TRANSACTIONS OF THE WAGNER FREE INSTITUTE OF SCIENCE OF PHILADELPHIA

VOLUME XI

APRIL, 1927

THE BIOCHEMISTRY OF THE AMERICAN PITCHER PLANTS

HEPBURN, ST. JOHN, AND JONES

WAGNER FREE INSTITUTE OF SCIENCE OF PHILADELPHIA

MONTGOMERY AVE. AND SEVENTEENTH STREET
PHILADELPHIA

BIOCHEMICAL STUDIES OF THE NORTH AMERICAN SARRACENIACEAE

By

JOSEPH SAMUEL HEPBURN, A.M., B.S. IN CHEM., M.S., PH.D.

FRANK MORTON JONES, F.E.S.

and

ELISABETH QUINTARD ST. JOHN, M.D.

CONTRIBUTIONS FROM THE CONSTANTINE HERING RESEARCH LABORATORY, HAHNEMANN MEDICAL COLLEGE OF PHILADELPHIA

INTRODUCTION

THE observations and experiments forming the basis of the following papers on the North American *Sarraceniaceæ* had their beginning in 1892 when *Sarracenia flava, S. rubra,* and *S. purpurea* came under observation in Richmond County, North Carolina. In succeeding years all the species have been under frequent observation in their native habitats, and have been made the subject of several papers on insect-plant relations by one of us (Jones).

Field and laboratory experiments on the biochemistry of the *Sarraceniaceæ* were commenced in 1917, and have been continued until the present time. These researches form part of a general study of insectivorous plants, work on *Nepenthes* having been begun by Hepburn and St. John in 1914. *Dionaea* has been made the subject of a recent paper.

Observations and field experiments have been made or material for laboratory examination collected chiefly near the following localities for each species:

Darlingtonia californica.—Keddie, Plumas Co., and Mt. Eddy (the type locality), Siskiyou Co., Cal.

Sarracenia minor.—Summerville, Berkeley Co., S. C.; Jacksonville, Duval Co., Fla.

Sarracenia Sledgei.—Mobile, Theodore, and Bayou La Batre, Mobile Co., Ala.; Biloxi, and Wiggins, Harrison Co., Miss.

Sarracenia flava.—Southern Pines, Moore Co., Hamlet, Richmond Co., and Wilmington, New Hanover Co., N. C.; Summerville, Berkeley Co., S. C.; De Funiak Springs, Walton Co., Fla.; Bay Minette, Baldwin Co., Ala.

Sarracenia Drummondii.—De Funiak Springs, and Freeport, Walton Co., Fla.; Bayou La Batre, and Theodore, Mobile Co., and Bay Minette, Baldwin Co., Ala.

Sarracenia rubra.—Southern Pines, Moore Co., and Hamlet, Richmond Co., N. C.; De Funiak Springs, Walton Co., Fla.

Sarracenia purpurea.—Tolland, Tolland Co., Conn.; Whitings, and Toms River, Ocean Co., N. J.; Pocono Pines, Monroe Co., Pa.; and many other localities from Maine to Mississippi.

Sarracenia psittacina.—De Funiak Springs, Walton Co., Florida; Bayou La Batre, Mobile Co., and Bay Minette, Baldwin Co., Ala.; and Ocean Springs, Jackson Co., Biloxi, and Wiggins, Harrison Co., Miss.

Samples of pitcher liquor and aqueous solutions of the nectar, which were collected in the field for laboratory examination at Philadelphia, were preserved by addition of 0.2 percent of trikresol. The collection of material for bacteriological study and for chemical examination of the plant tissues is described in detail in the papers on these respective subjects.

In this series of papers the authors have followed Macfarlane[95] in the nomenclature of the *Sarraceniaceæ*. They are indebted to Dr. Macfarlane for many helpful suggestions in the course of these researches.

The authors record their indebtedness to the Franklin Institute of the State of Pennsylvania for permission to use portions of a paper on absorption of nutrients and allied phenomena in the pitchers of the *Sarraceniaceæ*, originally published in the *Journal of the Franklin Institute*, February, 1920. The Institute has also permitted the reprinting of several illustrations from its *Journal*.

The American Museum of Natural History has granted permission to reprint three illustrations from its journal, *Natural History*.

WORK OF PREVIOUS INVESTIGATORS ON THE BIOCHEMISTRY OF THE SARRACENIACEÆ

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

DIGESTION AND ABSORPTION

MACBRIDE[1] studied *Sarracenia adunca* (*S. minor, S. variolaris*) and *S. flava* in their native habitat. His observations were made chiefly during 1810 and 1811 and were communicated to the Linnean Society of London in 1815. He noted the masses of captured insects within the pitchers and wrote: "What purposes beneficial to the growth of these plants may be effected by the putrid masses of insects, I have never ascertained."

Hooker[2,3] found "a slight acid secretion" in young pitchers of *Darlingtonia californica*. He states that the pitchers of both *Sarracenia variolaris* (*S. minor*) and *S. flava* secrete a fluid. However, the pitchers of *S. variolaris* examined by him contained no secretion. He also examined both half-grown and full-grown pitchers from cultivated plants of *S. flava;* they contained no fluid except what may have been accidentally introduced. According to Hooker, secretion of "water" by the pitchers of *S. purpurea* had never been observed, and he suggested "the possibility of this plant either having no proper secretion of its own, or only giving it out after the pitcher has been filled with rain water." He considered it quite likely that the "digestive functions" in *Sarracenia* pitchers may be of short duration. Digestion experiments were not made with the *Sarraceniaceæ*.

Mellichamp[4] made observations on *Sarracenia variolaris* (*S. minor*) growing in its native habitat in the vicinity of Bluffton, South Carolina. Unopened pitchers usually contained from 3 to 5 drops of liquor, occasionally as many as 10 drops, rarely 15 drops. The liquor was bland and somewhat mucilaginous to the taste; it left an astringent taste in the mouth. Perfect, open pitchers usually contained from 10 to 15 drops of liquor, very rarely a half-drachm. Mellichamp adds: "*I have, however, since found the fluid much increased in quantity*, very frequently a drachm, and *sometimes as much as two*

drachms." He states that the secretion of pitcher liquor "appears to continue during the whole period of the entrapment, which I suppose does not last over two or three weeks at most."

"During this active period the 'water' is attracted to the top of the mass and permeates and percolates through every portion of it so that eventually all the soft parts of the insects are thoroughly dissolved. After these animal juices have been partially or entirely absorbed by the plant, or by the larva of the Sarcophaga fly which continues to grow and fatten upon this rich diet; the remaining portion, commences gradually to dry, until only the backs and legs and shells of the various insects remain."

The digestive action of the pitcher liquor was tested on venison, apparently *in vitro*, without a bactericide. Bits of fresh venison were immersed in the pitcher liquor and in a corresponding amount of pure water. After the lapse of 15 hours, the venison in the pitcher liquor was more changed, softened, and broken up, and far more offensive to the nostrils (being offensive to a disgusting degree) than was the venison in the water. Mellichamp concluded that this experiment perhaps showed that the liquor hastened the decomposition of the insects and their conversion into "liquid manure."

Edwards[5] considered that the pitcher liquor of *Darlingtonia californica* did not possess true digestive power, but was able to "cause decomposition" of the prey. He stated: "I do not attempt to speak authoritatively upon the subject, but I am inclined to think that no process similar to digestion goes on within the plant, but that the fluid mass derived from the decay of the imprisoned insects descends through the tube into the earth, and is taken up by absorption, through the roots, thus acting as a kind of liquid manure. It is true that in the dead leaves the hard integuments of insects, such as the elytra of beetles, and the bodies of wasps and hornets are to be found undecayed, but this may be because the liquid secreted by the plant is not powerful enough to cause decomposition of these parts before the plant itself decays." He commented on the "most disgusting" smell of the pitcher contents, and recorded that "after handling a number of specimens of the tubes, it is necessary to use some disinfectant like ammonia or chloride to remove the disagreeable odor."

The statements of Canby[6] concerning *Darlingtonia californica* were based in part on the observations of his correspondent, Lemmon. Canby mentions the secretion of a pitcher liquor by *Darlingtonia*. "Several inches

of the bottom of the tube are filled with a clear fluid (secreted by the leaves it must be). . . . Mr. Lemmon has kindly sent me an ounce phial completely filled with the fluid 'from two petioles' . . . It is scarcely necessary to say, that as it is certain no water can get into the tube by any ordinary means, and as the fluid is always present in healthy leaves, it must be secreted by the plant as Mr. Lemmon says."

Canby also mentions the offensive odor of a patch of *Darlingtonia* plants whose pitchers contained prey. "Mr. Lemmon further says 'I came upon a patch once in September and smelled it from afar so offensive was it. A portion of the leaves filled with insects to the depth of four to six inches, had fallen down apparently from the weight of the fluid and insects.'"

The response of *Darlingtonia californica* to food stimulation was discovered by Mrs. R. M. L. Austin who studied these plants in their native habitat, Butterfly Valley, Plumas County, California. Mary E. Pulsifer Ames[7] mentions Mrs. Austin's researches, and publishes the following extract from a letter received by herself from Mrs. Austin. "In July, 1875, I fed a great many of the leaves, some with fresh raw mutton and others with that which was boiled. The liquid, in the course of a week, would fill the tubes and flow out of the orifice." Asa Gray[22] states that Mrs. Austin, studying *Darlingtonia californica*, found that "the watery liquid in the pitcher, which must be wholly a secretion, is much increased in quantity after the capture of insects."

Batalin[8] described certain changes in the cell-wall of the pitcher-lining of *Darlingtonia californica* and of several species of *Sarracenia* after prey had been captured. He concluded that these changes facilitated the absorption, by both genera, of the products derived from the captured insects.

Pitchers of green-house plants of *Sarracenia flava* never contained any liquor, although their inner surface was frequently moist. The outer surface of the pitcher always was green. The inner surface of the lower part of the pitcher was green when insects had not yet been captured, and became a brownish yellow at those places where captured insects adhered to the wall. This change affected only the interior surface of the pitcher, and occurred only in its lower or detentive zone. Sections of the inner surface of this zone were cut. When the inner surface was green and free from adhering insects, the cavity surface of the epidermal cells was quite smooth, homogeneous, and free from markings of any kind. When insects were adhering to the epidermal cells, the cavity surface of each cell contained one or two paler areas surrounded

by a more or less broad, intense yellowish-brown margin which lay at a higher level. The epidermal cells are provided with a cuticula which forms the outermost layer of the cell membrane. In cells, to which insects had adhered, the cuticula had become detached from the paler colored areas; the margin was the remaining portion of the cuticula. Cells, which adjoined the altered cells but were free from insects, had not lost any of their cuticula and were free from paler areas.

Some sections revealed the manner in which the cuticula was detached. Bubbles appeared, as if some substance were secreted, between the cellulose membrane and the cuticula. The bubbles were of a brighter yellow than the cuticula. The secreted substance, the nature of which is unknown, gradually collected in greater amount, produced a further separation of the cellulose membrane from the cuticula, rendered the latter yellow and viscous or gelatinous, and finally caused detaching of the cuticula. At times not only the cuticula, but possibly the entire cuticular layer was cast off from the paler areas. The secreted substance owed its origin to a stimulus exerted on the epidermal cell by the adhering insect. Occasionally the cuticula was detached from as many as ten areas on a single cell; these areas were then surrounded by a yellow network of the remaining cuticula. The detentive hairs spring from cells which are smaller than those participating in these changes.

In *Sarracenia purpurea*, the change in the cuticula was almost the same as in *S. flava*. In the lower portion of the wide-open pitcher, each of the altered cells lost the cuticula from an oval area or from two areas. In the narrower portion of the pitcher, near its bottom, the cuticula might be cast off from as many as ten or twelve areas on a single cell and, at times, might become detached from a number of adjacent cells as a thin unbroken membrane which showed distinctly the contour of the individual cells.

In old pitchers of *Sarracenia variolaris* (*S. minor*) which had not captured insects, the cuticula was not homogenous in the lower or detentive zone, but was extremely thin or entirely lacking on certain regions, especially on the clefts which occurred at the outer edge of the cuticula where it met the inner border of the projection of the side wall of the cell. These clefts consisted of two or three rows of extremely small points or irregular, four-cornered, rounded areas, or of a single row of larger areas; occasionally these larger areas united into two or three still larger areas, between which were smaller areas or points. Other points and areas of this type occurred on the surface of the cuticula.

When the cuticula was cast off, detachment usually took place along these clefts so that only narrow strips of cuticula remained as scollops. At times, a cell would lose the cuticula from two or three isolated areas instead of a single large area. The remnants of the cuticula were usually yellow, and apparently gelatinized; the cuticula-free surface of the cell and its side walls were almost colorless.

Only old pitchers of *Darlingtonia californica* were available for study. In the detentive zone, the epidermis resembled that of *Sarracenia variolaris*. The areas, on which the cuticula was extremely thin or entirely lacking, were similar to those of *S. variolaris* with respect to both shape and distribution; occasionally they were so strewn over the entire surface of the cell that it resembled a sieve-plate. The cuticula was thus modified only in those parts of the pitcher cavity which contain long, stiff hairs. These modified areas were almost entirely lacking in the upper region with its short thin hairs. Clefts of large surface area predominated in the middle portion of the pitcher. In the lower, narrow portion, the modified areas were points, scattered over the cuticula in such large numbers that its surface was granular. Detachment of the cuticula could occur in all parts of the cavity of the pitcher; it was cast off from the entire surface of the cell along the line of the modified areas; only a remnant of cuticula was left between adjoining cells.

Zinc chloride plus iodine was of service as a stain in study of the sections.

Batalin drew the following conclusions from his research: "The physiological significance of the phenomena here described is quite clear. They can be considered as processes which facilitate for the plant the absorption of dissolved substances from without. It has been known for a long time that the cuticula is that part of the epidermis membrane which offers the greatest resistance to the penetration of substances into the cell. Therefore one can look upon its detachment as an adaptation for facilitating this entrance of substances into the plant. Accordingly in these two genera we have an interesting example of adaptation of the plants for particular purposes. . . . In *Sarracenia* and *Darlingtonia*, in consequence of the almost complete lack of glands in the pitchers, their function is here taken over by the entire inner surface; the epidermis is changed for this purpose in such a way that the absorption of solutions is rendered possible. From the described process, the manner and way in which the cuticula is cast off, it is seen that a substance is secreted between it and the cellulose membrane. How Nature has provided

this secretion is unknown to me; it is not improbable that it contains the solvent (Lösungsmittel) for the digestion of the proteins."

"These pitcher plants must be grouped with those which satisfy their nitrogen requirement through the captured insects. . . . They take up the nitrogen which they require directly from the insects . . . and not indirectly by manuring the soil on the surface of which the dead leaves plus the captured insects decay."

Schimper[9] studied *Sarracenia purpurea* growing wild on the Massachusetts coast. When the plants grew in the open, the pitchers usually contained water, i. e., liquor. Observations made on plants under cultivation led to the conclusion that a very small portion of this liquor is secreted by the pitcher itself; the major portion owes its origin to the rain. Secretion of liquor occurs in young pitchers, indeed long before opening. Acid-reacting droplets occur on the lower hairy region as well as on the middle region of smooth epidermis, and collect at the bottom of the pitcher.

Digestion experiments were made; small pieces of meat were introduced into several pitchers; other pitchers were permitted to catch insects. A control experiment was made at the same time by introducing small pieces of meat into water contained in a glass vessel. The meat dissolved very slowly in the liquor within the pitchers; in fact, the rate of solution did not exceed that in the control experiment; hence it was concluded that the pitcher liquor did not contain pepsin. Moreover, the presence of bacteria was definitely shown. Introduction of nitrogenous compounds did not increase the acidity of the pitcher liquor.

Schimper stated: "Innumerable worms were present in all the leaves studied, these possibly participate in the transformation of the animal bodies into soluble compounds." This may be construed to mean that the larvæ of the insect associates possibly play a part in the digestion of the prey.

The decomposition products of the prey were absorbed. This was definitely shown by certain changes which occurred in the epidermis cells in the bottom portion of the pitcher, and, to a lesser degree, in the cells of the adjacent subepidermal layer. As already stated, some pitchers were nourished by captured insects or by introduced meat. Other pitchers were, in a sense, starved by plugging the mouth with tissue paper as soon as the lid had opened. A striking difference was observed between the nourished and the starved pitchers. The cell sap of the cells in question is rich in tannin; it formed one,

two, or more very strongly refractive, glittering drops in the nourished pitchers; in starved pitchers it formed, at the most, a single drop which possessed these properties to a very slight degree and occupied a much larger space. The drops were not suspended in the cell sap, but represented the entire cell sap. Food caused a marked removal of the chlorophyll granules from the cell wall and their occurrence about the vacuoles or drops. The substances of animal origin in solution in the pitcher liquor acted as a stimulus; the protoplasm acquired greater power of imbibition, and withdrew water from the cell sap. The colloidal tannin was unable to pass through the protoplasm by osmosis and remained in the cell sap, which thus became more concentrated and acquired a higher refractive power, while the protoplasm became less refractive. These phenomena were not produced exclusively by nitrogenous compounds, but were obtained when either a dilute solution of sodium chloride, or sea water, or borax was used as a stimulus. Pure water was without stimulating action.

The "nourished" appearance was true of the cells at the base of the hairs even before the pitcher lid had opened. It was frequently noted at the border of a section in cells which had either been cut or rested directly on cut cells, and was then due to the disorganization of the cell contents. Therefore only somewhat thick sections were of value.

Schimper concluded that absorption certainly occurs through the entire interior surface of the hairy bottom portion of the pitcher. The thick-walled hairs have no function other than preventing the escape of the prey. If the pitchers contain very little liquor and many insects, the surface tissues become brown and decomposed. Pitchers of this type, which are extremely rare in plants growing in the open, apparently served Batalin in his studies. The property of the protoplasm to attain a greater degree of capacity for imbibition under the stimulus of certain substances appeared to be, in all likelihood, of direct significance for the nutrition of the plant. The very marked swelling would produce a widening of the micellar pores and a marked increase in the diosmotic properties of the protoplasm; as a result, the entrance into the tissues of the substances contained in the pitcher liquor would at least be facilitated, possibly be first made possible. Therefore the insect captures, in all probability, are actually of value to the plant.

Higley[10] made an extensive series of analyses of the pitcher liquor of *Sarracenia purpurea*, growing in the open, probably in Wisconsin.

His findings would indicate that the water content of the pitcher liquor is collected rain. "As a result of the examination of over 800 leaves I find that none contained any fluid before they had opened. . . . After opening there is no fluid till after the first rain except in a few cases when there has been a heavy dew."

One hundred analyses were made in order to ascertain the composition of the pitcher liquor before it contained any insects. Twenty-five of these analyses are published and may be summarized. The samples of pitcher liquor were collected from 2 to 20 days after the pitcher had opened to such an extent that rain could easily enter it, and from 1 to 18 days after rain had fallen. As to color, 13 samples were clear, 1 slightly yellow, 6 yellow, 3 tinged, and 2 dirty. The amount of solids and acids and bases, which were present, are reported in the table. The organic solids "consisted, to a great extent, of pollen, various other vegetable structures, Infusoria, algæ and the like." The reaction was "nearly neutral" in 4 samples, "slightly acid" in 11 samples, and "acid" in 10 samples.

CHEMICAL CONSTITUENTS OF THE PITCHER LIQUOR OF *SARRACENIA PURPUREA*, ACCORDING TO HIGLEY.

			Liquor prior to capture of insects.	*May samples.*	*June samples.*	*July samples.*	*August samples.*
Number of analyses published			25	10	10	10	10
Solids, parts per 1000	Organic..	Maximum	20	130	173	207	260
		Minimum	2	60	120	137	164
	Inorganic	Maximum	5	8	13	12	18
		Minimum	1	2	5	6	8
Number of samples containing	Ammonium*		25	10	10	10	10
	Sodium		12	9	10	10	10
	Potassium		24	10	10	10	10
	Magnesium		2	5	10	10	
	Calcium		19	8	10	10	10
	Aluminium		6	3	10	5	8
	Iron		4	3	7	7	10
	Silica		1		3	7	4
	Carbonates		25	10	10	10	10
	Chlorides		18	10	10	10	10
	Sulphates		9	3	10	5	8

On the last day of each month—May, June, July, and August—100 samples of pitcher liquor were collected, and analyzed; for each month, 10 typical analyses, including the extremes, were published. The data concerning solids and occurrence of acids and bases are summarized for each month in the table.

* A trace of nitric acid was probably present as ammonium nitrate in these samples.

The May samples were gathered from 1 to 23 days after rain had fallen. The reaction was acid in all 10 of the published analyses; the color of the liquor was "yellow" in 2, "dark" in 2, "dirty" in 3, and "wine" in 3 samples.

The June samples were collected from 1 to 7 days after rain had fallen; the reaction was acid in the entire 10 published analyses; 1 sample was "wine" color and 9 samples "dirty."

The July samples were obtained from 3 to 15 days after rain had fallen; the entire 10 published analyses were characterized by an acid reaction and a "dirty" color.

The August samples were collected from 5 to 20 days after rain had fallen; all 10 published analyses showed an acid reaction and a "dirty" color.

The pitchers, from which the liquor was procured for analysis, represented the growth of the season up to the date of collection. Each pitcher was cut open, and the contents were used only when they were of such consistency that they flowed easily.

The acidity of the pitcher liquor became greater each month, and was especially marked during July and August. "To just what acid, if any particular one, the reaction was due in the liquid of the earlier pitchers is not certain, but in the last two months both malic and citric acids appeared, the former in greater abundance."

The source of the malic acid is indicated: "It seems highly probable that the first lot of insects merely decay after maceration in the water first collected in the pitchers and that this mass acts not only as a stimulant to further decay but also to render the liquid more capable of absorbing certain organic principles from the leaf, such as Malic acid, which aid in the preparation of the abundant supply of food for absorption by the leaf. Thus the first mass might be called a digestive excitant."

Absorption occurred more rapidly during the last days of June and the months of July and August than earlier in the season; the cells then became filled with absorbed matter.

Ammonia was quite readily absorbed by the pitcher from the liquor. Several sets of experiments were made. In each set use was made of two pitchers of the same age and size, growing on the same plant, containing the same volume of liquor and approximately the same amount of insect remains; the pitchers chosen contained but a few insect remains. A quantitative determination of the "organic ammonia" was immediately made upon the fluid from

one of the pitchers. The other pitcher was so turned and propped that rain could not enter, and was left thus for a week; the "organic ammonia" content of its pitcher liquor was then determined, and compared with the amount found in the first pitcher. "The fluid showed a decided decrease in each case, from the amount found in the one used in comparison. Though these analyses were perhaps quite far from sure in every detail, yet the average difference on comparison, viz.: sixty parts in one hundred would indicate quite rapid absorption, for such an amount could not possibly be removed in any other way." Therefore the pitchers absorbed nitrogenous compounds derived from "the decomposing remains of the insects."

Chemical examinations were made of the soils upon which the plants were growing. Not all the inorganic salts which were found in the pitcher liquor could be accounted for as from the soil.

Zipperer[11] found that the pitcher liquor of *Sarracenia purpurea* corroded starch grains after several days' action, and dissolved coagulated egg white in a short time. These results indicated that both a diastase and a peptonizing enzyme are secreted into the pitcher. Zipperer also made experiments with isopods of the genus *Oniscus*, either *Oniscus scaber* or *O. murarius*. The pitcher liquor first exerted a narcotic action on the prey; then death occurred as a result of suffocation; and finally, of the entire body, only a residue of chitin armor remained. He concluded that *Sarracenia purpurea* is a "finely developed insect trap, in the interior of which the insect is killed by a secretion, and assimilated by ferments."

Goebel[12] demonstrated that water and various solutions, introduced into *Sarracenia* pitchers, decreased markedly in volume in a few days. The water or solution was introduced into a pitcher of *Sarracenia illustrata* (a hybrid between *S. flava* and *S. purpurea*) until the level of the pitcher contents was 10 centimeters below the orifice; the latter was closed with a cork; and paraffin of low melting point was poured over the cork to prevent evaporation of the water. The level was marked by a strip of paper pasted on the exterior surface of the pitcher. The pitchers were examined 48 hours later with the following results:

One pitcher had received 20 cc. of 1 percent solution of formic acid and some swollen fibrin; the decrease in volume, due to absorption, was 6.8 cc.; the remaining solution still had an acid reaction; the fibrin appeared entirely unattacked.

Another pitcher had received 10 cc. of water, of which 2 cc. were absorbed.

A third pitcher had received 10 cc. of very dilute meat infusion exactly neutralized with sodium carbonate; the decrease in volume was 2.5 cc.; the meat infusion became alkaline in reaction and cloudy, and was full of bacteria.

A similar experiment was made with a pitcher of *Sarracenia Drummondii*: A 5 percent peptone solution, which had been introduced into the pitcher, underwent a decrease of somewhat more than 7 cc. in volume; the remaining solution occupied a volume greater than 2 cc., was markedly cloudy, and possessed a faint odor of putrefaction.

A young, green pitcher of *Sarracenia purpurea* was used in another experiment. A piece of meat the size of a barley grain and 10 cc. of water were introduced into the pitcher; and the orifice was closed airtight. Two days later 2.8 cc. of the water had been absorbed; the piece of meat was scarcely attacked, it was not foul, though thickly covered with bacteria.

Pitchers, each of which had received 5 cc. of meat juice and a small fragment of meat, were characterized 3 days later by a foul odor and the presence of ammonia. Therefore, whenever the pitchers contain a sufficient quantity of substances which are capable of putrefaction, that phenomenon makes its appearance.

The plants used in these experiments had previously been kept rather moist, in a cool north room. Under other conditions, the absorption would have been greater.

These conclusions are drawn. *Sarracenia* pitchers secrete neither a proteolytic enzyme nor a substance which prevents putrefaction. Digestive glands are not present. The inner surface of the pitcher, especially its lower portion, is able to absorb water and substances dissolved in the latter. Absorption occurs through the inner wall of the pitcher. The plant, as a rule, is but little sensitive to putrefaction products, provided they do not form in the pitchers in too great a quantity.

Goebel states that secretion of a pitcher liquor has never been observed in cultivated specimens of *Darlingtonia*. He suggests that the liquor of pitchers of plants growing in their native habitat may possibly exert an antiputrefactive action, provided too many insects are not captured. This genus was included in the absorption tests. A fragment of meat the size of a barley grain and 5 cc. of distilled water were introduced into each of three young pitchers of *Darlingtonia*. The orifice of each pitcher was then closed as tightly

as possible with cotton. Two days later the greater portion of the fluid had been absorbed; a portion had evaporated; the remaining fluid contained bacteria and moulds, but did not have a putrid odor. However, an odor of putrefaction was distinctly noticeable in the dead prey of other pitchers.

The presence of larvæ amid the prey in pitchers of *Sarracenia* and *Darlingtonia* was considered by Goebel as evidence that digestion of the prey does not occur in these two genera as rapidly as in *Nepenthes*. The identity of the compound, produced by digestion of the prey and absorbed by the pitcher wall, is not known. From experiments on *Nepenthes*, it is very probable that ammonia is absorbed. The specific cause of the digestion, whether autolysis of the prey or the activity of micro-organisms, is not stated.

Goebel concluded that *Darlingtonia* like *Sarracenia*, does not secrete a proteolytic enzyme in its pitchers. Secretion of a substance, which prevents putrefaction, does not occur in either genus, except, possibly, to a slight extent.

Lambert[13] made experiments on the secretion of pitcher liquor by *Sarracenia purpurea*, and on absorption from the pitchers of that species. His work apparently was done on the isle of Saint-Pierre and on the coast of Newfoundland.

The plants used in the secretion tests were kept under normal conditions. The liquor was withdrawn from the pitchers by means of a fine pipette; and every trace of moisture was removed with bibulous paper. The plant was placed beneath a shelter to exclude rain water, and the soil was watered amply. The interior of the pitchers remained perfectly dry.

Neither the introduction of several drops of ether nor the movements of a living insect produced any secretion of liquor by the pitchers. It had been hoped that these stimuli would cause secretion of fluid by the "stomach zone" into the dry pitcher cavity.

These results showed that the pitcher by itself does not secrete any fluid.

In the absorption tests, several drops of a solution of a crystalloid stain, such as methylene-blue of fuchsin, were added to the pitcher contents. Approximately 2 hours later the pitcher was cut open. In the pitcher wall of the bottom region, absorptive zone, or "stomach," the interior epidermis and one or two layers of subepidermal "digestive cells," which are characteristic of this zone, were stained blue or red according to the dye used. Staining of the pitcher wall occurred only in this zone. Colored colloids, *e. g.*, tincture of cochineal, were not absorbed and did not stain the tissues.

A highly concentrated solution of methylene-blue was left in living pitchers for an entire week. Only the epidermis and digestive cells were stained. The result was the same at the end of several hours as at the end of the week. After absorption, the stain was localized; and a limit was thereby placed on the total amount of absorption.

Lambert evidently considered that enzymic digestion of the prey, and absorption of the products occurred in the pitchers. "The albuminoid mass formed in the pitcher by the cadavers of the drowned insects will be attacked little by little by the liquid contained in the wall of the stomach, a liquid which passes through the wall as through a dialyzer. Under the action of this pepsin-like liquid, the albuminoids will be transformed into peptones and rendered assimilable, *i. e.*, will then be able to pass through the thin membrane of the epidermal cells in the stomach region and of the digestive cells which localize them."

The liquor is constantly agitated by living worms or annelids (larvæ?) in pitchers containing prey. It is suggested that this agitation takes the place of peristalsis in promoting digestion and absorption of the prey.

In the researches of Fenner[14] on insectivorous plants, *Sarracenia flava* was the only species of the *Sarraceniaceæ* studied. In their normal condition, the pitchers contained no liquor. Secretion of a digestive fluid occurred after insects were present in the pitcher. Flies were introduced into a young pitcher in sufficient number to fill the lowermost part of the pitcher, and were pressed together so that the mass was in intimate contact with the wall of the pitcher. In the course of 2 or 3 hours, a slight amount of mucilaginous secretion was poured out from the pitcher lining, and digested the insect bodies which were in immediate contact with the lining. The products of digestion were absorbed. Each newly captured insect shoves together the cadavers already present, and again brings them into direct contact with the pitcher lining and the secretion. The mass of insect remains therefore is well moistened and thoroughly saturated with the secretion. When the insect remains were present only in the absorptive zone, putrefaction was entirely absent. When the amount of prey was so great that the absorptive zone was filled, and a large number of insects were present in the detentive zone, then a very distinct odor of decay emanated from the latter zone; this phenomenon occurred chiefly in older pitchers.

Certain changes in the cell-contents were attributed to absorption of the

products of digestion. When cadavers of freshly captured insects were present in a pitcher, the cells in the bottommost or absorptive zone were characterized by a typical aggregation and turbidity of their contents, due to absorption of organic substances. These changes were most marked in the innermost layer of the pitcher wall. Another indication of absorption was the frequent occurrence of a large number of dark masses in the second layer of cells of the pitcher lining in the absorptive zone.

Several tests were made of the absorptive power of the pitcher. A small volume of water was introduced into a pitcher so that its level did not extend above the absorptive zone; the water was absorbed in 2 or 3 days.

When a larger volume of water was used, absorption produced a slight sinking of its level, but soon ceased. Introduction of insects into such a pitcher was followed by development of a putrefactive odor within 4 to 6 days. The dilution of the secretion with much water had unfavorably influenced the digestive function of the absorptive zone.

Several drops of meat juice were introduced into a very young pitcher; they disappeared after a time, even though the orifice of the pitcher had been closed with a cotton plug to prevent evaporation. Absorption therefore took place. However, if the pitcher were half-filled with meat juice, and the level of the latter extended far above the absorptive zone, an odor of putrefaction appeared at the end of approximately 10 days. These experiments showed that the absorptive power is limited.

Fenner concluded that *Sarracenia flava* is an insectivorous plant, provided with a digestive enzyme, requiring and utilizing its prey for its nutrition, and able to absorb animal substances through a definite, though small, region of the pitcher.

Robinson[15] conducted greenhouse experiments on plants of *Sarracenia purpurea* which had been gathered recently at Poughkeepsie, New York, and near Lakewood, New Jersey. Various substances were introduced into the pitchers, usually as aqueous solutions; and the changes in both the pitchers and the introduced substances were noted.

"Before a solution was placed in a pitcher the contents were withdrawn by means of a pipette, the pitcher was thoroughly, though gently rinsed with tap-water and distilled water, and swabbed with absorbent cotton. After the solution had been placed in the pitcher, it was covered with lace net." Insects were thereby kept out of the pitchers; an additional precaution to

exclude ants from the pitchers was to place the crocks, containing the plants, in water.

Solutions, introduced into the pitchers, produced the following results:

A 0.5 percent solution of acetic acid caused pitchers to wither above the level of the liquid within a few hours; the pitchers were dead at the end of 6 days.

A $\frac{1}{1024}$ molar solution of potassium nitrate did not injure the pitchers in which it was kept, with frequent renewals, for 6 weeks. A 0.5 percent solution of this salt was not injurious; in one experiment, perceptible growth occurred in its presence. With a 1 percent solution, the pitchers withered in 6 days; with a 2 percent solution, they became dry and brown in 3 days. Both young and mature pitchers behaved in the same manner.

Sach's nutrient solution, each liter of which contained 1.0 gram calcium nitrate, 0.25 gram potassium nitrate, 0.25 gram dipotassium phosphate, 0.25 gram magnesium sulphate, and a trace of ferrous sulphate, caused pitchers "to begin to decay within a few days, the tissues being entirely dead in from two to three weeks."

A dilute solution of Liebig's meat extract produced partial withering of the pitcher in less than a week; and complete decay occurred in approximately 2 weeks.

Milk was diluted, 1 drop in 10 cc. of distilled water; the resulting solution was neutral to litmus; it remained odorless and neutral in reaction after 6 days in the pitcher. When the concentration of the milk was doubled, acidity developed, and the pitcher decayed almost completely in 2 weeks. With a still higher concentration of milk (20 percent by volume), the pitcher contents coagulated and acquired an unpleasant odor within 2 days. "It was inferred that the pitcher gave out an alkaline substance which reacted with the acid produced in the very dilute solution of milk but was not sufficient to neutralize the solutions of greater strength. There was nothing to indicate that the milk fat or protein was digested."

When a 10 percent solution of glucose was kept in the pitchers for periods varying from 4 days to 3 weeks, it retained the power to reduce Fehling solution on heating, and the presence of much carbohydrate was shown by the α-naphthol test. Some of the glucose underwent ordinary fermentation, but the products were without apparent detrimental influence on the plant.

Sucrose was used in concentrations between less than 1 percent and 33⅓

percent; no bad effect was noted. The 33⅓ percent solution did not injure the pitcher during a period of 2 months; young pitchers containing this solution grew at the same rate as those containing distilled water. After sucrose solutions of various concentrations had been in the pitchers from 3 to 7 days, they gave a reddish precipitate of cuprous oxide with hot Fehling solution, and also spontaneously produced a heavy reduction of that reagent without heating. Blank, or control, experiments on the reagents and on water, which had been kept in the pitchers, gave no reduction. Therefore the sucrose had been cleaved into invert sugar.

Starch paste was kept in the pitchers for from 3 to 13 days: it then reduced Fehling solution, but only on boiling: and it still gave a blue color with iodine. In some experiments toluene was introduced into the pitchers with starch paste; sufficient toluene was used to form a layer above the surface of the starch paste; the contents of these pitchers also acquired the power to reduce Fehling solution. Reduction of Fehling solution was never obtained in control experiments made on the reagents, and on tap-water plus toluene which had been permitted to stand in the pitchers.

Neutral olive oil was mixed intimately with either distilled or tap water, in the ratio of 0.4 cc. of oil and 9.6 cc. of water. The mixture was introduced into pitchers; in some experiments no bactericide was used, in others toluene was added as a bactericide; the results were the same in the two series of experiments. From 4 to 7 days later, the contents of the pitchers were removed and titrated with 0.01 molar potassium hydroxide solution, using phenolphthalein as an indicator. Control experiments were made on portions of the mixture which were not introduced into pitchers. The titrations showed that the oil had not been hydrolyzed by the pitchers.

Ethyl butyrate was also used as a reagent for a fat-splitting enzyme. Tap water, or 0.01 molar potassium hydroxide solution, or 0.01 molar acetic acid solution was kept in the pitchers for 1 day. The liquid was then removed and mixed with ethyl butyrate, 4 drops of the ester to 2 cc. of the liquid. After incubation at room temperature for 24 hours, the liberated butyric acid was titrated with 0.01 molar sodium hydroxide solution, using phenolphthalein as an indicator. Control experiments were made *in vitro* with the ester and portions of the standard solutions. The results showed the absence of enzymic cleavage of the ester.

Distilled water produced no change in the external appearance of pitchers in which it was kept, with frequent renewals for approximately 5 weeks.

In another series of experiments water was kept in the pitchers for 6 days, then removed, and tested for the presence of a protease. The water from the pitchers was divided into several portions, to each of which a granule of fibrin was added. The test was carried out in neutral, in slightly acid, and in slightly alkaline solution; each of these three sets of tests was made both in the presence and in the absence of toluene. "The result was quite uniform, for the fibrin granule remained apparently unchanged in each liquid."

The general conclusions reached by Robinson were:

"1. The pitchers of *Sarracenia purpurea* can adapt themselves to solutions of very different osmotic strengths.

"2. They give out an enzyme which hydrates sucrose and starch to reducing materials, presumably simple sugars.

"3. They have no fat-digesting power.

"4. They do not secrete a protein-dissolving enzyme."

Hepburn, St. John, and Jones[18] made a preliminary report on the presence of a protease in the liquor of both unopened pitchers and open pitchers of *Sarracenia flava*, on the bacterial sterility of the contents of unopened pitchers of *S. flava* and *S. minor*, and on the types of bacteria present in the contents of open pitchers of these two species.

In another paper, Hepburn, St. John, and Jones[19] made a preliminary report on the biochemistry of the pitcher liquor of the North American *Sarraceniaceæ*, and on the response by the pitchers to introduced substances. They also gave a detailed account of the absorption of various nutrient solutions which were introduced into the pitchers.

Certain flies of the genus *Sarcophaga* pass their entire larval stage within the *Sarracenia* pitchers, living on the cadavers of the captured insects. Hepburn and Jones[20] demonstrated the presence of antiproteases in these larvæ; this indicates that the proteases, which occur in the pitcher liquor, exert a digestive action on proteins present within the pitcher cavity.

Burnett[21] wrote of the pitchers of *Sarracenia:* "The water in these receptacles, impregnated by the half-decomposing animal matter, doubtless affords a highly nutritive and invigorating diet to the plant," and "the Sarraceniæ, if kept from the access of flies, are said to be less flourishing in their growth, than when each pouch is truly a sarcophagus." He mentioned digestion of the prey, and spoke of the pitchers as special organs "for the especial purpose of retaining food, and absorbing thence its nutritious particles." He

considered the pitchers the nearest approach in the vegetable kingdom to the stomach of animals. Burnett apparently believed that the prey underwent digestion within the pitchers, and that the products of digestion were then absorbed and used for the nutrition of the plant.

Wherry[04] has commented on the reaction (hydrogen-ion concentration) of the pitcher liquor of *Sarracenia purpurea*.

Values obtained by Wherry on the pitcher liquor of this and other species of *Sarracenia* have been placed at the disposal of the authors, and are given on page 69.

PHYSIOLOGICAL ACTION OF THE PITCHER LIQUOR AND OF THE NECTAR

The Pitcher Liquor

The physiological action of the pitcher liquor on insects was studied by Mellichamp.[4] Liquor was collected from pitchers of *Sarracenia variolaris* (*S. minor*). Insects were placed in a layer of the liquor which was not deep enough to immerse them completely. House-flies were used in approximately 20 experiments; they became anesthetized or intoxicated in from 0.5 to 10 minutes. When removed from the liquor, they recovered; the time required for recovery varied from 0.5 hour to one hour or longer. The liquor also exerted this action on a cockroach, a moth, and a common house spider. "Without doubt, therefore, the secretion found in the tubes of *Sarracenia variolaris* is intoxicating, or narcotic, or anesthetic, or by whatever word we may prefer to indicate that condition to which these small insects succumb." The liquor quickly saturated, clung to, and clogged the wings of house-flies, rendering flight impossible. Pure water does not exert this wetting action, but "runs" from the wings.

The following experiment showed that the pitcher liquor does not evolve a poisonous exhalation for overcoming the prey. Two house-flies were suspended in a cage of thin gauze within a large wide-mouth phial, which contained approximately one-half ounce of pitcher liquor; about 8 hours later, the flies were still struggling frantically to escape.

In a second communication on the stupefying action of the pitcher liquor of *Sarracenia variolaris* (*S. minor*), Mellichamp[16] wrote: "Pour out a teaspoonful or two of the fluid in an ounce measure or a small wine-glass.

Throw in a fly so that his wings will be wet or slimed. He will in a few minutes cease to struggle and will appear as if dead. Take him out after a while and let him dry, and in about half an hour he will revive."

Pitcher liquor was collected at a time when no rain had fallen for nearly two weeks, and, in part, from unopened pitchers; it was turbid, slightly acid in reaction, had very little, if any, taste, and was very active with respect to stupefying power.

A sample of pitcher liquor, which had been kept for 3 years, was clear, neutral in reaction, and without much sediment; it was nearly or quite inert with respect to the stupefying power.

Watson[17] repeated the experiments of Mellichamp, and confirmed the latter's results on the stupefying power of the pitcher liquor of *Sarracenia variolaris* (*S. minor*).

Zipperer[11] conducted experiments on the action of the pitcher liquor of *Sarracenia purpurea* on isopods of the genus *Oniscus*, and concluded that the liquor first exerted a narcotic action on the isopods, and that their death was due to suffocation.

From experiments made by Lambert[13] on millipeds, it appeared that the pitcher liquor of *Sarracenia purpurea* had no action on *living* animal organisms.

The Nectar

Mellichamp[4] permitted flies, large red ants, and smaller ants—both black and red—to feed on the nectar of *Sarracenia variolaris* (*S. minor*). The nectar was innocuous, and entirely without narcotic, stupefying, or intoxicating action on these insects.

This conclusion was confirmed by later experiments of Mellichamp,[16] made on pitchers (leaves) of this species. "While still fresh, the upper portions of these leaves were cut off and slit open, thereby exposing the honeyed secretion on the internal surface, which was very abundant and glistening, sweet to the taste and viscid to the touch. These were then flattened out on a large newspaper, the whole surface of which was covered with them. Many house flies were soon attracted and commenced to feed, and I carefully watched their motions without any interruption for the space of one hour. The result was precisely as previously stated. In no instance did I discover the slightest unsteadiness or tottering in any of the flies, although I watched some of them

feeding at one spot for at least ten minutes, at the expiration of which time they flew off apparently unhurt. They continued feeding and flying off from the leaves during the hour I watched them, and certainly not one fell, nor was there any indication at any time of either stupor or intoxication." The pitchers, which were used, were secreting nectar freely. The experiment was repeated twice, and the same result was always obtained. The nectar functioned simply as a lure.

CHEMICAL COMPOSITION OF THE TISSUES

Porcher[23] made various qualitative tests upon the cold infusion, the decoction, and the alcoholic extract of the rhizomes of *Sarracenia flava* and *S. variolaris* (*S. minor*). Meconic acid, morphine, narcotine, and quinine were not found in the rhizomes. Volatile, bitter and astringent principles are mentioned: "The bitter and astringent principles are volatile, for upon boiling a portion of the powdered root, the liquid was almost tasteless."

Porcher included in his paper the results obtained by Shepard in a preliminary examination of the dried rhizomes of the species mentioned. Sections of the rhizomes imparted an acid reaction (red color) to moistened blue litmus paper. Upon incineration, a white ash was obtained containing silica, calcium carbonate, and potassium carbonate. From qualitative tests, made chiefly on the extract in 80 percent alcohol and the extract in water acidulated with sulphuric acid, Shepard concluded that the rhizomes contained liguin, coloring matter, traces of a resin, an acid salt of calcium "(the acid being neither the tannic nor the gallic, but possibly one altogether new), and a salt of some alkaloid, related perhaps to cinchonia, which, should it prove new, may be called Sarracenia."

Both Porcher and Shepard noted the pigment. Shepard obtained a rich red precipitate (color of port wine) on the addition of potassium carbonate to the solution obtained by extraction of the rhizomes with water acidulated with sulphuric acid. The color was attributed to the pigment of the rhizomes. Porcher described the extraction of pigment from the rhizomes by spirits of ammonia with the production of a solution of blood red hue.

Björklund and Dragendorff[24, 25] made an exhaustive study of the chemical composition of the dried tissues of *Sarracenia purpurea*. The rhizomes and the pitchered leaves were studied separately. The various chemical compounds were separated from each other and then identified by appropriate

tests; and quantitative determinations were made of the amount of each compound present. The specimens examined apparently had been collected in the spring of the year.

The percentage composition of the tissues, as reported by these investigators, is given in the table. In addition to the compounds there enumerated, they state that the following substances were present in quantities "not determinable":

PERCENTAGE COMPOSITION OF THE DRIED TISSUES OF *SARRACENIA PURPUREA* ACCORDING TO BJÖRKLUND AND DRAGENDORFF

Constituents	*Rhizomes*	*Pitchers (Leaves)*
Hygroscopic moisture	12.08	8.60
Cellulose	19.82	14.55
Starch	25.55	..
Lignin, cuticular substance, and insoluble plant mucilage	3.17	19.90
Soluble plant mucilage	0.89	..
Sugar	9.56	3.95
Soluble plant albumin	5.70	1.02
Insoluble plant casein	7.10	1.40
Volatile amide	0.18	0.77
Volatile acid (acrylic acid)	1.49	0.12
Indifferent white resin	8.81	5.47
Wax	0.10	0.53
Ash	2.25	2.14
Silica	0.21	0.31
Ferric and phosphoric oxides	0.91	0.50
Calcium carbonate	0.09	..
Potassium sulphate	0.25	0.64
Sodium chloride	0.45	0.03
Magnesium carbonate	0.03	..
Sodium sulphate	0.15	..
Calcium sulphate	...	0.71

In the *rhizomes:* (1) an unknown substance which, on boiling in aqueous solution, furnishes a substance similar to cinchona red; (2) a non-volatile acid; (3) a tannic acid similar to caffetannic acid; (4) amorphous extractives; (5) a chromogen which yields a red pigment on treatment with hydrochloric acid, and (6) a volatile aromatic substance with an odor similar to that of "Rad. Carlinæ."

In the *pitchers (leaves):* (1) a non-volatile acid; (2) tannic acid; (3) plant mucilage soluble in boiling water; (4) non-crystalline extractives; (5) a red

pigment soluble in dilute hydrochloric acid, and (6) traces of carbonates and magnesium.

The volatile amide, which occurred in both the rhizomes and the pitchers, is also termed an alkaloid, but lacked the toxic properties of a true alkaloid as was shown by feeding it on bread to a mouse. It was a liquid at ordinary temperatures; its molecular weight was approximately 35; its odor resembled that of coniine; and it formed a hydrochloride.

In order to ascertain the physiological action of the volatile amide, approximately one centigram of its hydrochloride was dissolved in a very little water, absorbed in about one-half a gram of white bread, and given to a mouse. The mouse ate approximately one-half the mass of moistened bread greedily, then gradually stopped eating, and later showed no inclination to consume the remainder of the bread. In about one-half an hour, the animal became indolent, and remained so for about 20 minutes, then again behaved in a normal manner; these attacks of dullness and indolence returned several times. The mouse showed no inclination to drink, and now and then ate white bread, but refused to consume the remainder of the bread which had been treated with the hydrochloride. It urinated very frequently. Death occurred after 12 hours. Autopsy showed almost complete emptiness of the heart, marked extravasation of blood in the lungs, and slight quantities of blood in the brain. The stomach contained some viscous black material adhering to its walls. The intestines contained black feces alternating with gas; the gas was most abundant in the uppermost parts of the intestines. The bladder was completely filled. Björklund and Dragendorff decided that, while this single experiment did not permit a definite conclusion concerning the toxicological action of the volatile amide, yet it supported the view that this compound does not belong among the true poisonous alkaloids, and also indicated that the diuretic action of the rhizome may be due, at least partly, to the presence of this amide.

Both the rhizomes and the pitchers contained the same resin; but the chlorophyll content of the pitchers was included in the percent of resin reported for them.

The species of sugar present in the tissues was not determined.

The substance, similar to cinchona red, derived from the rhizomes, was soluble in alkalies with a red color.

The red pigment present in hydrochloric acid decoctions of the rhizome

was apparently derived from a chromogen occurring in the cambium layer, which dissolved readily in water, very difficultly in alcohol, and was converted into the pigment by cleavage by the acid. It was precipitated from its solution in hydrochloric acid as a violet pigment on addition of ammonia.

A proximate analysis of *Sarracenia purpurea* by Frohwein is included in a report on that plant by a committee of the New York County Medical Society.[26] Since the report deals with the use of the rhizome as a remedy for small-pox, rhizomes probably were the tissues analyzed. Frohwein reported the presence of gum, starch, vegetable albumin, tannin, resin, bitter principle with an acid reaction, extractive matter, traces of volatile oil, calcium, magnesium, potassium, sodium, iron, silica, phosphoric acid, sulphuric acid, and carbonic acid. "From this analysis it would seem that an alkaloid does not exist in the root of the *S. purpurea*, and it might be considered only a mild tonic, on account of the bitter principle which it contains."

Martin[27,28] found in the rhizomes of *Sarracenia purpurea* an alkaloid (sarracenine), a resin, a yellow coloring matter, extractives, and substances which form the skeleton of plants.

In order to obtain the alkaloid, sarracenine, the rhizomes were pulverized and made into a thin paste with distilled water acidified with sulphuric acid. This paste was kept in an oven until desiccation was complete. The powdery residue was extracted for four days in a flask with carbon disulphide, with agitation from time to time. The supernatant liquid was removed by filtration through linen under strong pressure. The woody residue on the filter contained the sarracenine; it was extracted with boiling distilled water in a capsule for one-half hour; the resulting solution was removed by filtration under strong pressure. The filtrate was again filtered, then evaporated on the water-bath to a syrupy consistency. This syrup was mixed with twice its volume of ethyl ether in a flask which was shaken vigorously at intervals for 1 or 2 days; the ether was then decanted, and permitted to evaporate spontaneously. The residue was dissolved in distilled water. The resulting solution was filtered if necessary, and was concentrated on the water-bath until the sarracenine sulphate crystallized. Pure sarracenine was obtained by mixing its sulphate with sodium bicarbonate, and isolating the free alkaloid by means of rectified alcohol.

Sarracenine is described as a white substance, soluble in alcohol and ether, and forming salts with acids. Its sulphate crystallizes in beautiful needles, and has a bitter taste which it imparts to its solutions.

Reference is at times made in the literature to an abstract of Martin's paper.[29]

Schmitt[30] carried out a series of experiments on dried *entire plants* of *Sarracenia purpurea*. They contained 11.43 percent of hygroscopic moisture (determined by drying at a temperature of 120° C.), and yielded 3.32 percent of a white ash. Analysis of the ash showed the presence of much potassium, also of calcium and the radicals of the following acids: sulphuric, phosphoric, silicic, and hydrochloric.

He speaks of the use of the decoction and the tincture of these plants in pharmacy, and of the occasional use of the powdered plant. The decoction was prepared by mixing 50 grams of the plant with 1 liter of water, and concentrating to a volume of 500 cc.; it had a brownish yellow color; the dose is given as one-half glass in 24 hours. The tincture was prepared by percolation or maceration of 1 part of the plant with 5 parts of 80 percent alcohol; it was green in color. Both the decoction and the tincture were acid to litmus; they were subjected to qualitative tests for the presence of various compounds.

As a result of his experiments, Schmitt reports the occurrence in the plant of the following substances: (1) Plant skeleton, (2) gums, (3) albuminous substances, (4) resins, (5) sarracenic acid, (6) sarraceno-tannic acid, (7) fats and waxes, (8) potassium and calcium salts of organic acids, (9) inorganic compounds, and (10) water.

Sarracenic acid is the name given to the coloring matter of the plant. It is described as almost, if not entirely, insoluble in water, ether, and petroleum ether, and dissolves most readily in alcohol. It is an acid with a bitter taste; and its compounds with the alkalies and alkaline earths have a characteristic yellow color. The yellow solution obtained by the action of the alkaline earths (lime or baryta) upon the tincture of the plant reacts with alum to form, as a precipitate, a beautiful yellow color-lake, while the filtrate from this precipitate is entirely colorless.

Sarraceno-tannic acid is described as a physiological tannin, belonging to the group of tannins which includes those occurring in coffee, catechu, and Peruvian bark.

When the dried entire plant was subjected to distillation with water, volatile products were not obtained.

Vines[31] made a study of glycerol extracts of the pitchers of *Sarracenia flava*. Some pitchers were extracted with glycerol without previous treatment

with acid; others were treated with 1 percent acetic acid for 24 hours in order to activate a zymogen (if present), then were extracted with glycerol. These glycerol extracts were then tested for the presence of a protease. A portion of the glycerol extract, a fragment of swollen fibrin, and 2 cc. of 0.2 percent hydrochloric acid were mixed and permitted to digest at a temperature of 40° C. The period of incubation is not stated, but probably was 6 or 8 hours. In this test, a partial or complete solution of the fibrin and a positive biuret reaction, yielded by the filtered digestion-fluid, were considered evidence of the presence of a protease in the glycerol extract. Vines found no evidence of the presence of a protease in the tissues of the pitchers of *Sarracenia flava*, although he was able to demonstrate the presence of a protease in the tissues of the pitchers of certain species of *Nepenthes* by means of this technic.

The glycerol extract of the pitchers of *Sarracenia flava* was found to contain sugar.

Hetét[32] examined "powder of the leaves," therefore dried pitchers, of *Sarracenia purpurea* from the isles of Saint Pierre and Miquelon. He found in them "an alkaline substance, whose properties are identical with those of veratrine. The crystallization is the same, in beautiful prisms and in octahedra of the orthorhombic system. It behaves in the same way with the principal neutral solvents; it gives the same reactions with the acids and the solutions used to distinguish the alkaloids, that is, particularly, the successive colorations with concentrated sulphuric acid, with sulphomolybdic acid, and especially hydrochloric acid and heat, which produce that beautiful, persistent, reddish violet coloration, quite peculiar to veratrine."

Hetét also found in the pitcher the amine previously discovered by Björklund and Dragendorff,[24, 25] and "another alkaline substance, soluble in water."

Lambert[13] reports experiments, apparently made on fresh plants of *Sarracenia purpurea* on the isle of Saint Pierre and on the coast of Newfoundland. He studied sarracenic acid, the yellow coloring matter of the plant, which had been observed previously by Schmitt.[30] Lambert writes: "We have very easily recognized this coloring matter in the plant and have isolated it. We have ascertained that it has the singular property of being very soluble in alcohol, which it slightly colors. It suffices to add several drops of an acid (nitric acid seems to serve best) for the alcoholic solution to acquire a beautiful red color. On the other hand, several drops of any alkali

make the red color pass, after neutralization, to a dull green; and the least trace of an acid suffices to restore the color to its original red. This coloring matter, then, indeed behaves like a veritable acid and compares singularly in its reactions with litmic acid. The sensitivity of the coloration of this acid is such that it has appeared to us as capable of replacing litmus or phthalein in the titration of alkalies and of acids."

Lambert also tested the plants for the presence of a volatile base. A tiny fragment of the plant was treated with a dilute potash solution in a test-tube. When the tube and its contents were heated, the characteristic mouse-like odor of coniine was at once recognized.

The text does not state whether these experiments on the pigment and the volatile base were made on the pitcher, the rhizome, or the entire plant. However, attempts were made to isolate the base from the leaves (pitchers), but were fruitless; for the base was present in so small an amount that only the odor was obtained. It is suggested that this base acts as a lure for insects.

Gies[33] used fresh pitchers of *Sarracenia purpurea* in his experiments. The pitchers were thoroughly macerated in glycerol; and the resulting extract was used in tests for the presence of a protease. The glycerol extracts obtained from one set of plants showed moderate but distinct digestive action on fibrin at a temperature of 38° C. in the presence of "slight amounts of hydrochloric or oxalic acids"; the control experiments showed no digestion. The glycerol extracts from a second set of plants entirely lacked digestive action. Gies interprets these results: "In view of the negative results in the second series it is impossible at present to draw a satisfactory conclusion in this connection. It may be that the positive results in the first case were due to a bacterium specially favored by the medium furnished by the constituents of the glycerin extract, or to enzyme in unobserved diseased portions of the plants. Again, the negative results may have been due to a less favorable degree of acidity, or the secreting cells of the pitchers may have been in a 'resting condition' without either enzyme or zymogen." The two sets of plants came from different localities.

The diluted glycerol extract was practically colorless when neutral, crimson with acids, and green with alkalies. Further experiments showed that "Sarracenia purpurea contains a pigment which in concentrated glycerin extract has a reddish color, but which when diluted is practically colorless. At such dilution, when scarcely any color is to be seen, a drop of dilute acid

produces a bright pink throughout the whole fluid; alkali in minute amounts turns it green. The pink is converted to green by alkali, *vice versa* by acid. Even in crude glycerin extract the pigment appears to be very sensitive and may be used to advantage in titrimetric work. I have named the pigment alkaverdin, because of the beautiful green produced on treatment with alkali. . . . Excellent 'test papers' have been made with the pigment in glycerin extract. Ordinary filter paper dipped into the red, concentrated extract is colorless wet or dry. The dry paper turns a bright pink when dipped into acid, a deep green is produced when in contact with alkali." The solutions of alkaverdin had no special influence on the spectrum.

Both the aqueous and the saline extracts of the pitchers contained "an abundance of dextrorotatory, reducing and fermentable substances."

Meyer and Gies[34] found that alcohol extracted 3 coloring matters from macerated purple pitchers of *Sarracenia purpurea:* green chlorophyll, purplish red alkaverdin, and a brownish black substance. Water extracted only alkaverdin and the brownish black pigment from the pitchers. When the aqueous extract was evaporated almost to dryness *in vacuo* and was then poured into absolute alcohol, the brownish black pigment was precipitated. The color of this pigment was not influenced by either acid or alkali; it did not react with ferric chloride, and had no reducing power.

The alcoholic solution now contained all the alkaverdin; it was evaporated almost to dryness *in vacuo*. The residue had the color and consistency of dark molasses, a bitter taste, and an odor which was sugary and also resembled the characteristic odor of the macerated pitchers. It was insoluble in ether and chloroform, but soluble in water and alcohol, was free from the halogens, nitrogen, and sulphur, and contained a large amount of fermentable dextrorotatory carbohydrate which had a marked reducing action on Fehling solution and yielded a phenyl osazone resembling in crystal form that derived from glucose (dextrose). Removal of the sugar by fermentation had no apparent injurious action on the alkaverdin.

The aqueous solution of this molasses-like syrup had a reddish color, but became colorless when very dilute. Such a colorless solution became green on addition of a drop of 0.2 normal alkali, and returned to its colorless condition on addition of a drop of 0.2 normal acid. A drop or two of the acid in excess produced a pink color, quite different from the color of the syrup, and intensified

somewhat on standing. These changes in color were due to the presence of alkaverdin; and delicate test papers were made with that pigment.

Alkaverdin was not obtained in the crystalline state; it was found to have no influence on the spectrum. When its aqueous solution was warmed for some time on the water bath, the alkaverdin was converted into a brownish substance which still gave a green color with alkali, but did not yield a pink color with acid. Hydration with 2 percent sulphuric acid caused alkaverdin to lose completely its tinctorial properties.

Clark,[35] in the course of a research on plant oxidases, examined the "pitcher-plant leaves" of *Sarracenia Drummondi*, using an aqueous extract of the fresh pitchers for his experiments. The extract did not contain *oxygenase;* presence of *peroxidase* was doubtful; *catalase* was present; *chromogens*, which are oxidized to colored compounds by the natural oxidase of the plant, were not found.

For oxygenase (direct oxidase) seven reagents were used: tincture of guaiac, tincture of guaiac previously boiled with bone black to remove peroxides, α-naphthol, 1,4 phenylenediamine hydrochloride, phenolphthalein, phenol, and the indophenol reagent (α-naphthol plus 1,4 phenylenediamine hydrochloride in the presence of sodium carbonate). Hydrogen peroxide was added to each of these reagents in testing for peroxidase. Catalase was detected by its action in liberating molecular oxygen from a dilute solution of hydrogen peroxide.

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 Sarracenias 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. Ames: *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: *Homœopathic 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: Homœopathic 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, Rehfuss, 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 courronnés 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.