

A BACTERIOLOGICAL STUDY OF THE PITCHER LIQUOR OF THE SARRACENIACEAE

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D. and
ELISABETH QUINTARD ST. JOHN, M.D.

Bacteriological studies have been made of the liquor from both closed pitchers and open pitchers of plants growing in their native habitat. Up to date, all the North American *Sarraceniaceae* except *Sarracenia psillacina* have been drawn into the scope of these studies. The liquor from each pitcher was studied separately. The procedure for the collection and laboratory examination of the pitcher liquor was an amplification of that in our study of *Nepenthes*.⁶⁸

CLOSED PITCHERS

Technic. The external surface of the pitcher, near the base of the cavity and slightly above the level of the pitcher liquor, was sterilized by passage through the flame of an alcohol lamp. The pitcher was then cut obliquely through the sterilized region by means of sterile scissors. The upper portion of the pitcher was discarded. The lower portion contained the pitcher liquor, which was immediately poured into a tube containing a "slant" of sterile plain nutrient agar. The usual precautions were observed in making this transfer, *i. e.*, to sterilize the scissors in the flame just before use, to flame the top of the tube and its cotton plug just before removal of the plug to make the transfer, and again before insertion of the plug after making the transfer. In making the transfer by pouring, the pitcher liquor came into contact only with a small area of the inner wall of the pitcher. The tubes were immediately sent to Philadelphia by express.

In a few instances, a sterile platinum loop was used to transfer the pitcher liquor to a tube of sterile gelatin. These tubes were brought to Philadelphia by one of us.

Since closed pitchers of *Sarracenia purpurea* contain so very little liquor, the technic was modified somewhat for this species.

The top of the closed pitcher was passed through the flame of an alcohol lamp. The top was then removed by means of sterile scissors. One cc. of sterile water was immediately introduced into the pitcher cavity by means of a sterile pipette. By imparting a gentle motion to the pitcher, this water

was made to wash the inner wall of the cavity. The water was then transferred, by use of another sterile pipette, to the agar slant.

Upon arrival at Philadelphia, the tubes were incubated for four days at a temperature of 37° C.

A total of 50 experiments were made, each on liquor from a single closed pitcher. These experiments were distributed as follows: *Darlingtonia californica* 3, *Sarracenia minor* 3, *S. Sledgei* 3, *S. flava* 15, *S. Drummondii* 3, *S. rubra* 3, *S. purpurea* 20.

Results. Neither colonies nor other evidence of proliferation of bacteria developed in any of the 50 experiments. Therefore, the pitcher cavity and liquor of closed pitchers is bacteriologically sterile.

OPEN PITCHERS

Technic. The procedure for the transfer of liquor from an open pitcher to a slant of sterile agar was exactly the same as was followed in the case of closed pitchers. However, in the case of open pitchers of *Sarracenia purpurea*, 1 cc. of the liquor was transferred from the pitcher cavity to the agar slant by means of a sterile pipette. A separate pitcher containing prey was used for each experiment.

A total of 39 experiments were made, distributed as follows: *Darlingtonia californica* 3, *Sarracenia minor* 5, *S. Sledgei* 4, *S. flava* 8, *S. Drummondii* 3, *S. rubra* 4, *S. purpurea* 12.

In all 39 experiments, colonies developed upon the agar slants. Therefore, bacteria were always present in the liquor of open pitchers. Sterile physiological (0.85 percent) sodium chloride solution was added to each agar culture, and an emulsion of the bacteria was prepared. This emulsion was then used for the inoculation of the sterile media enumerated below. No effort was made to isolate bacterial species and to test their action in pure culture upon the various media, for the sole purpose of the bacteriological experiments was to permit the bacteria to act on the media in as nearly as possible the same manner as they act on the prey in the pitcher cavity.

The following media were used. The figure in parentheses after each medium shows the number of separate experiments in which it was employed.

I. Substrates for proteolytic bacteria.

A. Gelatin (38).

B. Nährstoff-Heyden agar (28).

- C. Loeffler blood serum (35).
 - D. Dorset egg medium (32).
 - E. Aleuronat-protein agar (6).
 - F. Casein agar (6).
 - G. Fibrin agar (3).
 - H. Ovalbumin agar (3).
 - I. Litmus milk (28).
- II. Substrates for alkali-forming bacteria.
- A. Ammonium lactate rosolic acid agar (34).
 - B. Ammonium tartrate rosolic acid agar (31).
 - C. Acetamide rosolic acid agar (34).
 - D. Urea rosolic acid agar (31).
 - E. Asparagin rosolic acid agar (34).
 - F. Glycocoll rosolic acid agar (34).
- III. Substrates for acid-forming bacteria.
- A. Litmus lactose agar (19).
 - B. Litmus glucose agar (19).
- IV. Substrates for the colon-aerogenes group.
- A. Lactose bile salt bouillon (49).
 - B. Trypsinized peptone (28).

The following media are frequently used in routine bacteriological work, and were made in the usual manner: Litmus milk, litmus lactose agar, litmus glucose agar, lactose bile salt bouillon, Dorset egg medium, and Loeffler blood serum. The gelatin⁸² and the trypsinized peptone⁸³ were prepared and used according to the directions of Rivas. The various protein agars and rosolic acid agars were made as suggested by Crabill and Reed.⁸⁴ They had as their base a medium containing magnesium and ferrous sulphates, dipotassium phosphate, potassium chloride, and agar. To this base was added either a protein or a simple nitrogenous organic compound, to serve as the sole source of nitrogen and carbon for the bacteria. The various protein agars were made by addition of 1 percent of a protein (Nährstoff-Heyden, aleuronat-protein, casein, fibrin, ovalbumin) to this plain agar prior to sterilization, and thorough suspension of the protein in the sterile mass prior to use. The various rosolic acid agars were made by additions of (a) one-half percent by volume of a 2 percent solution of rosolic acid in 60 percent alcohol and (b) a simple nitrogenous compound (ammonium lactate or tartrate, acetamide,

urea, asparagin, glycocoll) to the plain agar. One percent of asparagin was used, the other compounds in molecular concentration equal to that of the asparagin. The rosolic acid agars were always sterilized by the discontinuous method. The production of basic compounds (amines or ammonia) by bacteria growing on these media was shown by the red color imparted to the medium beneath and around the colony. Sterile plates of rosolic acid media were always poured, to serve as controls for the determination of the change in color in the experiment proper.

The media, after inoculation with the suspension of the bacteria, were incubated at a temperature of 37° C., and examined at intervals for evidences of bacterial action. Unless otherwise stated, observations were made during a period of 30 days.

Results. The results obtained with each medium may be summarized briefly.

Gelatin. The gelatin was completely liquefied in all 38 experiments. In approximately three-fourths of the experiments, liquefaction was complete by the end of the first week of incubation.

Nährstoff-Heyden agar. Colonies developed in 26 of the 28 experiments. Clearing of the cloudy medium about the colonies occurred in 7 of these experiments, and was apparent on some plates as early as the fifth day of incubation. This clearing of the medium was due to the action of proteolytic bacteria on the Nährstoff-Heyden, which, according to Gotschlich,⁸⁵ is a mixture of albumoses (proteoses).

Loeffler blood serum. Digestion of this medium occurred in four-fifths of the 35 experiments. The digestion was slight in 2 experiments and marked in 26 experiments; it became apparent as early as the fifth day of incubation, and tended to become more pronounced as the period of observation lengthened. A putrid odor was evolved in many of the experiments; it became apparent as early as the tenth day of incubation.

Dorset egg medium. Distinct digestion of the medium occurred in 15 experiments. It was noted as early as the fifth day of incubation, and tended to become more marked as the period of incubation increased. Incipient digestion of the medium was observed in 4 additional experiments. Therefore evidence of digestion was present in 59.4 percent of the 32 experiments. A putrid odor developed in many of the experiments, and became apparent as early as the tenth day of incubation.

Aleuronat-protein agar. While colonies developed in all 6 experiments, digestion of the protein with clearing of the medium about the colonies occurred in but 2 experiments.

Casein agar. The bacteria proliferated in all 6 experiments. Digestion of the casein with clearing of the medium about the colonies took place in 3 experiments.

Fibrin agar. Colonies developed in all 3 experiments, but the fibrin was not digested to any appreciable extent.

Ovalbumin agar. Growth of the bacteria occurred in all 3 experiments. However, digestion of the coagulated albumin did not occur to an appreciable extent.

Litmus milk. Coagulation of the milk, *i. e.*, the casein, occurred in all 28 experiments. The coagulum was then digested in 22 experiments; evidence of digestion became apparent as early as the fifth day of incubation; usually from fifty to eighty percent of the coagulum had been dissolved by the end of the period of observation. The litmus was bleached in three-fourths of the experiments.

Rosalic acid agars. The number of experiments in which each of these media was used has been recorded above (see page 77). The entire group of experiments may be summarized in tabular form, reporting for each substrate: (a), the percent of experiments in which colonies developed, and (b) the percent of experiments in which alkalinity was produced.

Substrate	Percent of experiments showing	
	Growth	Alkalinity
Ammonium lactate.....	.91.2	88.3
Ammonium tartrate.....	.96.8	83.0
Acetamide.....	.91.2	91.2
Urea.....	.77.4	52.3
Asparagin.....	.97.1	97.1
Glycocoll.....	.94.1	91.2

This alkalinity characterized the colonies and the medium about them, and frequently extended over the entire plate. It could be produced in any one of four ways:—

1. By oxidation of the organic nitrogenous compounds, including the ammonium salts, to ammonium carbonate.

2. By hydrolysis of an amide, *e. g.*, acetamide, and oxidation of the resulting ammonium salt to ammonium carbonate.

3. By desaminization of an amino acid with liberation of ammonia.
4. By decarboxylation of an amino acid with the formation of an amine.

Of the substrates used, acetamide and urea are amides, glycocholic acid is an amino acid, asparagin is both an amino acid and an amide, and ammonium lactate and tartrate are ammonium salts of organic acids.

A grand total of 198 plates of these rosolic acid agars were inoculated. In 22 percent of them an odor of ammonia or amines developed during the earlier portion of the period of incubation.

Toward the end of this period, a fading or bleaching of the rosolic acid occurred in approximately 40 percent of all the plates inoculated.

Litmus agars. The litmus lactose agar and the litmus glucose agar were used in the same 19 experiments. With the lactose medium, a permanent acidity developed in 2 experiments, a primary acidity followed by a secondary alkalinity in 1 experiment, and a permanent alkalinity in 16 experiments. With the glucose medium, a permanent acidity developed in 4 experiments, a primary acidity followed by a secondary alkalinity in 5 experiments, and a permanent alkalinity in 10 experiments.

These results indicate that the bacterial flora of the liquor in open pitchers attacked the peptone of the medium in preference to its carbohydrate. This was especially true of the lactose medium.

Lactose bile salt bouillon. In 38 experiments, the emulsion of bacteria was sown into lactose bile salt bouillon contained in Dunham fermentation tubes. In each of 11 additional experiments, a 2 cc. sample of liquor from an open pitcher was sown into a sterile tube of this medium in the field, using a sterile pipette to collect the liquor from the pitcher and transfer it to the tube. The inoculated tubes were carried to Philadelphia.

The period of incubation with this medium was 5 days at a temperature of 37° C. The production of gas and its collection in the inner inverted tube (positive reaction) was presumptive evidence of the presence of the colon-aerogenes group of bacteria, commonly called the *Bacillus coli* group.

Of the 49 experiments in which this medium was used, gas was produced in 26 experiments, or 53 percent of the total. The test was always negative (no gas-formation) with liquor from open pitchers of *Darlingtonia californica*. The test was also applied to liquor from open pitchers of each species of *Sarracenia* except *S. psittacina*; with each species, some pitchers yielded positive, some negative results, although they were growing near each other. These

results indicate that the *Bacillus coli* group, if present, is introduced by the captured insects and not from the bog water of the habitat. This conclusion is further supported by the experiments on *Sarracenia purpurea*. The 11 field experiments, mentioned above were made with this species. Two bogs lay on opposite sides of a railroad, and a stream of water flowed through both bogs. Of 7 samples of pitcher liquor, taken in one bog, 5 gave positive and 2 negative results. Of 4 samples, taken in the other bog, all yielded negative results. The liquor from 12 open pitchers growing in these bogs was also studied, using the agar slant technic. The bacterial emulsions thus obtained gave 9 positive and 3 negative tests for the presence of the *B. coli* group. Therefore, of 23 open pitchers in these bogs, 14 (61 percent) presumptively contained members of the colon-aerogenes group while 9 did not. Duplicate samples of water, each containing 2 cc., were taken from the stream, and from each bog in the immediate vicinity of the pitchers whose liquor was used in the field tests. Sterile pipettes were used to collect the water and transfer it to the sterile tubes of the medium. The samples of water and of pitcher liquor were taken at the same time. The stream water and the water of both bogs did not produce gas in the medium. Therefore the organisms of the *B. coli* group, which were present in 61 percent of the *Sarracenia purpurea* pitchers examined, did not come from the surrounding water, and must have been carried into the pitchers by the prey. The absence of this group of microorganisms from the open pitchers of *Darlingtonia californica* was doubtless due to the very sparsely settled region in which that species grows, and the consequent failure of its prey to become infected by members of the group.

Trypsinized peptone. With this medium, the period of incubation was 5 days at a temperature of 37° C. The test for the presence of indol was then made by means of para-dimethylaminobenzaldehyde and hydrochloric acid. A purplish red color developed if indol had been produced in the medium by the bacteria. A positive reaction for indol was obtained in 21 experiments, *i. e.*, in 75 percent of the 28 experiments in which this medium was used.

Now in these 28 experiments, the lactose bile salt bouillon had also been employed, and gas had been produced in 15 experiments, *i. e.* in 53.6 percent.

Since indol formation occurred in a higher percent of these experiments than did gas formation, the conclusion may be drawn that indol-forming bacteria, other than members of the *B. coli* group, may be present in the liquor of open pitchers.

GENERAL SUMMARY

Closed pitchers. The pitcher cavity and liquor of closed pitchers invariably was bacteriologically sterile.

Open pitchers. The liquor from open pitchers, which had captured prey, invariably contained bacteria. The bacterial flora of this liquor always included species which digest proteins. Gelatin was always liquefied. Loeffler blood serum and the casein of litmus milk were digested in approximately 80 percent of the experiments in which these respective media were used. The proteins of Dorset egg medium were attacked in about 60 percent of the experiments in which it was employed. The proteins in the various protein-agars were not markedly attacked, but supported growth of the bacteria. As a rule, the bacteria from a given open pitcher produced proteolysis of at least 2 or 3 substrates and frequently of 4 or 5 substrates.

However, the bacteria digested the proteins so slowly that their part in the digestion of the prey must be a minor one on the genus *Sarracenia*, the protease of the pitcher liquor playing the leading rôle. The bacteria apparently live in symbiosis with the *Sarracenias*, drawing their nutriment from the digested insects, and aiding, to a certain extent, in the digestion of the prey. Since the pitcher liquor of *Darlingtonia californica* does not contain a protease, the proteolytic bacteria, present in the open pitchers of this species, must be the chief factor in the digestion of the prey. Doubtless, in the pitchers of both genera (*Sarracenia* and *Darlingtonia*), tissue enzymes of the captured insects participate in the digestion by virtue of their autolytic action.

A putrid odor was frequently produced by the action of the bacteria on the Loeffler blood serum and the Dorset egg medium. This phenomenon recalls the observations concerning the presence of hydrogen sulphide in the pitcher contents of *Darlingtonia californica* several days after egg white had been introduced, see page 73.

The bacteria exhibited a marked tendency to attack protein (peptone) in preference to carbohydrate. This was shown by the production of an alkaline reaction in the litmus agars, especially litmus lactose agar. When the bacteria from a given pitcher produced an alkaline reaction in the litmus agars, they also produced an alkaline reaction in the rosolic acid agars.

The bacteria were able to use amino acids, amides, and the ammonium salts of organic acids as their sole source of nitrogen and carbon, and, as a result, render the reaction alkaline to rosolic acid; and, at times, they produced

an odor of ammonia and amines. As a rule, the bacteria from a given pitcher grew and produced an alkaline reaction in 5 or 6 of the rosolic acid media. An alkaline reaction to rosolic acid means a hydrogen-ion concentration, pH, numerically equal to or greater than 8.0,⁸⁶ or a specific alkalinity numerically equal to or greater than 10. However, the pitcher should be able to maintain the normal reaction of its liquor even if basic compounds be produced in its cavity, just as it maintains the normal reaction when dilute acid or dilute alkali is introduced into the cavity (see page 44).

Members of the colon-aërogenes group of bacteria were present in the liquor of over half of the open pitchers. They apparently were introduced into the pitcher cavity by the prey.

Indol-producing bacteria, not members of the colon-aërogenes group, also were frequently present in the liquor of open pitchers.

BIBLIOGRAPHY

By JOSEPH SAMUEL HEPBURN, A.M., B.S. in Chem., M.S., Ph.D.

(Citations 1 to 68, both inclusive, also 87, 89, 94, 95, refer to insectivorous plants. Those from 36 to 63, both inclusive, refer only to the use of the Sarracenia in medicine and have not been reviewed in this series of papers.) The rest of the citations refer to methods of research.

1. Macbride: *Transactions of the Linnean Society of London*, London, 1818, 12, 48-52.
2. Hooker: *Nature*, London and New York, 1874, 10, 366-372.
3. Hooker: *Report of the Forty-third Meeting of the British Association for the Advancement of Science*, 1874, *Notes and Abstracts of Miscellaneous Communications to the Sections*, London, 1875, 102-116.
4. Mellichamp: *Proceedings of the American Association for the Advancement of Science*, Twenty-third Meeting, 1874, Salem, 1875, B, 113-133.
5. Edwards: *Proceedings of the California Academy of Sciences*, 1875, San Francisco, 1876, 6, 161-166.
6. Canby: *Proceedings of the American Association for the Advancement of Science*, Twenty-third Meeting, 1874, Salem, 1875, B, 64-72.
7. Amcs: *California Horticulturist and Floral Magazine*, San Francisco, 1880, 10, 225-229.
8. Batalin: *Acta Horti Petropolitani*, St. Petersburg, 1880, 7, 343-359.
9. Schimper: *Botanische Zeitung*, Leipzig, 1882, 40, 225-233.
10. Higley: *Bulletin of the Chicago Academy of Sciences*, Lake Geneva, Wisconsin, 1885, 1, 41-55.
11. Zipperer: *Inaugural-Dissertation*, Erlangen, 1886; *Botanisches Centralblatt*, Cassel, 1887, 29, 358-359.
12. Goebel: *Pflanzenbiologische Schilderungen*, Elwert, Marburg, 1893, 2, 166-170. The work on the genus *Sarracenia* but not that on *Darlingtonia* is summarized in *Naturwissenschaftliche Rundschau*, Braunschweig, 1893, 8, 566-568.
13. Lambert: *Annales d'hygiène et de médecine coloniales*, Paris, 1902, 5, 652-662.
14. Fenner: *Flora oder allgemeine botanische Zeitung*, Marburg, 1904, 93, 335-434; especially pages 351-357.
15. Robinson: *Torreya*, New York, 1908, 8, 181-194.
16. Mellichamp: *American Naturalist*, Boston, 1877, 11, 432-433.
17. Watson: *American Naturalist*, Boston, 1877, 11, 564.
18. Hepburn, St. John, and Jones: *Proceedings of the American Philosophical Society*, Philadelphia, 1918, 57, 127-129.
19. Hepburn, St. John, and Jones: *Journal of the Franklin Institute*, Philadelphia, 1920, 189, 147-184.
20. Hepburn and Jones: *Contributions from the Botanical Laboratory of the University of Pennsylvania*, Philadelphia, 1919, 4, 460-463.
21. Burnett: *Quarterly Journal of Science, Literature, and Art*, London, 1829, July to December, 279-292.
22. Gray: *Darwiniana*. Appleton, New York, 1876, 330; copyright 1876.
23. Porcher: *Charleston Medical Journal and Review*, Charleston, 1849, 4, 1-13.
24. Björklund and Dragendorff: *Pharmaceutische Zeitschrift für Russland*, St. Petersburg, 1863-1864, 2, 317-325, 344-352, 369-378.
25. Björklund and Dragendorff: *Archiv der Pharmacie*, Hannover, 1864, 169 (2 Riehe, 119), 93-124. Reprint of citation 24.
26. New York County Medical Society. Report of Committee on *Sarracenia purpurea*: *American Medical Times*, New York, 1864, 8, 7-8, 18-19.
27. Martin: *Bulletin générale de Thérapeutique médicale et chirurgicale*, Paris, 1865, 63, 308-311.

28. Martin: *Journal dem édecine, de chirurgie et de pharmacologie* publié par la Société des Sciences médicales et naturelles de Bruxelles, Bruxelles, 1865, 40, 470-471. Reprint of citation 27.
29. Martin: *Annuaire de Thérapeutique de matière médicale de pharmacie et de toxicologie*, 1866, 26, 72-73. Abstract of citation 27.
30. Schmitt: *Gazette Médicale de Strasbourg*, Strasbourg et Paris, 1871-1872, 31, 78-79.
31. Vines: *Journal of Anatomy and Physiology*, Cambridge and London, 1876-1877, 11, 124-127.
32. Hetét: *Comptes rendus des séances de l'Académie des Sciences*, Paris, 1879, 88, 185.
33. Gies: *Journal of the New York Botanical Garden*, Lancaster, Pa., 1903, 4, 37-39.
34. Meyer and Gies: *American Journal of Physiology*, Boston, 1905, 13, xxxiii-iv.
35. Clark: *Dissertation*, Faculty of Pure Science, Columbia University, Easton, Pa., 1910, 79.
36. Palmer: *American Journal of Pharmacy*, Philadelphia, 1869, 41 (3 Series, 17), 292, 396.
37. Roe: *Therapeutic Gazette*, Detroit, 1880, 4 (New Series, 1), 224.
38. Rusby: *Therapeutic Gazette*, Detroit, 1884, 8 (New Series, 5), 546-548.
39. Miles, H. C.: *Transactions of the Epidemiological Society of London*, London, 1863, 1, 278-281; read November 4, 1861.
40. Miles, H. C.: *Lancet*, London, 1862, 2, 430-431.
41. Morris: *American Medical Times*, New York, 1862, 4, 297-298.
42. Morris: *Chicago Medical Examiner*, Chicago, 1862, 3, 438-440.
43. Morris: *Journal of Materia Medica*, New Lebanon, N. Y., 1862, New Series, 4, 346-347.
44. Morris: *American Journal of Pharmacy*, Philadelphia, 1862, 34 (3 Series, 10), 362-364.
45. Miles, H. C.: *Lancet*, London, 1862, 2, 665.
46. Miles, C.: *Lancet*, London, 1862, 2, 241.
47. Morris: *Lancet*, London, 1862, 2, 638.
48. Logie: *American Journal of Pharmacy*, Philadelphia, 1866, 38 (3 Series, 14), 308-310.
49. Cigliano: *Il Dinamico*, 1871. Translation by Lilienthal in *American Observer*, Detroit, 1871, 8, 467-470.
50. Editorial Note: *Medical and Surgical Reporter*, Philadelphia, 1862, 8, 391.
51. Roe: *Therapeutic Gazette*, Detroit, 1880, 4 (New Series, 1), 6-7.
52. The Homeopathic Pharmacopœia of the United States Published under the Direction of the Committee on Pharmacopœia of the American Institute of Homœopathy, 3d edition, Revised, Otis Clapp & Son, Agents, Boston, 1914, 510-511; copyright 1914.
53. The American Homœopathic Pharmacopœia, 8th edition, Boericke & Tafel, Philadelphia, 1906, 402-403; copyright 1904.
54. Bute: *Hahnemannian Monthly*, Philadelphia, 1867, 2, 424.
55. Oehme: *Hahnemannian Monthly*, Philadelphia, 1876, 11, 535.
56. Macfarlan: *Homeopathic Physician*, Philadelphia, 1892, 12, 100, 527; 1893, 13, 376, 382, 441, 535.
57. Allen: *Encyclopedia of Pure Materia Medica*, Boericke and Tafel, New York and Philadelphia, 1878, 514-526; copyright 1878.
58. Hering: *Guiding Symptoms of Our Materia Medica*, Estate of Constantine Hering, Philadelphia, 9, 222-228; copyright 1890.
59. Crica: *Journal of Homœopathic Clinics*, Philadelphia, 1869, 3, 78. Also cited as *American Journal of Homœopathic Materia Medica*.
60. Berridge: *North American Journal of Homœopathy*, New York and San Francisco, 1874, 22 (New Series, 4), 194.
61. Mouremans: *Revue homœopathique belge*, Bruxelles, 1874, 1, 61-69. Abstracts of this paper appeared in *Hahnemannian Monthly*, Philadelphia, 1874, 10, 178, and in *Annual Record of Homœopathic Literature* (Raue), New York and Philadelphia, 1875, 6, 287-288.
62. Hale: *Homeopathic Materia Medica of the New Remedies*, 2d edition, Lodge, Detroit, 1867, 937-961; copyright 1866.
63. Houat: *North American Journal of Homœopathy*, New York, 1869, 18, 70-86.
64. Hepburn: *Contributions from the Botanical Laboratory of the University of Pennsylvania*, Philadelphia, 1919, 4, 419-442.
65. Hepburn: *Journal of the Franklin Institute*, Philadelphia, 1922, 194, 771-781.

66. Hepburn: *Contributions from the Botanical Laboratory of the University of Pennsylvania*, Philadelphia, 1919, 4, 442-451.
67. Dakin: *Journal and Proceedings of the Royal Society of Western Australia*, 1917-1918, Perth, 1919, 4, 37-53.
68. Hepburn and St. John: *Contributions from the Botanical Laboratory of the University of Pennsylvania*, Philadelphia, 1919, 4, 451-457.
69. Browne: *A Handbook of Sugar Analysis*, 1st Edition, Corrected, Wiley, New York, copyright 1912, 197, 391.
70. Spencer, Meyer, Reh fuss, and Hawk: *American Journal of Physiology*, Baltimore, 1916, 39, 459-479.
71. du Noüy: *Journal of General Physiology*, New York, 1919, 1, 521-524.
72. Central Scientific Company, *Bulletin* 82, Chicago, 1921.
73. Wherry: *Journal of the Washington Academy of Sciences*, Baltimore, 1916, 6, 672-679.
74. Wherry: *Journal of the Washington Academy of Sciences*, Baltimore, 1918, 8, 589-598.
75. Wherry: *Journal of the Washington Academy of Sciences*, Easton, 1919, 9, 305-309.
76. Wherry: *Journal of the Washington Academy of Sciences*, Easton, 1920, 10, 217-223.
77. Wherry and Adams: *Journal of the Washington Academy of Sciences*, Easton, 1921, 11, 197-202.
78. Wherry: *Ecology*, Lancaster, Pa., 1920, 1, 160-173.
79. Wherry: *Annual Report of the Board of Regents of the Smithsonian Institution* for the year ending June 30, 1920, Washington, 1922, 247-268.
80. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Second Edition. Washington, 1925, 115, 180.
81. *Pharmacopœia of the United States of America*, 10, Philadelphia, 1925, 352-354.
82. Rivas: *Journal of the American Medical Association*, Chicago, 1908, 50, 1492-1495.
83. Rivas: *Centralblatt für Bakteriologie, Parasitenkunde, und Infektionskrankheiten, Erste Abteilung, Originale*, Jena, 1912, 63, 547-550.
84. Crabill and Reed: *Biochemical Bulletin*, New York, 1915, 4, 30-44.
85. Gotschlich: *Kolle und Wassermann, Handbuch der pathogenen Mikroorganismen*, 2. Auf., Jena, 1912, 1, 102.
86. Clark: *The Determination of Hydrogen-Ions*, Williams and Wilkins Company, Baltimore, 1920, 67.
87. Clautriau: *Mémoires couronnés et autres mémoires publiés par l'Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique, Collection in 8°*, Bruxelles, 1899-1900, 59, third memoir.
88. Van Slyke and Cullen: *Journal of the American Medical Association*, Chicago, 1914, 62, 1558-1559.
89. Pfeffer: *Landwirtschaftliche Jahrbücher*, Berlin, 1877, 6, 969-998.
90. Levy, Rowntree, and Marriott: *Archives of Internal Medicine*, Chicago, 1915, 16, 389-405, especially 392.
91. Sörensen: *Biochemische Zeitschrift*, Berlin, 1909, 22, 352-356.
92. Sörensen: *Ergebnisse der Physiologie*, Wiesbaden, 1912, 12, 393-532, especially 437.
93. Giles: Sutton, *Systematic Handbook of Volumetric Analysis*, 10th edition, Philadelphia, Blakiston, 1911, 309, footnote.
94. Wherry: *Proceedings of the Academy of Natural Sciences of Philadelphia*, Philadelphia, 1920, 72, 84-111, especially 87.
95. Macfarlane. Sarraceniaceæ, in Engler, *Das Pflanzenreich*, 34 Heft, 4, 110. Leipzig, Engelmann, 1908.