667.

tion of the nitrate. The protoplasm is, therefore, colored a pronounced black. (*Cf.* p. 41.)

Since protoplasm is a mixture of different protein compounds, it gives their characteristic reaction. Living protoplasm does not, however, imbibe coloring matters as dead protoplasm does. A solution of caustic potash or concentrated ammonia water increases its transparency; acetic acid produces the opposite effect. Absolute alcohol affects it very characteristically, rapidly hardening it. This is of especial value in various investigations of protoplasmic structure, the changes which occur in the embryo sac, the division of the nucleus, etc. An aqueous solution of perosmic acid, even when very dilute (1:800), has a similar effect.

Aqueous solutions of sugar, table-salt, alcohol, glycerine, etc., by the abstraction of water, cause the protoplasm of cells to contract and separate from the wall. This frequently happens without killing the protoplasm, though this of course is not the case with alcohol unless greatly diluted with water.

The metaplasm of Hanstein,¹ *i. e.* that part of the protoplasm which holds the formative material, is colored almost scarlet by Hanstein's aniline violet. De Bary's epiplasm,² a special modifica-

¹ Organe der Harz- und Schleimabsonderung. — *Bot. Zeitung*, 1868, p. 709.

² *Morphol. u. Phys. d. Pilze etc.* — Hofmeister's *Handb.*, p. 103.

tion of protoplasm in the asci of ascomycetes [containing much glycogen], assumes a deep reddish-brown or violet-brown color with even a very dilute solution of iodine.

The preparation of the nucleus, which has been so zealously studied of late, has formed the subject of many contributions.¹ Acetic acid, alcohol, and perosmic acid differentiate it sharply, and are therefore used to render it evident. Coloring matters are taken up and condensed by the nucleus. The effect of haematoxylin, aniline green, Grenacher's alum-carmine, ammonia-carmine, and picro-carmine, is to color the nucleus deeper than the rest of the protoplasm in the cell. Iodine acts in exactly the same manner. Successive treatment with alcohol, picro-carmine, and glacial acetic acid is recommended by Maupas.

Among the various substances of the protein group, nuclein has been especially studied by Zacharias² and others. To detect it, immerse the preparation for an hour in a mixture of one volume of a ten per cent. solution of the yellow ferrocyanide of potassium in distilled water, and two volumes of acetic acid, prepared by diluting the glacial acid with an equal bulk of water. Wash the sections in sixty per cent. alcohol. The

¹ Strasburger, Hanstein, Ranvier, Treub, Schmitz, and others have done much in this direction. Cf. further. — Treub: Actes du Congrès International des Botanistes, etc., à Amsterdam en 1877, Leide, 1879, p. 146. — Maupas: Comptes rendus, July 1879, Vol. LXXXVIII., p. 250.

² Zacharias: Bot. Zeitung, 1881, p. 169; 1883, p. 209.

masses of nuclein are characterized by the blue color they assume, which was discovered by Hartig.¹

STARCH²

Is colored blue by the tincture of iodine, iodide of potassium, and other substances which contain free iodine, since, although the so-called starch cellulose cannot take up this element, granulose does so. The blue color which results is not due to a chemical compound, but is rather to be considered as the result of a (molecular?) solution of the iodine in granulose.³ In this connection we must remind the reader that the presence of water is a *conditio sine qua non* for the reaction. Dry starch treated with the vapor of iodine, or with iodine in anhydrous alcohol or chloroform, is only colored brown like pure starch cellulose.

All substances which combine directly with iodine destroy the blue color. Gentle warming in water has the same effect; but in cooling, the starch resumes its color.

When slowly heated in water starch grains swell considerably, usually after the temperature has reached 50° C. In this way starch paste is formed,

¹ Hartig: Bot. Zeitung, 1854.

² Nägeli: Beiträge zur näheren Kenntniss der Stärkegruppe, 1874.

³ The compound resulting from the long treatment of starch with salt-solutions containing an excess of free iodine is somewhat different. After being raised to a red heat, this still contains about three per cent. of iodine. — Cf. E. Sonstadt: Note on the compound of starch with iodine. — Chemical News, 1873, Vol. XXVIII., p. 248.

which may be diffused in the water, but does not form a true solution. This paste gives the same reaction as unaltered starch. Solutions of chloride of calcium, chloride of zinc, potassic hydrate, and concentrated iodide of potassium, as well as the mineral acids, carbolic acid, acetic acid, and trichloroacetic acid cause this conversion of starch into paste in varying degrees, depending upon their concentration. The beginning of the change may always be recognized by the more evident stratification of the grains. Dilute chromic acid produces this effect in a marked degree, and has been used to demonstrate stratification in the peculiar starch bodies which are found in the latex of *Euphorbiaceæ*.

Alcohol has an opposite effect, the stratification often completely disappearing under its action. Cuprammonia causes the grains to swell, and colors them pale blue, but it does not change them into paste.

For directions for demonstrating the starch in chlorophyll bodies the reader is referred to page 7.¹

¹ Crié announces a new substance found by him in the asci of *Sphaeria Desmazierei*, Berk., which he calls amylomycin, and which is said to have the same reaction as starch. (*Comptes rendus*, T. LXXXVIII., pp. 759, 985.) We mention this here merely to make our account complete. The substance is so insufficiently characterized by its discoverer that one may be pardoned some doubts as to its independent nature.

[GLYCOGEN.]

This substance, which occurs in cells of fungi in a semi-fluid, amorphous form, and more or less intimately united with albuminoid structures, is soluble in water as well as in acids and alkalies, but insoluble in alcohol and ether. It does not reduce Trommer's reagent, and gives no reaction with osmic acid, Millon's reagent, or the salts of iron. It may usually be distinguished from gums and mucilage by not forming gelatinous masses in water.

The characteristic test for vegetable glycogen is the red-brown or mahogany color it assumes when treated with iodine in the presence of water, the latter being essential, as in the corresponding reaction for starch. On heating the preparation the color fades, reappearing when it is cooled.

Errera¹ recommends crushing the cells to be tested in a drop of water under the cover-glass, and immediately adding the iodine dissolved in water containing a little iodide of potassium.

Vigorous young plants of *Phycomyces nitens* and *Coprinus evanidus* are recommended for this test, though the reaction may be obtained with

¹ Errera : L'Epiplasma des Ascomycètes et le glycogène des Végétaux. — Thèse présentée pour l'obtention du grade de Docteur Agrégé pres la Faculté des Sciences de l'Université de Bruxelles, 1882 ; Sur le glycogène chez les Mucorinées. — Bull. Ac. roy. de Belgique, Nov. 1882, 3 Sér., IV., No. 11.

many fungi, and is shown especially well in asci of *Peziza vesiculosa*, and various other ascomycetes. — W. T.]

DEXTRIN.

This transformation-product of starch may be recognized in the vegetable cell by Trommer's test (p. 36). The vermilion precipitate is finely granular, and shows the Brownian movement very plainly (*Cf.* grape sugar).

GRAPE SUGAR (Dextrose, Glucose)

May be recognized by either Trommer's or Fehling's test (p. 36). The reaction, which in general is not very sure, is manifested by a reddish-yellow precipitate of cuprous oxide. Barfoed's test,¹ *i. e.* heating with an aqueous solution of neutral acetate of copper, gives after long standing a red precipitate. Such a precipitate is not formed with dextrine. At ordinary temperatures glucose gives a precipitate with neutral acetate of copper, while dextrine remains clear for a long time. With very dilute alkaline nitrate of silver (1 : 100,000) glucose gives a brown color (p. 41).

¹ Zeitschr. f. anal. Chemie, Bd. XII., p. 27. Sachsse: Farbst., Kohlenhydrate u. Proteinsubst., 1877, p. 192. — I have not myself tested this reaction, but it is deserving of mention here, since macro-chemical reactions are often useful in micro-chemistry.

CANE SUGAR (Saccharose).

Cells which contain this substance do not give a precipitate with the Trommer reagent, but assume a pure deep violet color. If much saccharose is present in the tissue it can be caused to crystallize out by the use of absolute alcohol (p. 26).¹

INULIN (Sinistrin,² Synantherin)

Occurs dissolved in the cell sap, like the sugars which have just been mentioned. If a tissue containing it is treated with alcohol or glycerine the inulin separates as sphæro-crystals, which are insoluble in cold water, but easily soluble in water heated to 50°–55° C., in dilute acids, and in cuprammonia. A tincture of iodine colors the sphæro-crystals brown by penetrating into the fine clefts and fissures which they contain; when boiled with dilute acids or under pressure inulin is changed into levulose.

HESPERIDIN.

The sphæro-crystals of this substance are insoluble in most acids, glycerine, and absolute

¹ See also Kraus' glycerine test (p. 28).

² Not to be confounded with the carb-hydrate of the same formula which Prof. Schmiedeberg has lately described under this name, and which occurs in the bulb-scales of *Urginea Scilla*. — *Zeitschrift f. physiol. Chemie*, 1879, p. 112. — *Bot. Zeitung*, 1879, p. 513. — *Journal of the Royal Micro. Society*, 1879, Vol. II., p. 916.

alcohol, as well as in cold and boiling water. They are easily soluble in an aqueous or alcoholic solution of potassic hydrate, assuming a yellow or reddish color. They are also dissolved, but with more difficulty, in hot concentrated acetic acid, ammonia, and the alkaline carbonates.

Unripe oranges may be used in testing for this substance.¹

GUMS.

At present we have no certain micro-chemical reaction for gums. The different sorts are insoluble in alcohol. They swell strongly in water and are not colored blue by iodine, either alone or followed by sulphuric acid. Cell-walls which contain gum assume a red color when treated with Hanstein's aniline violet.

VEGETABLE MUCUS²

Is a comprehensive name used to designate a number of different substances which are closely related to the gums, but are still imperfectly known in many respects. They are distinguished from gums by the yellow or blue color which they

¹ Pfeffer: Hesperidin. — Bot. Zeitung, 1874, p. 481. Mika: Beiträge zur Morphol. u. mikroskop. Nachweisung des Hesperidins. — Magyar növénytany lapok, I., p. 93. Known to me only through Just's Jahresbericht.

² Kirchner and Tollens: Untersuchungen über den Pflanzenschleim. — Ann. d. Chem. and Pharm., Bd. CLXXV., p. 205.

assume with iodine, and the blue or violet brown imparted by iodine and sulphuric acid. Many of them swell considerably in water. Barcianu¹ gives the red color induced in tissues which contain mucus, by successive treatment with creosote, chloride of tin and aniline (?), as a reaction.

The so-called amyloid (Schleiden, 1844) must belong here.² Leguminous amyloid is colored blue by iodine in alcohol, and yellow by iodine in water. It is soluble in dilute alkalies and in boiling water.

Hanstein's aniline violet colors amyloid substances red, but of a shade different from that obtained in the gum and tannin reactions.³

TANNIN (Tannic Acid).

Cells containing this substance are colored deep blue or green by treatment with ferric acetate (p. 42) or chloride (p. 33). They are colored reddish brown by bichromate of potassium (p. 40), fulvous by Hanstein's aniline violet (p. 60), and red or violet by dilute chlor-iodide of zinc (p. 9). All of these reactions demand a prolonged stay in the

¹ Blütenentwicklung der Onagraceen.—Schenk and Luerssen's Mittheil. aus der Bot., II., Heft 1, p. 85. I have not made myself familiar with this reaction.

² Vogel and Schleiden: Amyloid.—Schleiden's Beitr. z. Bot., 1844, Bd. I., VIII.

³ Cf., further, Léon-Marchand: Gélatine produite par les Algues.—Bulléin de la Soc. bot. de France, 1879, p. 287.

fluid.¹ An alkaline solution of nitrate of silver (1 : 10,000) gives a black color with tannin (p. 41).

PECTIN

Often replaces the intercellular substance or certain layers of the cell-wall. It is recognized by the swelling of these layers in hot water and alkalies, and their solubility in the latter as well as in concentrated oxalic acid (p. 24). With cuprammonia (p. 15), pectate of copper is formed, and in thin sections this remains after the complete solution of the other parts of the wall.

ASPARAGIN

Is insoluble in absolute alcohol or a concentrated solution of itself, but soluble in water. On drying sections which contain it or treating them with absolute alcohol, acicular crystals of asparagin form in the cells or the fluid about them (p. 45).

CRYSTALLOIDS²

Is a name used to designate protein bodies having a crystalline form. They are characterized

¹ Höhnel (Die Gerberinden, Berlin, 1880) gives more particular information as to tannin in its technical bearings. The very characteristic reaction of a decoction of galls with iron was known to Pliny, and was used by the ancients to detect the adulteration of verdigris with sulphate of iron — the oldest chemical reaction.

² A. Schimper: Untersuchungen über die Proteinkristalloide d.



by giving the protein reaction, by their solubility in ammonia, dilute potash, or acetic acid, and by swelling in water. They are often insoluble in a potash solution of above ten per cent. There are also some which are soluble in a solution of table-salt (p. 32). As a characteristic which is always useful, though not of a chemical nature, we mention further the inconstancy of their angles.

The red, somewhat doubly refractive, tabular crystalloids of certain Florideæ that have been kept in the herbarium or mounted in glycerine are especially noteworthy. They are the so-called rhodospermin crystals of Cramer, and are insoluble in sodic-chloride, in which they lose their color.

The crystalloids contained in protein grains become visible only after treatment with warm glycerine.

CAOUTCHOUC¹

Occurs in the latex of different plants in the form of small homogeneous globules, which swell in ethereal oils, and are soluble in carbon bisulphide, chloroform and benzole, but are not attacked by dilute acids or alkalies.

Pflanzen, Strassburg, 1879. J. Klein: Krystalloide der Meeresalgen.—Flora, 1880, p. 65. Cohn's Beiträge zur Biol. d. Pfl., 1880, III., p. 163 (Crystalloids in the nuclei of *Pinguicula alpina*).

¹ Weiss: Allg. Bot., I., p. 191. Duchartre: Éléments de Bot., 1877, p. 74.

CHRYSOPHANIC ACID.

Cells which contain this acid are colored deep red by potassic hydrate. The hyphae of lichens are also colored red by the reagent, and are dissolved by it. On the other hand, calcic hydrate or barium water colors them without dissolving them. Ammonium carbonate gives no color with this substance, and hydrochloric acid does not affect it. When gently warmed, chrysophanic acid reduces the ammonium nitrate of silver.¹

FATTY OILS

Form strongly refractive spherical masses, soluble in ether, carbon bisulphide, benzole and oil of turpentine. They form soap with potash or sodium lyes. Perosmic acid, if not too dilute, colors them black or brown.

When very closely united with protoplasm, fats can be separated by concentrated sulphuric acid or an aqueous solution of chloride of calcium, when they collect in drops of varying size, especially at the margin of the preparation.

VOLATILE, ETHEREAL, OR ESSENTIAL OILS

Form ropy, motile, refractive masses, often very long in proportion to their diameter. They are

¹ Cf. Schwarz: Cohn's Beitr. z. Biol. d. Pfl., 1880, III., p. 249.

soluble in cold alcohol, and in the substances already mentioned as solvents of fatty oils; but they are insoluble in water.

RESIN.

The best marked reaction is the red color imparted by Müller's tincture of alcannin (p. 41). Hanstein's aniline violet colors it blue (p. 60). The different varieties of resin are soluble in alcohol and ether, but insoluble in water. The Unverdorben-Franchimont reagent, an aqueous solution of acetate of copper (p. 42), colors the resin a beautiful emerald green in masses of tissue which are allowed to lie in the fluid for several days.

WAX

Forms solid crusts, or characteristically-shaped secretions, on the surface of cells. It is insoluble in water, whether cold or hot, but melts in the latter case. It is insoluble, or hardly soluble, in boiling alcohol, but soluble in ether, benzole, chloroform and carbon bisulphide.

SILICIC ACID (Silica).

Silica frequently incrusts the cell-wall (grasses) or the pedicel of cystoliths, and it is sometimes found in the interior of cells in amorphous masses

(Podostemaceæ).¹ It may be recognized in the following way: A very thin section of the tissue to be tested is heated on platinum-foil, by which means all of its organic constituents are destroyed, leaving the inorganic elements, which consist of silicic acid and salts of lime, in a somewhat distorted skeleton. The calcareous portion is then removed by adding a drop of hydrochloric acid, the silica remaining as a network, which is soluble in hydrofluoric acid.

It may happen that the silica and lime salts fuse together in the process of heating, which interferes with the reaction. It is, therefore, desirable to remove these salts by the Schultze maceration before incineration. When the section has been boiled in this it is washed in hot distilled water. After the final addition of hydrochloric acid the silica alone remains. The operation needs to be skilfully performed. The epidermis of *Equisetum* is to be recommended for experiment.

Sachs has proposed a somewhat modified method. Larger tissue masses are moistened with concentrated sulphuric acid on platinum-foil, and heated over a Bunsen burner. The acid is at once blackened, while there is a violent evolution of gas. The heating is continued only until the pure white ashes remain, — a result which is reached somewhat more rapidly by this method than by the other.

¹ Cario: Bot. Zeitung, 1881, p. 31. Warming: Naturh. Forenings vidsk. Meddel., Copenhagen, 1881, p. 89.

LIME SALTS

Occur partly as invisible, amorphous incrusting substances in the cell-wall, and can then be detected in the ashes. Sometimes they occur as well-developed crystals, which are found in the wall itself and elsewhere.¹

Micro-chemical investigations show that calcium occurs in the cell in the form of carbonate, oxalate, phosphate, and sulphate :—

a. The compounds with carbonic acid are dissolved by dilute acids with violent effervescence. If the solution has been effected by sulphuric acid, gypsum needles crystallize out in the fluid as in the case of other salts of lime.

(Cystoliths ; Corallina, Melobesia, Chara, etc.)

b. Oxalate of lime, the most widely-distributed of these compounds, is not dissolved by potassic hydrate or acetic acid, but it is soluble in hydrochloric acid without effervescence.

(It occurs as an incrusting material in the hairs of *Asclepias*,² and in raphides, free crystals, Rosanoff's crystal glands, etc.)

c. Calcium phosphate³ has recently been shown to occur in plants. It is insoluble in water, alco-

¹ Holzner : Beitr. z. Kenntn. der Pflanzenkrystalle.—Zeitschr. f. Mikroskopie, 1877, I., p. 236, where there is a synopsis of the literature. — Van der Ploeg : De oxalsure Kalk in de Planten, Leiden, 1879.

² Kabsch : Bot. Zeitung, 1863.

³ Nobbe, Hanlein, and Councler : Landwirtsch. Versuchsstationen, Bd. XXIII., p. 471 (*Robinia Pseudacacia* and *Soya hispida*).

hol, ether and alkalies, but soluble without effervescence in acetic and other acids. The characteristic reaction, however, is the yellow color produced in a dilute neutral solution of nitrate of silver.

d. Calcium sulphate (gypsum) is insoluble or hardly soluble in hydrochloric, nitric and acetic acids. When crystals of this salt are placed in an aqueous solution of barium chloride they are soon covered by a granular crust of barium sulphate.¹

IRON

Has been detected in the cell-wall.² The sections must be made with a platinum or silver knife. They are then treated with an alcoholic tincture of sulpho-cyanate of potassium (p. 40), and the appearance of a red color, either immediately or on the further addition of hydrochloric acid, indicates the presence of a ferric compound. In case no reaction occurs the sections are treated with hydro-

¹ N. J. C. Müller claims to have found this salt in Guaiacum wood. (Allg. Bot., 1880, Theil 1, p. 557.) Hanbury and Flückiger, however, only mention calcium oxalate in this plant. (Pharmacographia, 1874, p. 94.) Nägeli (Mikroskop, 1877, p. 486) is of the opinion that it does not exist in the cell at all; while Wiesner (Techn. Mikroskopie, p. 85) figures the twin-crystals of gypsum from the mesophyll of Iris. Holzner, however, in his classic work on the crystals of plants, shows that the crystals taken for sulphate of lime in Guaiacum, Iris, etc., are in reality the oxalate. The existence of crystals of the sulphate in plants is, therefore, not demonstrated.

² Weiss and Wiesner: Sitzungsber. der wiener Akad., 1860, Bd. XL. — Cf. Bot. Zeitung, 1860, p. 357.

chloric or nitric acid, together with the sulphocyanate (p.40), by which ferrous compounds which may be present are oxydized, when their presence is indicated by the above red color.

SULPHUR

Has been found in the pure crystalline state only in those bacteria which live in thermal springs or on rotting algæ, and which liberate sulphuretted hydrogen.

Sulphur is soluble in carbon-bisulphide. For the violet reaction with the nitro-prussiate of sodium in alkaline fluids see page 39. It gives no reaction in an aqueous solution of perosmic acid, and thus may be easily distinguished micro-chemically from the fatty oils, to which it frequently bears no small resemblance.

COLORING MATTERS.¹A. — *Of Protoplasm.*

CHLOROPHYLL (Leaf Green)

Is a green substance which is insoluble in water, dilute acids, and alkalis, but soluble in ether, benzole and alcohol. It is bleached by Labarraque's solution. When treated with dilute acids it assumes a yellowish color, while concentrated hydrochloric and sulphuric acids change it to a bluish-green or blue.²

Methyl green (p. 63) colors chlorophyll bodies a more intense green, and by prolonged treatment with hydrochloric acid masses of hypochlorin may be extracted from them.

According to Pringsheim, the following pigments may be regarded as modifications of chlorophyll:—

a. ETIOLIN,

The yellow matter of etiolated plants, which is soluble in alcohol and ether, but insoluble in water. Its solution is colored emerald or verdigris green,

¹ Nägeli: *Das Mikroskop*, 1877, p. 528. — *Cf.*, further, the literature of vegetable coloring matters, *supra*, p. xviii.

² *Cf.* Bommer: *Bull. de la Soc. bot. de France*, 1873, Session extraordinaire.

and later, often after a number of hours, blue, by hydrochloric or sulphuric acid.

b. XANTHOPHYLL (Berzelius, Pringsheim),

The yellow coloring matter of autumnal leaves, is insoluble in water, but soluble in alcohol and ether. It is only colored emerald green by the acids which have been named.

c. ANTHOXANTHIN (Marquart, Pringsheim),

The yellow pigment of yellow flowers, fruits and seeds, occurs, like chlorophyll, diffused through protoplasmic bodies; less frequently in the form of yellow oily drops; and very rarely diffused in the cell-sap. The latter variety (anthochlor, Prantl; xanthein, Frémy) is soluble in water, in which the other forms are insoluble, though they are soluble in ether and alcohol. The varieties of anthoxanthin (xanthein, Frémy; lutein, Thudichum), which are soluble in the latter reagents, are colored green like xanthophyll, but later, like etiolin, blue, by the action of acids; those soluble in water, however, are not affected in this way. Anthochlor becomes brownish yellow when treated with potassic hydrate, but the original color returns when the solution is neutralized.

d. FLORIDEA-GREEN (Pringsheim).

The chlorophyll of Florideæ, which gives the same chemical reactions as the true chlorophyll of higher plants, is to be regarded, according to Pringsheim, as a variety of this, on account of its optical properties.

e. FLORIDEA-RED (Phycoerythrin, Kützing, Cohn)

Is soluble in water, by which it may be removed from the dead plasma-bodies. When allowed to stand in the light, exposed to the air, it fades, and the same effect is produced when it is treated with potassic hydrate. Sulphuric acid does not change the color.¹

HYPOCHLORIN.²

After chlorophyll grains have been treated for some hours with hydrochloric acid a substance separates from them, appearing either as a crystal-

¹ Nägeli and Schwendener (*Mikroskop*, 1867, p. 498) understand by "Floridea-Red" the entire coloring mass of the Florideæ, — chlorophyll-phycoerythrin. Sachsse used the name in Kützing's sense, as we do. Kützing's phycohæmatin from *Rhytiphloea tinctoria*, which needs further investigation, is omitted here.

NOTE. — To distinguish the colors which have been mentioned, properly, they must be investigated by the spectroscope. Cf. R. Sachsse: *Farbstoffe, Kohlenhydrate and Proteinsubstanzen*, Leipzig, 1877.

² Pringsheim: *Untersuchungen über Chlorophyll, IV., Das Hypochlorin und die Bedingungen seiner Entstehung in der Pflanze.* — *Monatsber. d. berl. Akad.*, Nov. 1879. (Cf. *ibid.*, July 1879; also *Comptes rendus*, Jan. 1880, p. 161.); *Jahrb. f. wiss. Bot.*, 1882, XIII. A. Hansen: *Assimilation und Chlorophyllfunction*, 1882.

line deposit, in brown oily drops, or in the form of ropy, semi-fluid masses, from which, after a while, needle or staff-shaped bodies, or fine crumpled threads separate. According to Meyer¹ the hypothetical matter called hypochlorin by Pringsheim is identical with the chlorophyllane of Hoppe-Seyler,² which is but a transformation product of the green pigment of the living plant, and perhaps also identical with the "crystallized chlorophyll" of Gautier.

It is insoluble in water, salt solutions, and dilute organic or mineral acids. It is easily soluble in ether, benzole, volatile oils and carbon bisulphide. An aqueous solution of chloral dissolves the crystals, leaving a drop which is soluble in alcohol. Heat volatilizes it so that green cells, which have been warmed to 50° C., give no trace of hypochlorin when subsequently treated with hydrochloric acid. When hypochlorin needles that have been formed by the aid of this acid are heated in water they lose their crystalline nature, and unite to form oily masses of a greener hue.

CHLORORUFIN (Rostafinski).³

The oospores of several algæ (*Oedogonium*, *Vaucheria*), the antheridia of *Chara*, and the cells

¹ A. Meyer: *Das Chlorophyllkorn*, Leipzig, 1883.

² *Zeitschr. f. phys. Chemie*, III.

³ Rostafinski: *Bot. Zeitung*, 1881, p. 461.

of certain fleshy fruits (*e. g.* Capsicum) are colored red by this substance. Sulphuric acid gives it a very intense blue color,¹ quite characteristic. Fuming nitric acid dissolves chlororufin, while ordinary nitric acid does not. This reaction indicates a striking analogy with the chrysoquinone of Liebermann. Chlororufin is, perhaps, identical with the solanorubrin of Millardet,² and probably is a constituent part of the xanthein of Frémy.

CAROTIN (Wachenroder, 1832).³

This substance forms red or yellowish-red crystalline pigment bodies, without any organized base, in the cells of *Daucus Carota*. It has also been obtained in the free state. It is easily soluble in benzole, carbon bisulphide and fatty and volatile oils. Ether and alcohol dissolve it with difficulty. Chloroform dissolves it readily; but an aqueous solution of chloral (5:2) or acetic acid does not produce any alteration. Sulphuric acid produces a distinct blue color, and chloride of iron gives a greenish color with a solution of this substance.

¹ De Bary: Ber. d. naturf. Ges., Freiburg, 1856, No. 13.

² Millardet: Note sur une substance colorante nouvelle, Nancy, 1876.

³ A. Husemann: Ueber Carotin und Hydrocarotin, Diss., Göttingen, 1860. A. Meyer: Das Chlorophyllkorn, 1883, p. 48. Fritsch: Ueber körnige Stoffe des Zellinhaltes, Diss., Königsberg, 1882, p. 39. Zeise: Jahrb. f. Chemie, XL., p. 297. Berg: Pharmaceutische Waarenkunde, 1879.

PHYCOCHROMIN (Sachsse = Nägeli's phycochrome—
chlorophyll)

Is a pigment which occurs in connection with chlorophyll in the bluish-green algæ. It is soluble in water, but insoluble in alcohol, and probably consists of phycoyanin (Sachsse¹), and a variable quantity of phycoerythrin (Kützing). Cells which contain phycochrome are colored yellowish-green or yellowish-brown by alkalies, and orange or brick-red by hydrochloric acid.

PALMELLIN (Phipson²)

Is the red pigment found in *Porphyridium cruentum* Naeg. It is soluble in water. The addition of alcohol, acetic acid or alkalies to this solution causes a flocculent precipitate, while the fluid assumes a blue color. Ammonium sulphide colors it yellow without forming a precipitate.

PHYCOXANTHIN (Millardet, Askenasy)

Is the yellow coloring matter of diatoms and Fucaceæ, and is more readily soluble in alcohol than chlorophyll is. In the diatoms it forms, with chlorophyll, the yellowish-brown endochrome

¹ Kützing's phycoyanin is the coloring matter of *Oscillariæ*, which is soluble in water.

² *Comptes rendus*, Aug. 1879, p. 316. Schnetzler: *Bull. de la Soc. vaudoise des Sc. nat.*, 2 Sér., Vol. XV.

masses,¹ and has been called diatomin (Nägeli, 1849). It is easily removed from the thallus of the Fucaceæ by 40 per cent. alcohol, which does not extract the chlorophyll. A small quantity of acid colors it bluish-green. Alkalies, like light,² have no pronounced influence on it.

PHYCOPHAEIN (Millardet)

Occurs in the thallus of Fucaceæ, mixed with chlorophyll and phycoxanthin. It is brown, soluble in water but insoluble in alcohol. Further investigations, however, are necessary.

B.—*Of the Cell Sap.*

(ANTHOXANTHIN. — For a form of this, see page 100).

ANTHOCYANIN (Marquart; Kyanin, Frémy and Cloez)

Is the blue coloring matter of many flowers. The erythrophyll, which is peculiar to the cell-sap of red and violet cells, is probably identical with this, or only a modification of it. Acids impart a red color to cells which contain anthocyanin, while alkalies restore the blue color, the same as with

¹ Petit: De l'Endochrome des Diatomées. — Brébissonia, 1880, Ann. II., No. 7, p. 81. — The composition of the endochrome was established in 1868 by Kraus and Millardet.

² Cf., however, Petit, *l. c.*

litmus. After the prolonged action of alkalies the blue passes into a green, yellowish-green, or yellow. But it has been thought that the latter tints are produced in consequence of the presence of tannin in the cell-sap, which gives a green color with iron, and which may give the yellow potassic hydrate reaction in the color mixture.¹

In *Strelitzia Reginae* and *Tillandsia amoena*, Hildebrand found anthocyanin diffused in small grains, which were soluble in potash, alcohol, and ammonia; which iodine colored brown; and which were, therefore, probably of a protoplasmic nature.

It is doubtless worthy of mention here that anthocyanin may form, with metallic salts, an insoluble compound of a green (Frémy and Cloez) or blue (Wiesner) color. Certain cells (*Gentiana verna*) containing anthocyanin, which have first been reddened by acids that have then been removed by rinsing with distilled water, assume a blue color with a solution of chloride of iron. Since, however, the same phenomenon occurs with acetate of lead, tannic acid can have nothing to do with it.

ALIZARIN ²

Forms yellow masses in the fresh cells of madder-root. With the access of air it soon becomes red

¹ Sachsse's objections, however, should be compared with this, *l. c.*, p. 76.

² Decaisne: *Recherches anat. et physiol. sur la Garance et le développement de sa matière colorante*, 10 pl., Bruxelles, 1837. Rosenstiehl: *Ann. de Chim. et de Phys.*, 5 Sér., T. XIII., p. 148.

and flocculent and passes into the cell-walls. Potassic hydrate colors it purple, and causes its escape into the surrounding medium. Chloride of iron colors it orange and finally brownish-red. Alcohol dissolves the fresh yellow pigment, but not that which has been reddened by exposure to the air.

INDICANE, INDIGOTIN.

Although the chemical investigation of this substance in the living cell has so far been very unsatisfactory, it will, no doubt, be of interest to histologists for us to call attention to it again.

In the cells of the flowers of certain orchids, especially *Phajus*, a blue substance is formed when the plant dies. It occurs either as distinct crystals or groups of crystals, and as small but numerous granules. These may be readily produced by crushing the parts of the flower, or by treating them with alcohol. Micro-chemical analysis shows this substance to be indigotin; it is, therefore, probable that indicane previously existed in the cells, possibly in small protoplasmic (?) granules (Trophoplasts?). The only micro-chemical reaction for this substance at present known is its formation by alcohol. It can be sublimated, and afterward forms small crystals.¹

¹ Cf. Bommer: *Bleuissement des fleurs de Phajus, etc.*; Just's bot. Jahresbericht, 1874, II., 2, p. 868.

C.—*Of the Wall.*THE PIGMENTS OF DYE-WOODS (Brazil Wood,
Sandal-Wood, etc.)

Occur in the cell-contents as well as in the wall, especially in the middle lamella, which may not improbably have derived them from the former. They are soluble in warm water, glycerine, acids and alkalies. "Alkalies dissolve the pigments very easily, with the production of a carmine or violet color. Alcohol and ether give either a colorless (Brazil wood, etc.) or a yellow, orange, or carmine solution. Glycerine and water dissolve them with a carmine, or, less frequently, with a blood-red, brownish-red, or violet color; cuprammonia, with a violet, or, more rarely, with a blue color; and acetic acid with a yellow color. Sulphuric acid either does not dissolve them at all (sandal-wood), or with the production of a carmine or blood-red color."¹ The coloring matter of species of *Pterocarpus* is exceptionally insoluble in hot water.

THE COLORING MATTER OF THE BARBERRY
ROOT

Is yellow, and occurs in the walls of the ducts, wood-cells, medullary rays, bast-cells and the external cortical cells. It is also found in the cell-

¹ Weiss.: *Allg. Bot.*, I., p. 137.

contents of all the tissue systems, with the exception of the spiral ducts.

Dilute acids induce at first the separation of small yellow drops, which exhibit the Brownian movement. Hot potash produces a brownish-yellow color. Glycerine and water dissolve it. Dried sections are rapidly extracted by water. In this yellow fluid hydrochloric acid forms yellow, often radiate aggregates of chloride of berberidin.

GLOCOCAPSIN

Is a red or blue pigment occurring in the cell-walls of *Gloeocapsa* and certain filamentous algæ. It changes to a red, or brownish-red, under the action of hydrochloric acid, and to blue or violet when acted upon by potash.

SCYTONEMIN

Is a yellow or brown coloring matter which occurs in the cell-walls of many *Phycochromaceæ*. It is changed to a verdigris-green by hydrochloric acid, the yellow color reappearing on the addition of alkalies.



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