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THE ABSORPTION OF NUTRIENTS AND ALLIED PHENOMENA IN THE PITCHERS OF THE SARRACENIACEÆ.*

BY

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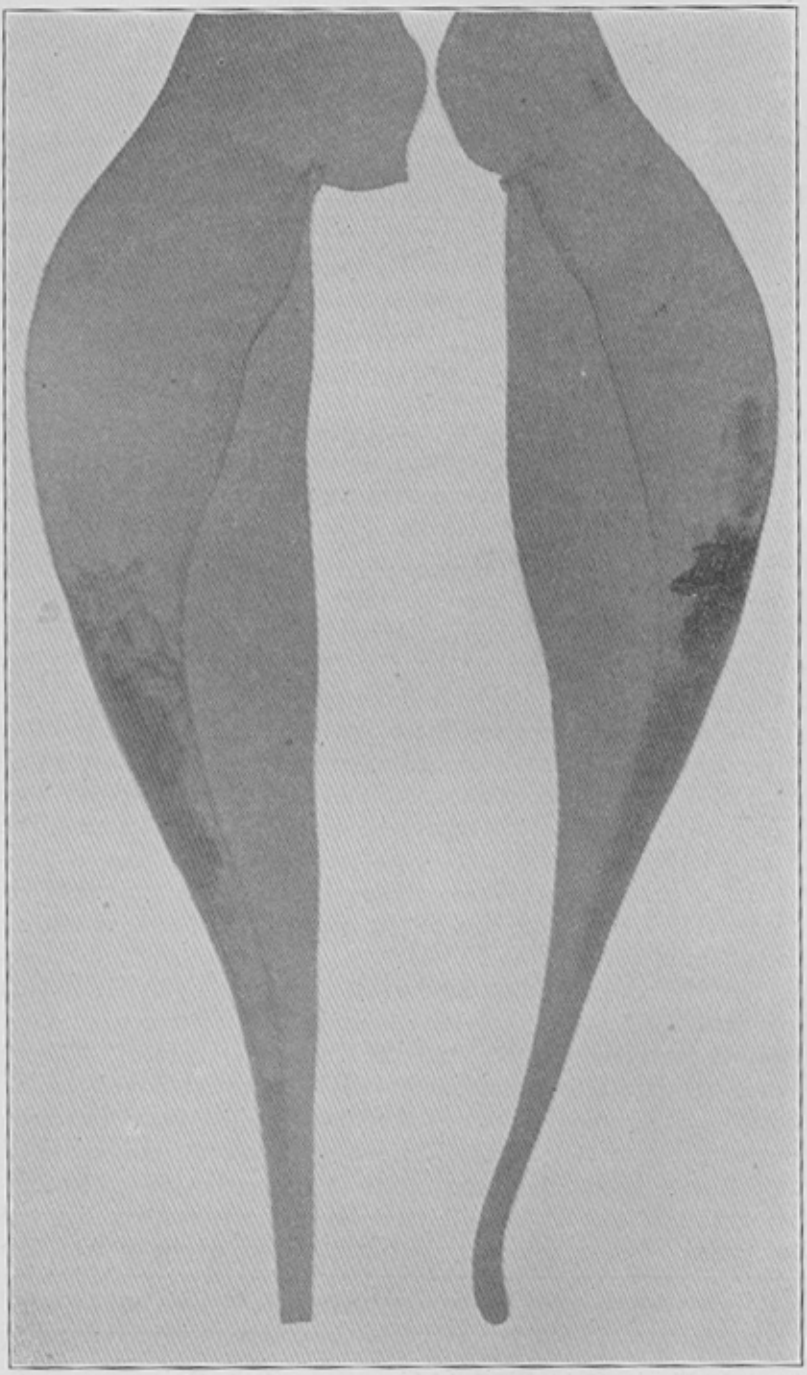
THE AMERICAN PITCHER PLANTS.

THE *Sarraceniaceæ* or American pitcher plants are bog plants. In this family of plants, the entire leaf has become a pitcher with terminal lid or bilobed flap. The pitcher proper is the hollowed-out midrib of the leaf, in front of which is the wing that represents the fused halves of the blade in *Darlingtonia* and the Sarracenias; the halves are distinct but closely opposed in *Heliamphora*. The lid in *Heliamphora* and the Sarracenias and the bilobed flap in *Darlingtonia* represent a portion of the blade early separated off from the wing below as a result of the hollowing-out of the midrib between the two separated parts of the blade.[1] The developing pitcher and lid form an envelope about a hermetically sealed space—the pitcher cavity. As maturity is reached, the lid and the lips of the pitcher open, and remain in that position permanently.

The pitcher and lid or bilobed flap form a highly developed insect trap. The entire outside epidermis of the pitcher is dotted with nectar glands and forms the "alluring surface." These glands are especially abundant on the "attractive zone," which consists of the inner surface of the lid or bilobed process and the edge of the recurved, thickened rim that surrounds the orifice of the pitcher. In *Heliamphora* and the Sarracenias, the inner surface of the lid is provided with numerous hairs which are directed downward. In *Darlingtonia*, the inner surface of the bilobed flap

* Communicated by Dr. Hepburn, member of the Institute, and Secretary of the Section of Physics and Chemistry. Presented before the Graduate Botanical Club of the University of Pennsylvania on November 3, 1919, and before the Physics Club of Philadelphia on November 14, 1919.



Radiograph of open pitchers of *Sarracenia purpurea* (full size) containing prey. The *liquid* pitcher contents have been removed; the dense shadow represents the mass of captured insects in the lower part of the pitcher; moths, beetles and flies were identified among the captures. Radiograph by Goodspeed.

is provided with similar hairs which are directed toward the mouth of the pitcher. The interior of the pitcher may be divided into two regions. The upper or "conducting" zone has an extremely smooth lining. The lower or "detentive" zone contains long, tapered, downwardly directed hairs.

The pitcher secretes a fluid or pitcher liquor even before the lid and the lips have opened. This secretion varies in amount with the species; it may be merely a studding of minute drops of

Section of open pitcher of *Sarracenia purpurea* according to Macfarlane, showing the wing at the left, the hairy lid, the smooth conducting surface below the rim, the smooth glandular area (characteristic of this species), and the detentive zone with its downward-pointing hairs.

fluid on the lining in the lower portion of the pitcher; or it may fill the pitcher cavity to the depth of several inches. The pitcher liquor increases in volume after the lid and lips have opened, and may be further increased in certain species by rain water or by floods in their native habitat.

Great numbers of insects are attracted by the exuding nectar, probably also by the brilliant color and flower-like shape of the pitcher, and a very perceptible fruity or honey-like fragrance during the period of nectar-exudation. A creeping insect is tempted upward by the nectar on the alluring surface, and finds its way to the attractive surface. A flying insect alights on some

part of the expanded upper portion of the pitcher. The insects sip the nectar, either at the rim of the pitcher, or on the inner surface of the lid or bilobed flap, where their course is directed toward the pitcher cavity by the hairs. In either case, they step upon the smooth conducting surface, which affords no, or a very insecure, foothold, and are precipitated into the pitcher cavity. Their escape is prevented by the hairs of the detentive zone and by the smooth lining of the conducting zone. In some species, the pitcher liquor apparently exerts either a wetting or a stupefying action on the prey. The captured insects then are drowned and undergo digestion.

In this paper the species of the *Sarraceniaceæ* will be enumerated, and their geographical distribution given. Observations on the nectar, and on both the wetting and the digestive power of the pitcher liquor will be reported. A full account will then be given of the studies on absorption, by the pitchers, of compounds introduced into the pitcher cavity. Lastly, the increased secretion of liquor by the pitchers in response to stimulation by introduced substances, and their response to introduced acid and alkali will be described.

CLASSIFICATION AND GEOGRAPHICAL DISTRIBUTION.

The family, *Sarraceniaceæ,* is divided by Macfarlane [1] into three genera which include nine species:

GENUS.	SPECIES.
Heliamphora	*H. nutans*
Darlingtonia	*D. californica*
Sarracenia	*S. minor*
	S. Sledgei
	S. flava
	S. Drummondii
	S. rubra
	S. purpurea
	S. psittacina

The plants of this family have their native habitat in bogs.

The genus *Heliamphora,* with its single species *H. nutans,* has been reported only from Mount Roraima between British Guiana and Venezuela.

The genus *Darlingtonia,* with its single species *D. californica,* occurs only in the mountain bogs of northern California and southwest Oregon.

The genus *Sarracenia* is found in eastern North America. *Sarracenia purpurea* ranges from Labrador, Newfoundland, and Manitoba to the Gulf of Mexico. None of the other six species of this genus occurs north of Virginia or west of Texas; their southern limit is the Gulf of Mexico.

THE NECTAR.

Chemical tests show the presence of sugar in the nectar. Each of the following species was studied separately: *Darlingtonia californica, Sarracenia minor, S. Sledgei, S. flava, S. Drummondii,* and *S. purpurea.* An aqueous solution of the nectar was obtained by washing with distilled water the tops of either 25 or 50 pitchers which were exuding nectar. Each of the six solutions thus prepared reduced Benedict's copper solution, showing the presence of a reducing sugar; each yielded the characteristic crystals of phenylglucosazone with phenylhydrazine; and each gave a distinct crimson solution when heated on the water bath with resorcin and hydrochloric acid (the Seliwanoff reaction for ketose sugars). These reactions would suggest the presence of either fructose or invert sugar in the nectar. The solution of the nectar of *Darlingtonia californica* was lævorotatory; both the optical rotatory power and the copper reducing power of this solution were determined quantitatively; the results indicated the sugar to be *d*-fructose.

ACTION OF THE PITCHER LIQUOR ON LIVING INSECTS.

Mellichamp [2, 3] found that the pitcher liquor of *Sarracenia variolaris* (*S. minor*) exerted an intoxicating, anæsthetic, or narcotic action on house flies, a cockroach, a moth, and a common house spider. His results were confirmed by Watson.[4]

One of us (Jones) has found during the present research that the liquor from both closed and open pitchers of *Sarracenia flava* exerts a wetting or stupefying action on large ants. He has also found that the liquor from both closed and open pitchers of *Darlingtonia californica* does not exert such an action on large ants, bumble bees, or locusts ("grasshoppers"). The wetting or narcotic principle—when present—apparently is not a saponin, for we have obtained a negative result when the hæmolysis test for a saponin was applied to liquor from closed pitchers of *Darlingtonia californica, Sarracenia flava, S. Sledgei,* and *S. Drummondii,* and from open pitchers of *S. flava,* and *S. purpurea.*

PROTEOLYTIC ENZYMES AND BACTERIA OF THE PITCHER LIQUOR.

Zipperer [5] found that the pitcher liquor of *Sarracenia purpurea* dissolved coagulated egg white. He decided that a peptonizing enzyme was secreted by the pitcher and digested the prey.

Fenner [6] concluded that the pitchers of *Sarracenia flava* likewise secrete an enzyme which digests the captured insects.

Batalin,[7] who studied *Darlingtonia californica, Sarracenia variolaris* (*S. minor*), *S. flava,* and *S. purpurea,* speaks of the secretion, by the pitchers of these species, of a solvent for digestion of proteins.

Lambert [8] apparently considered that the pitchers of *Sarracenia purpurea* secrete a proteolytic enzyme similar to pepsin.

Hepburn, St. John, and Jones [9] detected, in the pitcher liquor of *Sarracenia flava,* a proteolytic enzyme which digested carmine fibrin. They also found, in the contents of open pitchers of *S. minor* and *S. flava,* proteolytic bacteria which digested various proteins. These studies have been extended to the other North American pitcher plants *growing in their native habitat.* The results may be summarized briefly. *Darlingtonia californica* apparently does not secrete a proteolytic enzyme, for its pitcher liquor does not digest carmine fibrin in neutral solution, or in the presence of either 0.2 per cent. hydrochloric acid or 0.5 per cent. sodium carbonate. In the genus *Sarracenia,* proteolytic enzymes (proteases) occur in the pitcher liquor of *S. minor, S. Sledgei, S. flava, S. Drummondii, S. rubra,* and *S. purpurea.* The protease in the pitcher liquor of *S. minor* and *S. flava* digests carmine fibrin, best in the presence of a dilute acid (0.2 per cent. hydrochloric); the protease in the pitcher liquor of the other species mentioned digests carmine fibrin, best in an alkaline solution containing 0.5 per cent. or less sodium carbonate. The enzyme usually is present in the liquor before the pitcher has opened. On account of the small size of the pitchers, and the small volume of their contents in *Sarracenia psittacina,* tests for a protease in the pitcher liquor and a bacteriological study of the pitcher contents have not yet been undertaken with this species.

Certain flies of the genus *Sarcophaga* pass their entire larval stage within the *Sarracenia* pitchers, living on the captured insects. These larvæ contain antiproteases;[10] this indicates that the proteases which occur in the pitcher liquor exert a digestive action on proteins present within the pitcher cavity.

In *closed* pitchers, the pitcher cavity and liquor are bacteriologically sterile. This conclusion is based on the bacteriological examination of 44 pitchers, the lids of which had not yet begun to open. Each pitcher was examined separately. These pitchers were obtained from the following species: *Darlingtonia californica, Sarracenia minor, S. Sledgei, S. flava, S. Drummondii,* and *S. purpurea.*

Bacteriological study was also made of the contents of *open* pitchers of the six species just mentioned. All the pitchers used contained captured insects. The contents of 31 pitchers were studied separately, and three examinations were made on composite samples obtained from four or five pitchers; hence 34 samples in all were examined. These pitchers always contained proteolytic bacteria which produced some or all of the following changes when sown on suitable media: Liquefaction of gelatin, coagulation and digestion of the proteins in milk, digestion of coagulated serum albumin (Loeffler's blood serum medium), digestion of coagulated proteins of whole egg (Dorset's egg medium).

ABSORPTION FROM THE PITCHER CAVITY.

Our studies show that the pitchers of the Sarracenias secrete a proteolytic enzyme, and that proteolytic bacteria are present in the pitchers of both *Darlingtonia* and the Sarracenias, in the captured insects and the liquor surrounding them. Therefore at least two means exist for the digestion of the prey. A third possible factor is the autolysis of the insect cadavers, *i.e.,* their digestion by the enzymes of their own tissues. Since digestion of the prey occurs in the pitchers, the question arises whether the pitcher absorbs the products of the digestion, and thereby makes them available for the nutrition of the plant. Several previous investigators have endeavored to determine whether absorption occurs in the pitcher cavity.

Edwards [11] considered that absorption does not occur in the pitchers of *Darlingtonia californica.* He wrote: "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."

Batalin [7] observed certain changes in the pitcher lining of *Darlingtonia californica, Sarracenia variolaris* (*S. minor*), *S. flava,* and *S. purpurea,* after insects had been captured. The pitcher cavity is lined with epidermal cells. The outer layer of the wall of these cells is the cuticular layer, and its outermost portion the cuticula. When the body of a captured insect adhered to the lining of the pitcher, the cuticula and, at times, the entire cuticular layer was cast off from the underlying cells. Batalin concluded that this change in their cell-wall occurred so that the cells might absorb nitrogenous compounds from the captured insects, and thereby satisfy the nitrogen requirement of the plant.

Schimper [12] compared the microscopic structure of pitchers of *Sarracenia purpurea* which contained captured insects or into which meat had been introduced, with that of pitchers of the same species which had been starved by plugging the mouth with tissue paper as soon as the lid had opened. The "nourished" and the "starved" pitchers differed markedly with respect to the histological structure of the protoplasm in the epidermal cells in the bottom portion of the pitcher and, to a lesser degree, in the cells of the adjacent subepidermal layer. This difference in structure was taken as evidence of absorption.

The experiments of Fenner [6] were made on *Sarracenia flava.* He noted a typical aggregation and turbidity of the contents of the cells in the bottommost zone when the pitcher contained freshly captured insects. This was most marked in the innermost layer of the pitcher-wall. A large number of dark masses occurred in the second layer of cells of the pitcher lining in this zone. These changes in the cell contents were attributed to the absorption of organic substances. When water or meat juice was introduced into a pitcher, it decreased in volume after a time, even though the mouth of the pitcher was closed with a cotton plug to prevent evaporation; therefore absorption occurred.

Goebel [13] conducted experiments on *Darlingtonia californica, Sarracenia illustrata* (a hybrid between *S. flava* and *S. purpurea*), *S. Drummondii,* and *S. purpurea.* A definite volume of water or of *one* of the following solutions was introduced into each pitcher studied: 1 per cent. formic acid, 5 per cent. peptone, dilute neutral meat infusion. In some of the experiments a minute frag-

ment of meat was introduced with the water; fibrin was introduced with the formic acid solution. Precautions were taken to prevent evaporation. A marked decrease in the volume of the pitcher contents occurred in two days, and was taken as evidence of absorption.

Lambert [8] tested the absorptive power of the pitchers of *Sarracenia purpurea* by means of certain dyes. Several drops of a solution of a crystalloid stain, such as methylene blue or fuchsin, were added to the pitcher contents; and the pitcher was cut open approximately two hours later. Staining had occurred only in the wall of the bottom region, also called the absorptive zone or "stomach." The blue or red color, according to the stain used, was found only in the internal epidermis and one or two layers of subepidermal "digestive cells" which are characteristic of this zone. A highly concentrated solution of methylene blue, introduced into living pitchers, produced the same result whether its period of action was several hours or an entire week. The epidermis and digestive cells alone were stained. The stain was absorbed, then localized; and a limit was thus placed on the total amount of absorption. Lambert concluded that the prey is digested with the production of peptones, which are absorbed by the epidermal cells and localized by the digestive cells.

Higley [14] applied the methods of quantitative chemical analysis in studying absorption from the pitcher cavity in *Sarracenia purpurea*. He made several sets of experiments. In each set he used two pitchers of the same age and size, growing on the same plant, and containing the same volume of liquor and approximately the same amount of insect remains; the pitchers selected contained but few captured insects. A quantitative determination of the "organic ammonia" was immediately made on the fluid from one of the pitchers. The other pitcher was protected so that rain could not enter, and left thus for one week; then the "organic ammonia" content of its fluid was determined, and compared with that of 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."

Darwin [15] cites Burnett [16] as stating that "*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." This observation would indicate that these plants absorb, and are nourished by, products formed in the pitchers from the captured insects.

To sum up, previous investigators have given as evidence of absorption: (1) histological changes in either the cell wall or cell contents of the pitcher lining; (2) a decrease in the volume of solutions introduced into the pitcher cavity; (3) absorption of stains, and (4) changes in the "organic ammonia" content of the pitcher liquor.

A decrease in the volume of an introduced solution indicates absorption of water, but not necessarily of the solute or dissolved substance. Apparently no one, as yet, has introduced a *known weight* of a nutrient compound into a pitcher of any of the North American pitcher plants, and studied its absorption quantitatively. However, one such experiment has been made by Clautriau,[17] who demonstrated by this means the absorption of the proteins of egg-white by a pitcher of *Nepenthes Mastersiana*, which belongs to the *Nepenthaceæ* or oriental family of pitcher plants.

ABSORPTION TESTS.

Absorption tests have been made in the field on the following species and at the following places:

Darlingtonia californica at Keddie, Plumas County, California.
Sarracenia Sledgei at Theodore, Mobile County, Alabama.
Sarracenia flava at De Funiak Springs, Walton County, Florida; and at Summerville, Berkeley County, South Carolina.
Sarracenia Drummondii at Theodore, Mobile County, Alabama.
Sarracenia purpurea in Tolland County, Connecticut.

Vigorous plants of *Sarracenia purpurea* were obtained at Whitings, Ocean County, New Jersey, and used in laboratory experiments on absorption; they were planted in sphagnum moss; and the pots were kept filled with water. The plants were kept in the laboratory for several weeks; they retained their vigor and produced new pitchers. While our experiments were conducted on freshly gathered plants, we have maintained plants in the laboratory for as long as five months.

A separate series of experiments was made concerning the absorption from the pitcher cavity of *each* of the following:

1. Water.
2. Nitrogenous compounds—chiefly organic.
3. Organic nitrogenous compounds in the presence of a "buffer."
4. Phosphates.
5. The lithium ion.

Absorption of water was studied by observation of the changes in the volume of the pitcher contents after introduction of a definite volume of water.

Absorption of nitrogenous compounds and of phosphates was measured by the retention of nitrogen or phosphates by the plant. For the study of nitrogen retention, a solution containing a known weight of a nitrogenous compound was introduced into a pitcher; the fluid remaining in the pitcher was withdrawn at the end of a given time; and the amount of nitrogen present in it was determined. The difference between this weight and the weight of nitrogen introduced was the weight of nitrogen absorbed by the pitcher and retained by the plant. Retention of phosphates was studied in exactly the same manner, using a solution of phosphates, and making determinations of phosphoric oxide.

All the nitrogen compounds used in these experiments were non-volatile, hence could not escape from the pitcher. In one series of tests of nitrogen absorption, a "buffer" solution was added to the pitcher contents to prevent the escape of any volatile nitrogenous compounds, possibly formed by reactions occurring in the pitcher cavity.

In the experiments on the absorption of the lithium ion, lithium, which does not normally occur in the tissues of the pitcher, was found in those tissues after its absorption from the pitcher contents to which it had been added.

ABSORPTION OF WATER.

Technic.—The average volume of the true pitcher liquor (*not* diluted by rain) was determined for certain species by actual measurement of the amount in large, vigorous pitchers. The upper portions of the pitcher were clipped off with scissors until the pitcher liquor was within reach of a plain pipette, the lower portion of which was of fine bore. After each portion of liquor was removed with the pipette, another section of the pitcher was cut away; and this procedure was repeated until all the liquor had been collected from the pitcher. The total liquid contents of a

definite number of pitchers were thus collected in a stoppered vial; the total volume was then determined in a narrow graduated cylinder; and the average volume was calculated. This technic was found most satisfactory for field work in the bogs which are the native habitat of these plants. The average volume, the number of lots of pitchers studied and the total number of pitchers used in obtaining the average are given in Table I.

TABLE I.

Average Volume of Liquor in the Pitchers of Certain Sarraceniaceæ.

Genus and species.	Condition of pitchers.	Number of lots measured.	Total number of pitchers used.	Average volume of pitcher liquor.
				c.c.
Darlingtonia californica....	Closed.....	10	353	1.08
Darlingtonia californica....	Open.....	5	161	2.80
Sarracenia Sledgei.....	Open.....	3	74	0.38
Sarracenia flava.....	Closed.....	6	566	0.66
Sarracenia flava.....	Open.....	4	160	1.50
Sarracenia Drummondii...	Open.....	4	138	0.58

Water was introduced into pitchers of the same size and vigor as those used in the determination of the average volume of the pitcher liquor. With *open* pitchers, a definite volume of water was poured from a narrow graduated cylinder into the mouth of the pitcher; the top of the pitcher was folded over; and one or more pins were passed through the double fold. The pitchers were thus sealed, but were not injured; none of the tissues withered, not even the folded-over tops. Entrance of rain water and of insects was prevented. Identification labels of paper were inserted in the double fold, and were held in place by the pins. With *closed* pitchers, the top of the pitcher was cut off just beneath the lips or pitcher rim; a definite volume of water was poured into the pitcher cavity from a narrow graduated cylinder; and the upper portion of the pitcher was folded over and sealed as with open pitchers; the pitchers remained green and vigorous.

After the lapse of a definite period of time, the contents of these pitchers were collected, and their average volume determined. The technic was that used in the determination of the average volume of pitcher liquor as described above.

Results.—These experiments are summarized in Table II. The "shrinkage" was obtained as follows: From the sum of

the volume of water introduced plus the average volume of pitcher liquor was subtracted the average volume of the pitcher contents at the end of the experiment. For instance, open pitchers of *Sarracenia Drummondii* contain, on the average, 0.58 c.c. of pitcher liquor; each pitcher received 5.00 c.c. of water; and the average volume of the pitcher contents at the end of the experiment was 3.60 c.c. The shrinkage was $0.58 + 5.00 - 3.60 = 1.98$ c.c.

The pitcher contents of these species were so protected from rapid evaporation that the shrinkage in volume must be considered as due to absorption by the pitchers. The results indicate that but little, if any, absorption occurred in closed pitchers of

TABLE II.

Absorption of Water from the Pitcher Cavity in Certain Sarraceniaceæ.

Species.	Condition of pitchers.	Number of pitchers used in experiment.	Volume of water introduced into each pitcher.	Period of absorption.	Average volume of pitcher contents at end of experiment.	Shrinkage (allowance made for average volume of pitcher liquor).
			c.c.	hours	c.c.	c.c.
Darlingtonia californica..	Closed..	12	3.00	24	4.16	none
Darlingtonia californica..	Closed..	5	3.00	72	3.94	0.14
Darlingtonia californica..	Closed..	5	3.00	144	3.94	0.14
Darlingtonia californica..	Just open	5	3.00	72	5.16	0.64
Darlingtonia californica..	Just open	4	3.00	144	4.37	1.43
Sarracenia Sledgei.....	Open...	20	5.00	7	2.95	2.43
Sarracenia flava.....	Closed..	4	10.00	48	6.95	3.71
Sarracenia flava.....	Open...	4	10.00	48	6.80	4.70
Sarracenia Drummondii..	Open...	15	5.00	7	3.60	1.98

Darlingtonia californica; in open pitchers of this species, water was absorbed, and the volume absorbed increased with the period of absorption. The volume of water absorbed was greater in open than in closed pitchers of *Sarracenia flava,* other factors being equal. Absorption of water also occurred in open pitchers of *S. Sledgei* and *S. Drummondii.*

In a laboratory experiment, seven young, vigorous, fully opened pitchers of *Sarracenia purpurea,* approximately equal in size, were freed from insect remains by thorough washing with distilled water, which was then completely removed with a pipette. Ten (10.00) c.c. of distilled water were introduced from a pipette into each *empty* pitcher, which was then closed with a tightly fitting cotton plug. Ten days later, the water was removed from

each pitcher with a serological pipette. The volumes obtained from the individual pitchers were 0.00, 6.00, 7.30, 8.10, 7.90, 6.95, and 6.10 c.c., respectively, the average volume 6.05 c.c. This experiment was repeated with six pitchers of approximately the

Mature plant of *Darlingtonia californica*, showing the twisted pitcher, and expanded hood with the orifice below, the translucent window-like spots, and the pendant bilobed flap. Photographed in Plumas County, California, where the pitchers commonly attain a height of 20 to 30 inches.

same size, each pitcher receiving 10.00 c.c. of distilled water; twenty-one days later, four pitchers contained no liquid, the other two contained 1.10 and 6.90 c.c., respectively; therefore the average volume remaining in the pitchers was 1.33 c.c. The average shrinkage in volume increased with the time, *i.e.*, was greater in twenty-one days than in ten days. The decrease in volume of the

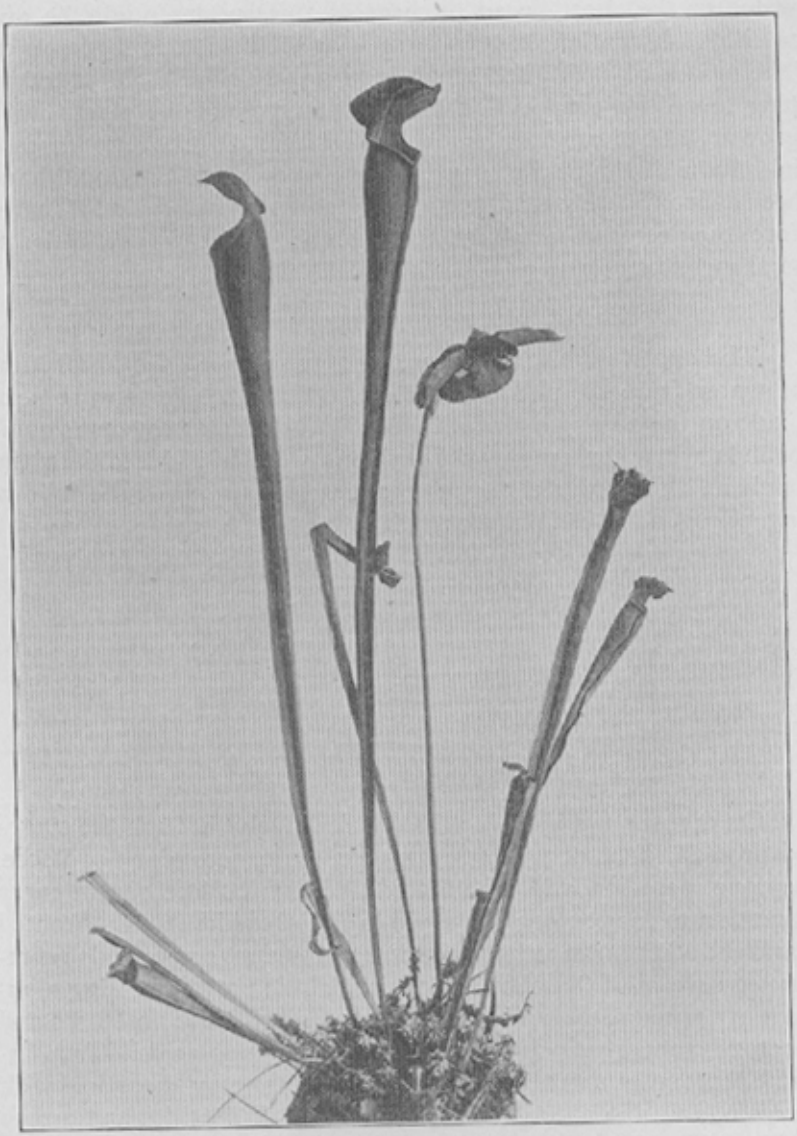
water in the short broad pitchers of this species might be due to either absorption or evaporation, or to their combined action. The mouth of each pitcher had been tightly closed with a cotton plug to reduce evaporation to a minimum; and the plants were growing above an open surface of water. Moreover, decrease in volume of the pitcher contents due to evaporation would have been approximately equal in all the pitchers of each series. Therefore, the decrease in volume must have been due, in part at least, to absorption of water.

ABSORPTION OF NITROGENOUS COMPOUNDS.

The experiments just described demonstrated the absorption of water from the pitcher cavity by the plant. Tests were next made on the absorption of various non-volatile nitrogenous compounds when their aqueous solutions were introduced into the pitchers. Each nutrient solution contained but *one* of the following nitrogenous compounds:

Ammonium chloride
Ammonium tartrate
Acetamide
Urea
Asparagin
Glycocoll
Trypsinized peptone
Peptone (Witte)
Egg albumin

On digestion, a protein passes successively through the stages of protean, meta-protein, primary proteose, secondary proteose, peptone, peptides, and amino acids. Enzymes like pepsin carry digestion to the peptone stage, while enzymes like trypsin proceed farther and produce amino acids. The nutrient compounds represented several of these groups of proteolytic products. Egg albumin is a true protein; Witte peptone represents the proteoses and peptones; and glycocoll and asparagin are amino acids. Trypsinized peptone is prepared from Witte peptone by digestion with trypsin, and probably is chiefly a mixture of peptides and amino acids. Closely related to the amino acids are the acid amides such as acetamide and urea (the diamide of carbonic acid), and the ammonium salts such as ammonium chloride and ammonium tartrate. The pitchers, therefore, received nitrogenous compounds of all stages of molecular complexity from the simple



Mature plant of *Sarracenia Sledgei*, showing recently opened pitchers, the flower from which the petals have fallen, and the shrivelled pitchers of the preceding season. Photographed at Biloxi, Mississippi, where the pitchers commonly attain a height of 16 to 24 inches.

inorganic ammonium chloride and the simple organic ammonium tartrate to the highly complex egg albumin.

Each nutrient solution was prepared immediately prior to use, by dissolving the finely divided compound in distilled water, and

Group of *Sarracenia flava*, showing open pitchers, photographed in midsummer at Southern Pines, North Carolina. The pitchers of this species attain a maximum height of nearly 40 inches, though commonly between 20 and 30 inches tall.

filtering if necessary. Heat was used only in the preparation of the solution of Witte peptone; the filtered solution was cooled to atmospheric temperature prior to use. The solution of trypsinized peptone was made according to the directions of Rivas.[21] As a rule, the solutions were about tenth normal for nitrogen, *i.e.*,

contained approximately 1.4 grams nitrogen per litre. A definite volume of a nutrient solution was always introduced into a pitcher; the same volume of the same solution was used for the determination of the exact nitrogen content of the solution by the Gunning modification of the Kjeldahl method.

Technic.—The procedure with the tall slender pitchers of *Sarracenia Sledgei, S. flava,* and *S. Drummondii* was somewhat different from that with the short broad pitchers of *S. purpurea.*

In plants of the first three species, the shape and size of the pitcher made it impossible to wash out the pitcher cavity and remove the wash water before introduction of the nutrient peptone solution. Therefore, the peptone solution was introduced directly into the untreated pitcher cavity. Pitchers of three types were used: closed, open, and plugged. The technic for *closed* pitchers and *open* pitchers was that used for such pitchers in the experiments on the absorption of water; however, a peptone solution was substituted for the water. *Plugged* pitchers were pitchers which, in age and development, were open and active, but had been kept free from insect captures. Closed pitchers, which were about to open, were selected; the top of each pitcher was folded over, and one or more pins were passed through the double fold; a paper identification tag was placed in the fold. The pitcher, thus sealed, was not injured, but continued to grow, and to expand its top even above the fold; withering of the tissues never occurred. After the lapse of seven or more days, the pitcher was used in an absorption experiment; the procedure was that followed with closed pitchers.

Three days after introduction of the peptone solution, the liquid remaining in the pitcher was collected as in the experiments on absorption of water. At times, its volume was measured in a narrow graduated cylinder. The liquid was then preserved with trikresol. It and the sample of the peptone solution (likewise preserved with trikresol) were placed in stoppered vials, and brought to Philadelphia for determination of the nitrogen content.

The open pitchers of *Sarracenia purpurea* were gently flushed with distilled water from a wash bottle; the macerated mass of insects was scraped from the pitcher walls with a fine loop of platinum wire; the pitcher contents were then removed with a pipette. This process of washing was repeated several times until the pitchers were as free as possible from insect remains. A

Plant of *Sarracenia Drummondii*, showing fully open pitchers, and younger pitchers with closed lids and lips. Photographed in Mobile County, Alabama, where the mature pitchers commonly attain a height of 18 to 28 inches.

definite volume—usually, though not invariably, 10.00 c.c.—of a nutrient solution was introduced by means of a pipette into the *empty* pitcher, and the mouth of the pitcher was tightly closed with a cotton plug. From three to twelve days later, the plug was removed; it was always dry, and not soiled by the pitcher contents. The liquid in the pitcher was drawn up into a serological pipette; its volume was noted; and it was transferred to a Kjeldahl flask. The interior of the pitcher was washed with several successive portions of distilled water; these were transferred in turn by means of the pipette to the Kjeldahl flask; and the nitrogen content of the combined liquid and washings, which had a total volume of approximately 250 c.c., was determined by the Gunning method. In the field experiments on this species, a measured volume of the nutrient solution was poured into the pitcher from a narrow graduated cylinder. The sample of the nutrient solution, and the combined liquid and washings from each pitcher were placed in a separate stoppered vial, preserved with trikresol, and brought to Philadelphia for analysis.

All determinations of nitrogen were made by the Gunning modification of the Kjeldahl method. If in a vial, the liquid was transferred to a Kjeldahl flask; and the vial was washed with several successive portions of distilled water which were transferred to the same flask. If necessary, the liquid from the open pitchers of *Sarracenia flava* was filtered to remove solid insect remains, then was analyzed. A crystal of cupric sulphate the size of a small grain of rice, 10 grams of potassium sulphate, and 25 c.c. of concentrated sulphuric acid were added to the contents of the flask, which were then heated until all the water had evaporated. The determination of nitrogen was then carried out in the usual way. Tenth normal solutions of sulphuric acid and sodium hydroxide were used for titration of the ammonia, using methyl red (orthocarboxybenzeneazodimethylaniline) as an indicator.

Action of the Nutrient Solutions on the Pitchers.—Of all the nutrient solutions used, only that of ammonium chloride exerted any injurious action on the containing pitchers. On the third day after introduction of the ammonium chloride solution, the pitchers were still normal. By the sixth day, the tip of the lid had withered; and by the tenth day the withered region included the

entire lid and the rim of the pitcher. On the other hand, ammonium tartrate solution had no injurious action on the pitchers.

Toluene was introduced into the pitcher with the peptone solution in two experiments recorded at the bottom of Table III. The pitchers withered above the level of their contents, but were not injured below that level.

Tabulation of the Results.—Details of the experiments are given in Table III. Since the weight of nitrogen, and not the weight of the nutrient compound, was determined, weights have been reported as grams of nitrogen. The analysis of the nutrient solution showed the weight of nitrogen introduced; analysis of the liquid remaining in the pitcher several days later gave the weight of nitrogen recovered; the former weight minus the latter weight equalled the weight of nitrogen absorbed. The weight of nitrogen absorbed was then calculated as per cent. of the weight of nitrogen introduced.

The volume of solution introduced and that of the liquid remaining in the pitcher at the end of the experiment are given; the difference between the two is the decrease in volume, which has also been calculated as per cent. of the volume introduced. In the experiments on *Sarracenia purpurea,* this per cent. is probably an index to the absorption of the water.

Absorption by the Southern Species.—Plugged pitchers of both *Sarracenia Sledgei* and *S. Drummondii,* and both closed and open pitchers of *S. flava* always showed a marked absorption of peptone in three days; some pitchers absorbed over 40 per cent. of the introduced peptone.

In the calculation of the absorption, the nitrogen content of the pitcher liquor itself and, in open pitchers, the presence of soluble nitrogenous compounds, derived from the prey, have been neglected. They would increase both the weight of nitrogen present at the beginning of the experiment and the weight of nitrogen absorbed, by exactly the same amount, and would therefore increase the value for the per cent. of nitrogen absorbed. The influence of nitrogenous compounds derived from the prey would depend on the size and number of captured insects.

The nitrogen content of the pitcher liquor is negligible. It amounted to 0.000036 gram nitrogen per 1 c.c. of liquor in a composite sample obtained from 100 closed pitchers of *Sarracenia flava.* A composite sample from 50 open pitchers of this

species contained 0.000060 gram nitrogen per 1 c.c.; another composite sample, also from open pitchers of this species, contained 0.000050 gram nitrogen per 1 c.c., giving an average of 0.000055 gram per 1 c.c. Each sample used for an analysis had a volume of either 25 or 50 c.c. The average volume of the pitcher liquor is 0.66 c.c. in closed pitchers and 1.50 c.c. in open pitchers of *S. flava* (Table I), 0.38 c.c. in plugged pitchers of *S. Sledgei* (average of 31 pitchers), and 0.66 c.c. in plugged pitchers of *S. Drummondii* (average of 20 pitchers). In the entire series of experiments, the influence exerted by the nitrogen content of the pitcher liquor would be greatest in the experiment on four open pitchers of *S. flava*, and would change the per cent. of nitrogen absorbed from 40.49 to 40.73, an increase of about one-quarter per cent.

The liquor in the plugged pitchers may be considered to have the same nitrogen content as that in the closed pitchers of *S. flava*. On account of the smaller volume of the pitcher liquor and its lower nitrogen content, the increase in the per cent. of nitrogen absorbed, due to the nitrogen content of the liquor, would be considerably less in both closed pitchers and plugged pitchers than in open pitchers.

The changes in volume observed in the contents of the pitchers of *Sarracenia flava* may be recalculated, taking into consideration the liquor present in the pitcher at the beginning of each experiment and using the average volume of pitcher liquor as given in Table I. The recalculated percentage decrease in volume is given below; it may be considered to represent the absorption of water. For convenience, the per cent. of introduced nitrogen absorbed is repeated.

	Per cent. of introduced nitrogen absorbed.	Percentage decrease in volume.
1 Closed Pitcher	30.79	37.15
3 Closed Pitchers	42.08	39.97
1 Open Pitcher	36.84	35.65
4 Open Pitchers	40.49	40.79

The results indicate that absorption of the solute (peptone) and of the solvent (water) were not strictly parallel, and did not proceed at exactly the same rate.

Absorption by Sarracenia purpurea.—The experiments on small, poorly developed pitchers of *Sarracenia purpurea* were

made in the field in Tolland County, Connecticut. The results showed that this species absorbs ammonium tartrate, acetamide, urea, and peptone from their aqueous solutions when these solutions are introduced into the pitcher cavity.

The experiments on large vigorous pitchers of *Sarracenia purpurea* were made in the laboratory; the pitchers had recently opened. Aqueous solutions of nine different nutrient nitrogenous substances were introduced into the pitchers; and all nine substances were absorbed to a marked degree. While the "individual difference" of each pitcher entered into each test and was beyond control, the results, as a whole, uniformly had the same trend, and support the following conclusions. The absorption of nitrogenous compounds was progressive, since the per cent. of introduced nitrogen absorbed increased with the time; from 50 to 90 per cent. or more of the nutrient compound was usually absorbed in twelve days. The rate of absorption was generally greatest during the first three or four days. With the exception of the experiments on egg albumin, the per cent. of the introduced nitrogenous compound absorbed in a given time was always greater than the percentage decrease in the volume of the pitcher contents; *i.e.*, the dissolved nutrient was absorbed more rapidly than the water. This conclusion, of necessity, holds true even if part of the water was lost by evaporation.

Absorption of water from the solution occurred. The experiments on ammonium tartrate, acetamide, urea, and asparagin were begun on the same day with pitchers of approximately the same size and with the same volume of nutrient solution. They ended twelve days later. During that time, the decrease in volume of the pitcher contents varied from 22.0 to 60.5 per cent. in different experiments, while it would have been approximately equal in all experiments if due entirely to evaporation.

Egg albumin was absorbed less rapidly than the simpler nitrogenous compounds. This indicates that possibly the complex protein was digested into simpler compounds prior to absorption.

Ammonium chloride was absorbed less rapidly than ammonium tartrate. The absorption of peptone was decreased when toluene was added to the pitcher contents. The decreased absorption was possibly due to the toxic action of the ammonium chloride and the toluene on the pitchers.

TABLE III.

Absorption of Nitrogenous Compounds from the Pitchers of Species of Sarracenia

Species.	Description of pitchers.	Number of pitchers	Nitrogen compound introduced.	Period of absorption, days.	Nitrogen absorption. Weight, in grams, of nitrogen. Introduced.	Recovered.	Absorbed.	Per cent. of introduced nitrogen absorbed.	Volume changes. Volume of nutrient solution introduced.	Volume of solution recovered.	Decrease in volume.	Decrease in volume calculated as per cent. of volume introduced.
									c.c.	c.c.	c.c.	
Sarracenia Sledgei....	Plugged	1	Peptone	3	0.01422	0.00792	0.00630	44.30	.....	.....	.....	.....
	Plugged	1	Peptone	3	0.01366	0.00960	0.00406	29.72	.....	.....	.....	.....
	Plugged	1	Peptone	3	0.01450	0.01044	0.00406	28.00	.....	.....	.....	.....
	Plugged	1	Peptone	3	0.01044	0.00651	0.00393	37.64	.....	.....	.....	.....
	Plugged	1	Peptone	3	0.01044	0.00609	0.00435	41.67	.....	.....	.....	.....
Sarracenia flava.....	Closed	1	Peptone	3	0.01387	0.00960	0.00427	30.79	10.00	6.70	3.30	33.00
	Closed	3	Peptone	3	0.04644	0.02690	0.01954	42.08	40.00	25.20	14.80	37.00
	Open	1	Peptone	3	0.01387	0.00876	0.00511	36.84	10.00	7.40	2.60	26.00
	Open	4	Peptone	3	0.08133	0.04840	0.03293	40.49	70.00	45.00	25.00	35.71
Sarracenia Drummondii	Plugged	1	Peptone	3	0.01142	0.01030	0.00112	9.81	.....	.....	.....	.....
	Plugged	1	Peptone	3	0.01478	0.00932	0.00546	36.94	.....	.....	.....	.....
	Plugged	1	Peptone	3	0.01478	0.00778	0.00700	47.36	.....	.....	.....	.....
	Plugged	1	Peptone	3	0.01044	0.00658	0.00386	36.97	.....	.....	.....	.....
	Plugged	1	Peptone	3	0.01044	0.00630	0.00414	39.66	.....	.....	.....	.....
Sarracenia purpurea.. (small, poorly developed pitchers)	Open	1	Ammonium tartrate	7	0.01352	0.00960	0.00392	28.99	.....	.....	.....	.....
	Open	1	Acetamide	7	0.01464	0.00736	0.00728	49.73	.....	.....	.....	.....
	Open	1	Urea	7	0.01430	0.00595	0.00841	58.57	.....	.....	.....	.....
	Open	1	Peptone	7	0.01415	0.00890	0.00525	37.10	.....	.....	.....	.....
Sarracenia purpurea (large, vigorous pitchers)	Open	1	Ammonium chloride	3	0.01499	0.01261	0.00238	15.88	.....	.....	.....	.....
	Open	1	Ammonium chloride	6	0.01499	0.01156	0.00343	22.88	.....	.....	.....	.....
	Open	1	Ammonium chloride	10	0.01499	0.01030	0.00469	31.29	.....	.....	.....	.....
	Open	1	Ammonium tartrate	4	0.01331	0.00953	0.00378	28.40	.....	.....	.....	.....
	Open	1	Ammonium tartrate	7	0.01331	0.00883	0.00448	33.66	.....	.....	.....	.....
	Open	1	Ammonium tartrate	12	0.01331	0.00658	0.00673	50.56	10.00	6.95	3.05	30.50
	Open	1	Acetamide	4	0.01464	0.00602	0.00862	58.88	.....	.....	.....	.....
	Open	1	Acetamide	7	0.01464	0.00329	0.01135	77.53	10.00	8.60	1.40	14.00
	Open	1	Acetamide	12	0.01464	0.00056	0.01408	96.17	10.00	3.95	6.05	60.50
	Open	1	Acetamide	4	0.01317	0.00616	0.00701	53.23	.....	.....	.....	.....
	Open	1	Urea	7	0.01317	0.00581	0.00736	55.88	10.00	9.50	0.50	5.00
	Open	1	Urea	12	0.01317	0.00336	0.00981	74.49	10.00	7.80	2.20	22.00
	Open	1	Asparagin	4	0.00588	0.00266	0.00322	54.76	.....	.....	.....	.....
	Open	1	Asparagin	7	0.01198	0.00462	0.00736	61.44	10.00	8.00	2.00	20.00
	Open	1	Asparagin	12	0.01198	0.00182	0.01016	84.81	10.00	6.30	3.70	37.00
	Open	1	Glycocoll	4	0.01422	0.01121	0.00301	21.17	.....	.....	.....	.....
	Open	1	Glycocoll	7	0.01422	0.00981	0.00441	31.01	10.00	8.35	1.65	16.50
	Open	1	Glycocoll	12*	0.01422	0.00217	0.01205	84.74	.....	.....	.....	.....
	Open	1	Trypsinized peptone	3	0.01786	0.01170	0.00616	34.49	10.00	9.60	0.40	4.00
	Open	1	Trypsinized peptone	7	0.01786	0.00778	0.01008	56.44	10.00	9.15	0.85	8.50
	Open	1	Trypsinized peptone	12	0.01786	0.00546	0.01240	69.43	10.00	8.60	1.40	14.00
	Open	1	Peptone	3	0.07061	0.06634	0.00427	6.05	25.00	23.50	1.50	6.00
	Open	1	Peptone	6	0.07061	0.05842	0.01219	17.26	25.00	22.50	2.50	10.00
	Open	1	Egg albumin	4	0.01765	0.01429	0.00336	19.04	10.00	8.30	1.70	17.00
	Open	1	Egg albumin	7	0.01765	0.01205	0.00560	31.73	10.00	5.80	4.20	42.00
	Open	1	Egg albumin	12	0.01765	0.00820	0.00945	53.54	10.00	1.90	8.10	81.00
	Open	1	Peptone *	3	0.07061	0.06760	0.00301	4.26	25.00	24.50	0.50	2.00
	Open	1	Peptone *	6	0.07061	0.06262	0.00799	11.32	25.00	23.00	2.00	8.00

* In these experiments, toluene was added to the contents of the pitcher.

ABSORPTION OF ORGANIC NITROGENOUS COMPOUNDS IN THE PRESENCE OF A "BUFFER."

The objection might be raised that the nitrogen, reported in Table III as absorbed, was actually converted into volatile nitrogenous compounds which escaped from the pitcher. While open pitchers of all the species used in the absorption tests have been found by us to contain bacteria which, in the Petri dish, act on simple nitrogenous compounds (glycocoll, asparagin, acetamide, urea, ammonium lactate, ammonium tartrate) with the production in the medium of a reaction alkaline to rosolic acid, and occasionally with the production of an odor of ammonia or amines, nevertheless, such an odor was never noted in the pitcher contents when they were removed for analysis at the end of an absorption test.

Reference to Table III shows that, in the presence of toluene, a bactericide, peptone was absorbed.

Additional laboratory tests on absorption were conducted, in which a "buffer" was introduced into pitchers of *Sarracenia purpurea* with the nutrient solution. Any volatile bases, formed from the nutrient nitrogenous compound by bacterial action, would be retained by the buffer, and could not escape from the pitcher.

The buffer used was an aqueous solution containing 0.4 gram monopotassium phosphate (KH_2PO_4) and 0.6 gram dipotassium phosphate (K_2HPO_4) in each 10 c.c.; a buffer solution containing these two phosphates in this ratio has been used in urinalysis to neutralize the ammonium carbonate formed by cleavage of urea by the enzyme urease.[22]

Technic.—The procedure in these experiments was exactly the same as in those on the absorption of nitrogenous compounds by *Sarracenia purpurea* described in the preceding section. However, 10.00 c.c. of the buffer solution were introduced into each pitcher at the same time as the 10.00 c.c. of the solution of the nutrient nitrogenous compound. Likewise 10.00 c.c. of the buffer solution were added to each sample of the nutrient solution used for the determination of the exact weight of nitrogen introduced.

In those experiments in which the period of absorption was seven days, the technic was modified somewhat. The pitcher was cut from the plant at its base; its exterior was cleansed with

a stream of distilled water from a wash bottle; and the cotton plug was removed from the mouth of the pitcher; it was always dry and unsoiled. The bottom part of the pitcher was cut off at the very beginning of the pitcher cavity; and the pitcher contents and subsequent washings were caught in a beaker, and passed in turn through a filter into a Kjeldahl flask. In this manner, all chances for retention of the solution of the nitrogenous compound in the narrow, bottom region of the pitcher cavity were entirely eliminated.

Results.—All four compounds—urea, acetamide, asparagin, and peptone—were absorbed in the presence of the buffer. The per cent. of introduced nitrogen absorbed usually increased with the period of absorption. Although approximately the same weight of a given compound was introduced into the pitchers in both series of experiments, the per cent. absorbed in a given time was usually less in the presence of the buffer (Table IV) than

TABLE IV.

Absorption of Organic Nitrogenous Compounds by Individual Open Pitchers of Sarracenia purpurea in the Presence of a Buffer.

Nitrogenous compound.	Period of absorption, days.	Weight, in grams, of nitrogen.			Per cent. of introduced nitrogen absorbed.
		Introduced.	Recovered.	Absorbed.	
Acetamide	4	0.01548	0.00953	0.00595	38.44
Acetamide	7	0.01548	0.00077	0.01471	95.03
Urea	4	0.01471	0.01093	0.00378	25.70
Urea	7	0.01471	0.01247	0.00224	15.23
Asparagin	4	0.01422	0.00981	0.00441	31.01
Asparagin	7	0.01422	0.00637	0.00785	55.20
Peptone	3	0.01569	0.01037	0.00532	33.91
Peptone	15	0.01569	0.00743	0.00826	52.64

in its absence (Table III). In this connection, it should be noted that the plants used in the experiments with the buffer were procured several months later than those used in the experiments on the absorption of nitrogenous compounds and the absorption of phosphates, and possibly were less active though still vigorous.

In the presence of the buffer, the pitcher contents increased in volume, while they decreased in nitrogen content. The initial volume was 20 c.c. At the end of four days, the contents of

three pitchers were withdrawn for analysis, and had the following volume:

Pitcher containing acetamide	25 c.c.
Pitcher containing urea	24 c.c.
Pitcher containing asparagin	24 c.c.

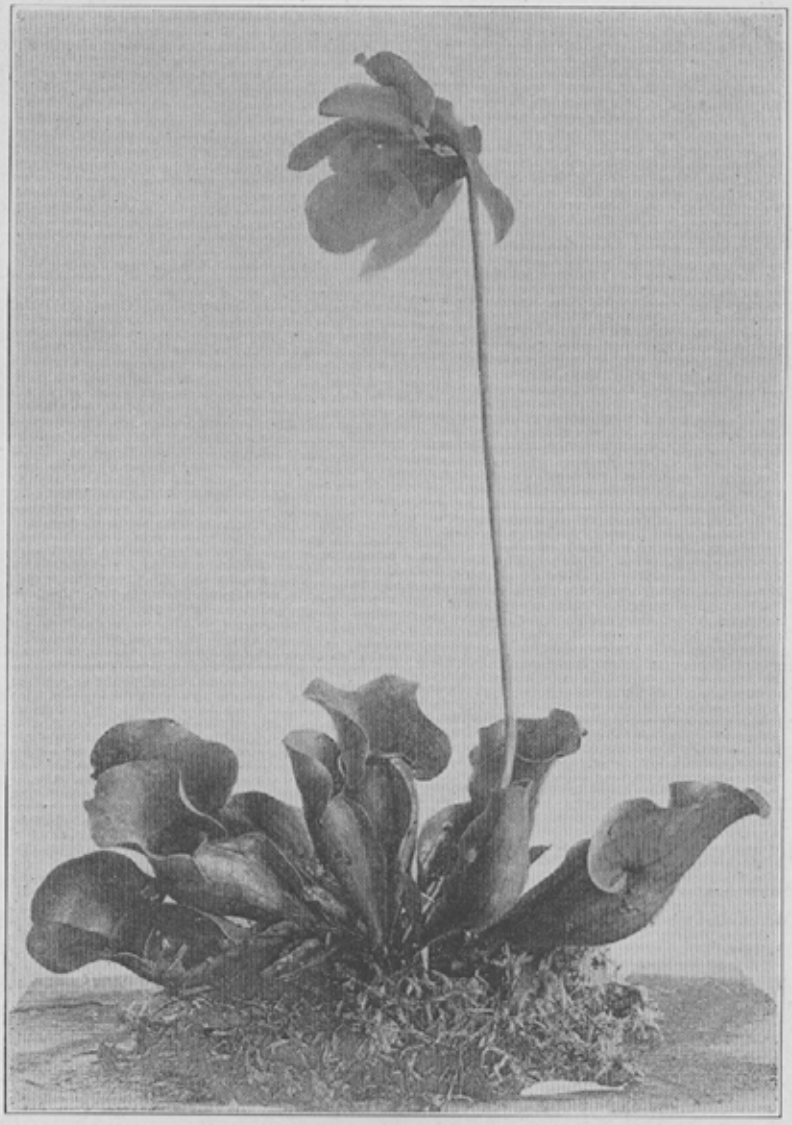
The increase in volume therefore ranged from 20 to 25 per cent.

ABSORPTION OF PHOSPHATES.

Pfeffer [18] has suggested that insectivorous plants derive both nitrogen and phosphorus from their prey. The following laboratory experiments were carried out to determine the absorption of orthophosphates from the pitcher cavity in *Sarracenia purpurea*. The plants formed part of the group secured at Whitings, New Jersey, for the laboratory experiments on absorption of nitrogenous compounds. The two series of experiments, on nitrogen compounds and phosphates, respectively, were made simultaneously but on different plants.

Technic.—The insect remains were removed from the pitchers as in the experiments on absorption of nitrogenous compounds. Then 10.00 c.c. of the phosphate solution were introduced with a pipette into each *empty* pitcher, the mouth of which was immediately closed with a tightly fitting cotton plug. The phosphate solution was exactly neutral in reaction, *i.e.*, had a hydrogen ion concentration of 10^{-7} or pH 7.0. It was prepared as directed by Levy, Rowntree, and Marriott,[23] whose procedure is based on that of Sörensen;[24, 25] 37 c.c. of a fifteenth molar solution of monopotassium dihydrogen orthophosphate and 63 c.c. of a fifteenth molar solution of disodium monohydrogen orthophosphate were mixed. The phosphate content of the resulting solution was determined by titration.

After this neutral phosphate solution had been in a pitcher for a definite period of time, varying between 7 and 20 days, the cotton plug was removed—it was always dry and not soiled by the pitcher contents; the liquid in the pitcher was drawn up into a serological pipette; its volume was recorded; and it was transferred to a beaker. The pitcher cavity was washed with several successive portions of distilled water; these were transferred in turn by means of the pipette to the beaker; and the phosphate content of the combined liquid and washings was determined by titration.



Plant of *Sarracenia purpurea* in full bloom, with open pitchers. Photographed in Ocean County, New Jersey, where vigorous pitchers attain a length of 10 or more inches.

All titrations of phosphate content were made with a standard solution of uranyl nitrate after the addition of acetic acid and sodium acetate; tincture of cochineal was used as an internal

indicator. The solution of uranyl nitrate was standardized against a definite weight of monopotassium dihydrogen phosphate as suggested by Giles.[20] Results were calculated as grams of phosphoric oxide, P_2O_5.

The difference between the weight of phosphoric oxide introduced into the pitcher and the weight of phosphoric oxide recovered in the fluid and washings from the pitcher represented the weight of that compound absorbed from the cavity by the pitcher.

Results of these experiments are given in Table V, and lead to the following conclusions. Absorption of phosphates occurred in all the pitchers. The individual pitchers do not show any

TABLE V.

Absorption of Phosphates from the Pitchers of Sarracenia purpurea.

Number of pitchers.	Period of absorption, days.	Weight, in grams, of phosphoric anhydride.			Per cent. of introduced phosphoric anhydride absorbed.		Volume changes.			
		Introduced.	Recovered.	Absorbed.			Volume of phosphate solution introduced.	Volume of solution recovered.	Decrease in volume.	Decrease in volume calculated as per cent. of volume introduced.
							c.c.	c.c.	c.c.	
1	7	0.04681	0.03515	0.01166	24.91	Average 21.68	10.00	3.50	6.50	65.00
1	7	0.04681	0.03818	0.00863	18.44					
1	12	0.04681	0.03636	0.01045	22.32	Average 22.98	10.00	6.00	4.00	40.00
1	12	0.04681	0.03575	0.01106	23.63		10.00	5.90	4.10	41.00
1	20	0.04681	0.03303	0.01378	29.44	Average 26.86	10.00	5.45	4.55	45.50
1	20	0.04681	0.03545	0.01136	24.27		10.00	5.85	4.15	41.50

definite relation between the period of absorption and the per cent. of the introduced phosphoric oxide absorbed. When the two experiments of each period are averaged, and the averages are compared, the absorption appears to have been progressive throughout the entire 20 days, but to have been most rapid during the first 7 days. In a given time, the pitcher contents usually showed a greater percentage decrease in volume than in phosphoric oxide content; this indicates that water was absorbed more rapidly than phosphates from the solution in the pitcher cavity.

ABSORPTION OF THE LITHIUM ION.

These experiments were also made on plants of *Sarracenia purpurea* in the laboratory. A double normal solution of neutral lithium citrate ($Li_3C_6H_5O_7$) was prepared from lithium car-

bonate and citric acid. The solution was filtered; and 20 c.c. of the filtrate were introduced into each of four open pitchers, which were then closed with tightly fitting cotton plugs. One pitcher was examined at the end of seven days, the other three pitchers at the end of fourteen days. Each pitcher was cut from the plant at its base; and its exterior was well washed with a stream of distilled water from a wash bottle. The cotton plug was removed; the bottom portion of the pitcher was cut off just above the beginning of the pitcher cavity; the contents were permitted to run out; all insect remains were removed with a platinum needle; and the entire interior of the pitcher was repeatedly flushed, and its lid and exterior washed with distilled water until the wash water no longer imparted the lithium color to the non-luminous Bunsen flame.

The pitcher was next dried at 100° C., then ashed, in a platinum crucible. The ash was treated with 0.5 c.c. of concentrated hydrochloric acid and 2.0 c.c. of distilled water; and the resulting solution was filtered through a small, moistened, ashless filter-paper.

Lithium was identified in this solution of the ash by the crimson color imparted to the non-luminous Bunsen flame, and by spectroscopic examination of this colored flame by means of a Hofmann direct vision spectroscope with a scale previously calibrated for the lithium and sodium lines. The ash from all four pitchers yielded the same result.

As a control, examination was made of a composite sample of dried tissue obtained from fifty-nine open pitchers of *Sarracenia purpurea* which had been thoroughly cleansed from insect remains. These pitchers were obtained at the same time and place (Whitings) as the plants used in the tests on absorption of lithium. The dried tissue was ground so that it passed completely through a sieve with 20 meshes to the linear inch. A five-gram sample of the ground tissue was ashed in platinum. A loop of platinum wire was dipped into concentrated hydrochloric acid, then into the ash, and finally was placed in the non-luminous Bunsen flame. Neither the flame reaction (color) nor the spectral line of lithium appeared.

These results show that lithium is not a normal constituent of the tissues of *Sarracenia purpurea*, and that the pitchers of

this plant absorb the lithium ion from its neutral solution when such a solution is introduced into the pitcher cavity.

The spectroscopic examinations were made, in collaboration with Dr. Charles B. Bazzoni, in the Randal Morgan Laboratory of Physics of this University.

RESPONSE OF THE PITCHERS TO STIMULATION.

In 1875 Mrs. R. M. L. Austin, a California botanist, discovered that the introduction of meat into the pitcher cavity causes the pouring out of an additional supply of pitcher liquor in *Darlingtonia californica.* The following quotation is from a letter written by Mrs. Austin to Mary E. Pulsifer Ames, and was published by the latter:[19] "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." According to Asa Gray,[20] 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."

In certain of our experiments in the field, a definite volume of a solution was introduced into pitchers which were then closed in the manner described in the sections on absorption. After the lapse of a definite period of time, from one to ten days, the average volume of the pitcher contents was determined in the usual way. From this average volume was subtracted the sum of the volume of solution introduced plus the average volume of the pitcher liquor in pitchers of the same species and same type (closed, open, or plugged); the remainder was the average increase in volume of the liquid pitcher contents, due to outpouring of additional liquor as the result of stimulation of the pitchers by the introduced solution. This average increase in volume was then calculated as per cent. of the average volume of the pitcher liquor of such pitchers.

Fresh skim milk was diluted with an equal volume of water, sterilized by heat, cooled to atmospheric temperature, and used in stimulation tests. The *average increase amounted to as much as* 1200 per cent. in 6 days in closed pitchers of *Darlingtonia californica,* 1000 per cent. in 4 days in closed pitchers of *Sarracenia flava,* and 900 per cent. and 500 per cent., respectively, in

5 days in plugged pitchers of *S. Sledgei* and *S. Drummondii.* With a given species and pitchers of a given type, the increase became more marked, the longer the time elapsed between introduction of the diluted milk and withdrawal of the pitcher contents, and the greater the volume of milk introduced.

Beef broth produced an *increase* of over 300 per cent. in pitchers of all three types in *Darlingtonia californica* in 5 days. It also produced increases of over 1700 per cent. and 600 per cent., respectively, in closed and open pitchers of *Sarracenia flava* in 4 days; the increase in one closed pitcher was over 2400 per cent.

Increases in the volume of the pitcher secretion were produced by certain reagents: dilute solution of potassium ferrocyanide, dilute solution of potassium ferricyanide, 2 per cent. solution of ferrous sulphate. The ferrous solution was introduced into pitchers of *Sarracenia Sledgei, S. flava,* and *S. Drummondii,* the other solutions into pitchers of *S. flava* only. A 2 per cent. solution of ferric ammonium sulphate, to which sodium acetate had been added, killed the pitchers of all three species within a few days after its introduction.

When introduced into the pitchers, raw egg-white produced no change in the volume of the pitcher liquor. This was also true of certain solids: coagulated egg-white, fibrin, casein, and cheese.

In the experiments on *Darlingtonia californica,* cubes of lean beef were introduced into the pitchers by means of forceps, and fell to the level of the pitcher liquor. Each edge of a cube was one-eighth inch long. When raw beef was used, an increase in volume of the pitcher liquor occurred in pitchers of all three types —closed, open, and plugged—but was always less than 200 per cent. in 7 days. When cooked beef was used, little, if any, increase took place in the volume of the liquor in either open or plugged pitchers.

This phenomenon of secretion of an abnormal volume of pitcher liquor is not in conflict with that of absorption. The decrease in volume of the pitcher contents, as a rule, occurred less rapidly than the absorption of nitrogenous compounds, and more rapidly than the absorption of phosphates from their respective solutions. Moreover, in the absorption experiments in which a buffer was added to the solutions of the nitrogenous compounds,

those compounds were absorbed while the pitcher contents increased in volume. An increase in volume of the pitcher contents also occurred in the experiments on the absorption of the lithium ion. In the plant economy, this increased secretion probably represents a result of natural selection, in which there has gradually developed a rise in the level of the pitcher liquor that insures the covering of captured insects which otherwise would lie in a dry and undigested state above the normal level of that fluid.

RESPONSE OF THE PITCHERS TO INTRODUCED ACID AND ALKALI.

Dilute aqueous solutions of acids (hydrochloric and acetic) and of sodium hydroxide were also introduced into the pitchers of certain species, and the pitcher contents examined 5 days later.

The pitcher liquor in open pitchers of *Darlingtonia californica, Sarracenia Sledgei,* and *S. Drummondii* and in plugged pitchers of *D. californica* is neutral to litmus, that in closed pitchers of *S. flava* acid, and that in open pitchers of *S. flava* faintly acid to neutral to litmus. The solutions introduced into the pitchers were tested, and possessed either a distinctly acid reaction (hydrochloric acid, acetic acid) or a distinctly alkaline reaction (sodium hydroxide) to litmus. Each pitcher received a definite volume (from 3 to 30 c.c., usually 5 c.c.) of a solution of *one* of the reagents.

The acetic acid was used as a 0.05 per cent. solution with the species of *Sarracenia* just mentioned, and as a 0.10 per cent. solution with *Darlingtonia californica.* Within 5 days, the reaction of the pitcher contents to litmus had become neutral in the plugged pitchers of *Darlingtonia californica,* in the open pitchers of that species, *Sarracenia Sledgei,* and *S. Drummondii,* and in the closed pitchers of *S. flava,* and was acid in the open pitchers of the last-named species. The volume of the pitcher contents showed a marked decrease in all the *Sarracenia* pitchers, and a slight increase in those of *D. californica.*

The hydrochloric acid was usually used as a 0.05 per cent. solution. Within 5 days, the pitcher contents had become neutral to litmus in open pitchers of *Darlingtonia californica,* and *Sarracenia Drummondii,* and in the majority of those of *S. Sledgei;* they were faintly acid to litmus in both open and closed pitchers of *S. flava.* The *Sarracenia* pitchers showed a decrease in the volume of their contents. A 0.2 per cent. solution of hydrochloric

acid was also introduced into both open and plugged pitchers of *D. californica;* some pitchers were injured, becoming partly shriveled and brown; their contents were faintly acid to litmus; other pitchers were unharmed, their contents were neutral to litmus at the end of the 5 days.

Sodium hydroxide was used as a 0.02 per cent. solution in both open and plugged pitchers of *Darlingtonia californica,* and as a 0.05 per cent. solution in open pitchers of *Sarracenia Sledgei* and *S. Drummondii* and in both open and closed pitchers of *S. flava.* Within 5 days, the contents of the pitchers had become neutral to litmus in all the experiments. The pitcher contents decreased in volume in the *Sarracenia* pitchers and increased slightly in volume in those of *Darlingtonia californica.*

It is of interest to note that, in the experiments with acids, an acid reaction was retained only in the pitchers of *Sarracenia flava;* the pitcher liquor of this species normally tends to have an acid reaction, and its protease acts best in an acid medium.

After the introduction of either dilute acid or dilute alkali into the pitchers, the normal reaction of the pitcher contents was restored, as a rule, in a few days. This return of the pitcher contents to their normal reaction recalls the behavior of the human stomach under somewhat similar conditions, as studied by Spencer, Meyer, Rehfuss, and Hawk.[27]

SUMMARY.

I. A résumé has been given of our observations on:

1. The occurrence of reducing sugar in the nectar.

2. The wetting action of the pitcher liquor on insects.

3. The occurrence of a proteolytic enzyme in the pitcher liquor of the Sarracenias and its absence from that of *Darlingtonia californica.*

4. The freedom from bacteria of the pitcher cavity and liquor in closed pitchers.

5. The occurrence of proteolytic bacteria in the contents of open pitchers, in which captured insects were present.

6. The secretion of additional pitcher liquor in response to stimulation by certain foods such as milk and meat broth, and by certain other substances.

7. The response by the pitchers to introduction of acid or alkali in dilute solution.

II. A detailed account has been given of our studies on absorption by the pitchers of substances introduced into their cavities. These studies led to the following general conclusions:

1. Water, which was introduced into the pitchers of *Darlingtonia californica* and the Sarracenias, underwent absorption.

2. When an aqueous solution of a nitrogenous compound was introduced into pitchers of the Sarracenias, both the nitrogenous compound and the water were absorbed, but at a different rate; absorption of the nitrogenous compound was usually more rapid than that of the water.

3. When a phosphate buffer was added to the aqueous solution of the nitrogenous compound, the latter was absorbed while the pitcher contents increased in volume.

4. When a neutral phosphate solution was introduced into pitchers of *Sarracenia purpurea,* both the phosphate and the water were absorbed, but at a different rate; absorption of the phosphate was less rapid than that of the water.

5. The per cent. of the introduced nitrogenous compound or phosphate absorbed usually increased with the period of absorption.

6. When a solution of neutral lithium citrate was introduced into pitchers of *Sarracenia purpurea,* the lithium ion was absorbed.

7. Absorption by the pitchers of substances introduced into their cavities in solution has been demonstrated (*a*) by the decrease in the nitrogen or phosphate content of the solution, and (*b*) by appearance in the pitcher tissues of lithium, an element not normally present.

8. These results indicate that the proteolytic products, formed in the pitcher cavity by digestion of the prey, are absorbed by the pitchers and are utilized for the nutrition of the plant. They also indicate that phosphates, and probably other mineral foods, derived from the prey, are absorbed and utilized in like manner.

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(Citations numbered 1 to 20 inclusive refer to insectivorous plants. The remainder of the papers cited deal with methods of research.)

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Method of Cleaning Coil Springs. DONALD A. HAMPSON. (*Machinery*, vol. xxvi, No. 4, p. 343, December, 1919.)—A certain manufacturer who produces coil springs from copper-finished steel wire had considerable trouble in keeping them bright and free from oil. These springs are two inches long and have an extension at each end that projects at right angles to the axis of the spring. In the production of these springs, the use of lubricant is imperative in the operation of the forming machine, which results in the presence of oil on the finished product. Unless this oil is removed, the bright copper finish of the wire becomes dull, and thus the selling qualities are lessened. This objection is now overcome by the method to be described.

The springs are packed in a wooden box having a removable cover. A one-inch shaft passes through the box and is secured to its sides by flanged collars. The shaft is supported by a bearing on each side of the box, and carries a driving pulley on one end by means of which the box is revolved at the rate of forty revolutions per minute. After the box has been filled with springs, a quantity of sawdust is dumped in and the cover fastened on. By revolving the box for about twenty minutes, the sawdust is circulated through the springs, during which process the oil is completely absorbed and the springs are given a uniform polish. The springs tend to become tangled if the box is revolved longer than the time stated, or if the box is only partly filled. R.

Test of Alpine Tanks.—The tank in peace-time is to be applied to transporting passengers and goods to hotels on mountains where carriage roads are lacking. A test of this method of mountain climbing was held September, 1919, in the Department of France in which Mt. Blanc is situated. The course was 4.7 km. long with an ascent of .7 km. over mule tracks, peaty ground, and meadow land, through fords and along deep ravines. The Renault caterpillar tractor made the ascent in 83 minutes, the descent in 66 minutes, and completed three trips in a day.

G. F. S.