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THE CAPTURE OF PREY BY THE BLADDERWORT[1]

A REVIEW OF THE PHYSIOLOGY OF THE BLADDERS

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(With Plate IX and 2 figures in the text.)

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I. Introduction

For more than fifty years which have elapsed since the publication of the classic researches by Darwin and Cohn, *Utricularia* has been included among the insectivorous or, more properly, carnivorous plants. However, it was not until quite recently that anything positive has been known about the mechanism by which the bladders of this plant effect the capture of their prey—the original explanation of Darwin and Cohn was hardly satisfactory. The first announcements of the discovery of the active engulfment of the victims by the springing of a set snare appeared in publications not claiming the attention of botanists generally, and so for some time remained unnoticed, even by those actively engaged in the study of the bladders. The best evidence of the truth of this statement lies in the fact that the active sucking-in of the quarry has been *discovered independently no less than four times*, by observers in widely separated parts of the

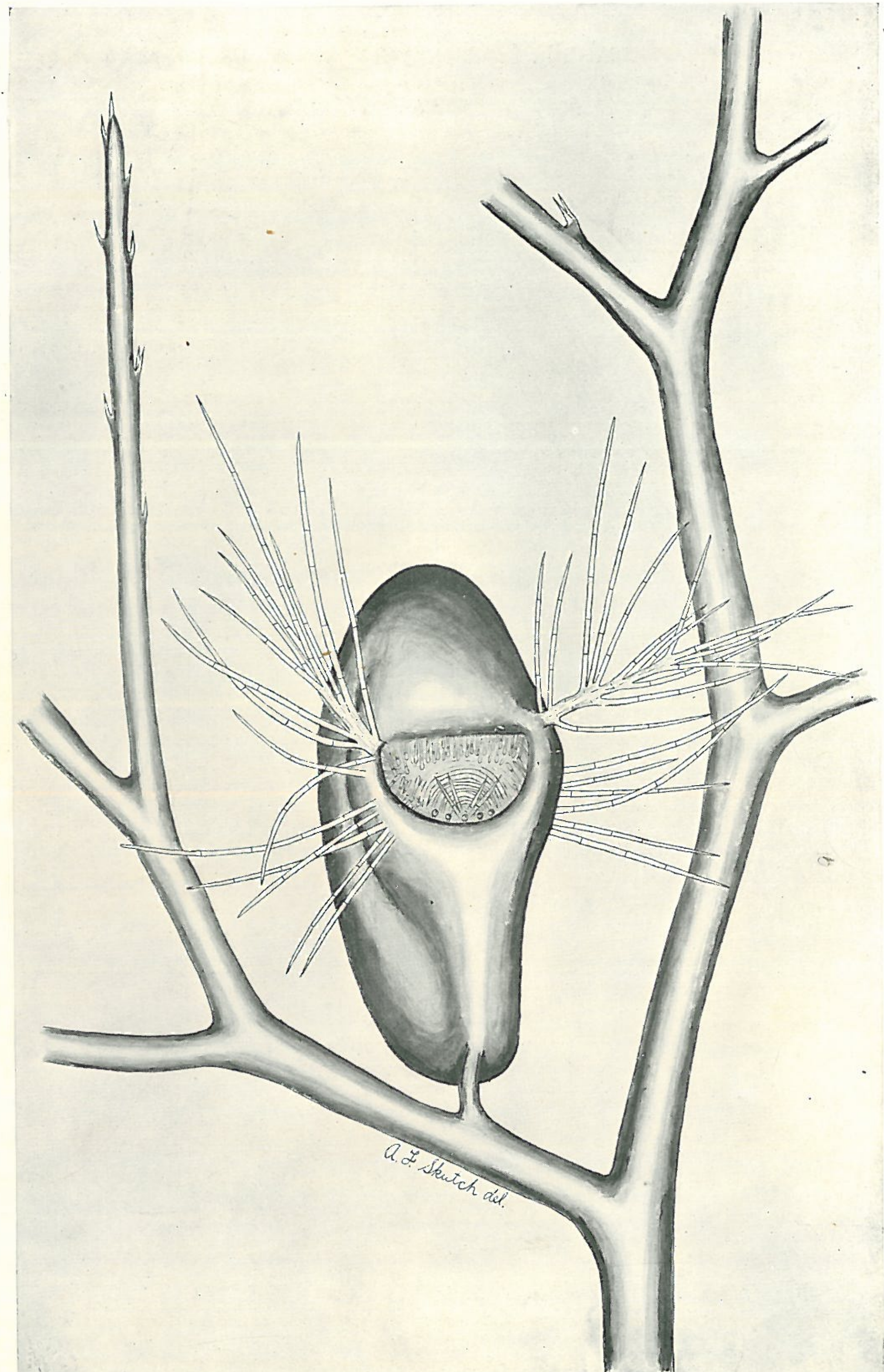
[1] Botanical Contribution from the Johns Hopkins University, No. 93.

world. In view of the very general biological interest of *Utricularia*, which appears to be one of the most peculiar and highly specialised of insectivorous plants, and of the scattered nature of much of the original literature, it seems to be of value to bring together into a single article and to make accessible the most important results of previous investigations. This interest appears to be broadening from the application of the bladders to several new uses—as an unique object adapted to the study of the nature of the external membranes of submerged aquatics, a research which the recent discovery of the significance of hydropotes promises to make of considerable importance; and as a possible agent for the prophylactic treatment of waters infested by the larvae of malarial mosquitoes.

The writer was led to the investigation of this problem at the suggestion of Professor R. W. Hegner who, while studying the bladders in another connection, had discovered their sudden increase in volume upon touching the valve, and recognised the need of research on the physiology of this reaction. At the same time that the subject was tackled in the laboratory, a thorough search was made of the literature, which revealed that the necessity of investigating this behaviour of the bladders was perhaps not so urgent as the promulgation in a form more readily accessible of the results of investigations already made on the subject. The present paper is, then, the outgrowth of plans for experiments which for this reason never materialised. The writer has, however, satisfied himself by personal observation of the correctness of the salient points of the story as retold here. He desires to acknowledge his great indebtedness to Professor Hegner and to Professor B. E. Livingston for many helpful criticisms and suggestions.

II. The Structure of the Bladder

The most complete accounts of the structure of the bladders of floating species of *Utricularia* are those of Cohn (5) and Meierhofer (27) for *U. vulgaris*, of Darwin (11) for *U. neglecta*, and of Goebel (17, 18) for *U. flexuosa*. Many other species have been more or less thoroughly studied and compared with these forms (see in particular Meierhofer, von Luetzelburg and Goebel). Withycombe (35) and Czaja (7) have given special attention to the structure of the valve and collar. The ontogeny of the bladders of four species has been carefully followed by Meierhofer, and Darwin, Kamienski (23) and Goebel have figured stages of their development. Perhaps the most detailed account of the structure of the bladder of a floating species is that by Meierhofer



of *U. vulgaris*, and this is certainly the most completely illustrated of all.

The bladder of *U. vulgaris* is roughly lenticular in shape, and may reach 2·5 or 3 mm. in length. It is attached by a short, slender stalk, inserted on its ventral or adaxial surface, to a spot near the inner angle of a fork of the leaf, which is dissected in a filiform manner. A single leaf may bear a dozen or more bladders, but often carries fewer. Occasionally, at the height of summer, a hundred bladders may be seen on one large leaf, and Glück (16) gives as a maximum 209. The bladder, which is the morphological equivalent of a segment of a leaf, or at times of a whole leaf (Kamienski (23)), is bilateral in symmetry (see Fig. 1 and Plate IX). The dorsal line is elongated and strongly arched, the ventral shorter and almost straight. At the anterior end is found the roughly semicircular aperture, which lies on the ventral surface. This aperture is closed by a valve, the free, ventral margin of which lies against a thickened pad of cells, the "collar" of Darwin. From the sides of the aperture, in continuation of the dorsal surface, spring two long, slender, branched, multicellular appendages, which Darwin termed the "antennae," from their resemblance to the antennae of an entomostracan crustacean. Below the antennae, there is on either side of the aperture a row of several long bristles, a single cell in thickness and several in length.

The walls of the bladder are everywhere two cells in thickness, except for the collar and the vascular bundle. The latter branches as it enters from the stalk, sending a posterior division along the dorsal wall and an anterior along the ventral wall. These vascular strands lie in the median plane of the bladder, and extend nearly to the aperture. In a truly median, vertical section of the bladder, the walls would therefore appear several cells thick. The ventral branch of the bundle gives off short, blind branches to the right and left as it enters the collar.

The roughly semicircular valve is attached dorsally by the arc, except for a short distance on either side where the arc joins the straight edge, which also is free. It consists of two regions, a central semicircular portion which is convex outward in both horizontal and vertical sections, and a ring-shaped outer portion which is flat. The outer of the two layers of the wall consists of a restricted area of small cells situated at the base of the four bristles, near the middle of the free margin, and around this of larger cells with angular, zig-zag anticlinal walls. The inner layer is of larger and less compactly arranged cells, their long axes radiating from a region of small cells

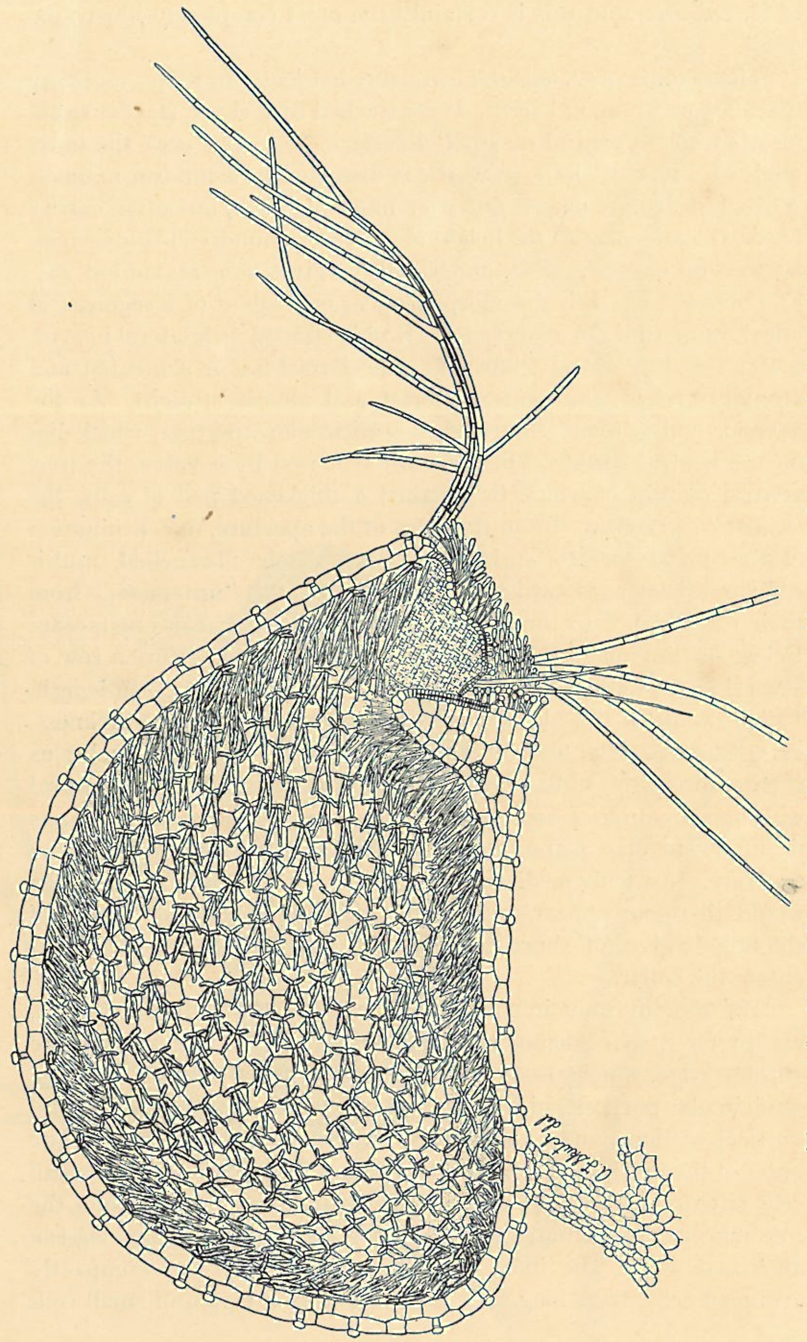


Fig. 1. A bladder of *Utricularia vulgaris* with one side removed to show the internal structure.

at the base of the bristles. Most of the cells of both layers have walls thickened by anticlinal ridges, which are more pronounced in the outer stratum, and the walls of the inner layer facing the cavity of the bladder are strengthened by broad thickenings which are continuous from cell to cell, and form conspicuous rings concentric about the bases of the bristles.

All parts of the bladder are studded with numerous trichomes, which are of great importance in the mechanism of the organ. These hairs may all be considered as modifications of a primary morphological type consisting of three cells, a stalk cell inserted in the wall, a short neck cell, and a head cell which may be variously modified. The whole trichome arises as a papillate outgrowth from a single superficial cell of the wall. The external walls of the bladder bear, in common with the leaves and the stem, glands in which the roughly spherical head is divided into two cells by an anticlinal wall. The greater portion of the inner wall is supplied with trichomes in which the head is divided into four cells, the "quadrifids" of Darwin. The arms, which are generally long and gently tapering, are grouped in pairs. Those of the longer pair point backward, away from the aperture, the shorter more or less sideways. The arms of all the quadrifids are longest near the aperture, and shortest near the stalk. On the inner side of the collar are found trichomes terminating in two long, tapering arms, which diverge from each other only slightly. The outer surface of the valve is richly supplied with hairs. Near its upper, attached margin are found the club-shaped hairs, with a very long stalk cell, short neck, and enlarged, cylindrical or clavate head. Proceeding toward the ventral margin the stalk cells become shorter, and the heads more spherical. Near this margin, the very large spherical or transversely elongated heads are practically sessile. In addition to trichomes of this type, the valve bears near its lower margin, and symmetrically placed on either side of the median line, two pairs of long, slender bristles, composed each of several uniseriate cells.

The collar, or sill, is composed of a cushion of large, parenchymatous cells, surmounted by a triple layer of specialised cells, which form a glandular epithelium. In a longitudinal section of the bladder, the epithelium appears to be made up of an upper and a lower layer of palisade-like cells, separated by a middle layer of flattened cells. Adjacent rows of cells are not fused by a middle lamella, for the pad is formed merely of closely packed trichomes of the typical structure. In surface view, the glandular heads are elongated parallel to the length of the collar, so that they present their broader walls to the edge of the valve.

From the above description, the trap-like nature of the bladder should be at once evident. The valve is so contrived that it is free to move inward, but pressure against its inner wall only pushes it more firmly against the resisting collar, and escape from the interior of the bladder is impossible. Various functions have been ascribed by different authors to the several types of trichomes. The appendages surrounding the orifice, consisting of an antenna and the several bristles situated on each side and converging toward their insertion, were supposed by Darwin to act in the manner of a funnel, conducting to the aperture any animal which may happen to swim in among them. On the other hand, Hegner (21) states that they "seemed rather to hinder than to guide paramoecia to the opening, since many specimens that might have entered gave the avoiding action and swam away when they encountered these bristles." They have also been supposed to facilitate the access to the valve of creeping animals. The glandular hairs situated on the valve and collar secrete a mucilage which probably serves to attract small aquatic animals. The four-armed trichomes on the inner wall have always been regarded as absorptive in function, taking up the nutritive substances derived from the disintegration of the prey, and they also remove water from the lumen in "setting" the bladder. The external, two-celled glands are perhaps hydropotes, at any rate, they readily absorb various dye-stuffs (Czaja (7)).

III. Historical Summary

During the vegetative season, the rootless shoots of the free-swimming, aquatic species of *Utricularia* float just beneath the surface of the pond or ditch in which they are growing. The conspicuous flowers are displayed in the air at the summits of long peduncles. Earlier botanists thought that the small bladders borne on the finely dissected leaves served merely to impart to the plant the buoyancy requisite to remain afloat and to sustain the weight of the aerial inflorescence. According to their observations, these bladders usually contained air. At the end of the vegetative season, when the compact turion or winter-bud was formed from the apical growing point, the bladders became filled with slime or water, or else dropped off, allowing the plant to sink to the bottom. In the following spring, air-containing bladders appeared on the newly expanding shoot, and lifted the plant to the surface once more (A. P. DeCandolle, quoted by Goebel (18)).

To those of this opinion the function of the bladders was the same as that of the peculiar flotation-apparatus at the base of the inflorescence of such highly specialised forms as *U. stellaris* and *U. inflata*. An expression of this view may be found in the earlier editions of Asa Gray's *Structural and Systematic Botany* ((20), p. 445). Even in 1879, after the appearance of the researches of Darwin and Cohn, Drude (12), while admitting that the bladders serve as pitfalls for animals, was inclined to regard their supposed function as a flotation apparatus as of equal importance, and consequently to view their ecological significance as two-fold.

However, if all of the bladders are removed from a bladderwort plant it will still float, as do many other aquatic plants without bladders; thanks to the air contained in the intercellular spaces of the stem and leaves, every portion of the plant is of itself buoyant. Moreover, one sometimes finds in the autumn plants which have shed all their bladders, but have not thereby lost their buoyancy (Brocher (1)), and in the spring turions sometimes rise to the surface before the young leaves have developed bladders (Goebel (18)). In some species, as *U. intermedia*, the bladders are not uniformly distributed over the plant, but are borne only on specialised leafless branches which are positively geotropic (von Luetzelburg (26)), and grow vertically downward beneath the surface of the water. Later students have shown that the *Utricularia* bladders do not usually contain gas-bubbles in their lumina, and hence cannot greatly decrease the specific gravity of the plant as a whole. Nor does the old explanation account for the presence of the bladders of the terrestrial bladderworts, which constitute the majority of the 250 or more species making up this genus, all except one of which possess bladders, albeit usually not in such abundance as in the case of the aquatic species. The single recorded species which is devoid of bladders is *U. neottioides*, which grows attached to rocks in the rapids of streams in Paraguay and Brazil (von Luetzelburg (26)).

According to Darwin, the Crouan brothers (6), pharmacists and amateur botanists of Brest, were the first to record, in 1858, the presence of small aquatic animals within the bladders of *Utricularia*. Ten years later, Holland (22), according to Darwin, observed the same thing on English plants. In 1875, Darwin (11) published an account of his experiments made principally on *U. neglecta*, and showed that the bladders of this plant often contain small crustacea, insect larvae, etc., which die in the prison, and he concluded that the dead bodies of these supply food to the plant. As it has turned out through later work, he rightly regarded *Utricularia* as a genuine carnivorous plant. In the same year, Mrs Treat (33) observed mosquito larvae and crustacea within the bladders of an American species, and followed the actual entrance of a larva into a bladder. Cohn (5) was stimulated by reports of Darwin's work on insectivorous plants to undertake the investigation of *U. vulgaris*, and satisfied himself that he was dealing with a carnivorous species. He fortified his deductions from structure and from analogy with other rootless plants by experiments and observations on living and herbarium material. Since the publication of the work of these pioneers, observations on the bladders of many species of *Utricularia* have multiplied to such an extent that there can be no doubt of their carnivorous habit.

The next step was to discover the method by which the bladders engulf their prey, and in this endeavour all of the earlier observers were equally unsuccessful. It is of much interest to follow Darwin's account of his efforts to clear up the problem. "As I felt much difficulty in understanding how such minute and weak animals, as are often captured, could force their way into the bladders, I tried many experiments to ascertain how this was effected. The free margin of the valve bends so easily that no resistance is felt when a needle or thin bristle is inserted. A thin human hair, fixed to a handle, and cut off so as to project barely $\frac{1}{4}$ in., entered with some difficulty; a longer piece yielded instead of entering. On three occasions minute particles of blue glass (so as to be easily distinguished) were placed on valves whilst under water; and on trying gently to move them with a needle, *they disappeared so suddenly that, not seeing what had happened, I thought that I had flitted them off*; but on examining the bladders, they were found safely enclosed. The same thing occurred to my son, who placed little cubes of green boxwood (about $\frac{1}{60}$ in., ·423 mm.) on some valves; *and thrice in the act of placing them on, or whilst gently moving them to another spot, the valve suddenly opened and they were engulfed*. He then placed similar bits of wood on other valves, and moved them about for some time, but they did not enter" ((11), p. 405. The italics are my own; how nearly Darwin came to the true explanation will appear in the sequel). He also mentions the eventual disappearance of particles of blue glass, lead shavings, etc., which had rested for some time on the valve without being swallowed. In one case a cube of green boxwood remained on the surface of a valve for 19·5 hours without entering, but was found inside 3 hours later.

Darwin already knew that *Dionaea*, *Drosera*, and *Pinguicula* execute active movements either in the capture or in the digestion of their prey, and acting upon the suggestion offered by these, he tried to discover whether the valve of *Utricularia* is irritable. He scratched the surface with a needle, or rubbed it with a fine camel-hair brush, but was unable to observe that it responded by opening. He observed the same lack of response when he maintained the bladders at unusually high temperatures (26–54° C.), expecting thereby to increase their irritability. He concluded "that animals enter merely by forcing their way through the slit-like orifice; their heads serving as a wedge." He supposed that minute animals habitually seek to intrude themselves into small cavities, in search of food or protection, but expresses his surprise that such small and weak creatures could push the valves inward.

Cohn (5) had already concluded, as a result of his own observations, that the *Utricularia* bladder is not endowed with irritability, and that the captured animals force their way into it, which he conceived they must do against an internal pressure which holds the valve against the collar and prevents their subsequent escape. The explanation of Büsgen (4) differed from this in detail only. In the natural orientation of the bladder the external surface of the valve often faces upward. Büsgen believed that the animal did not endeavour to force its way inward, as Darwin had supposed, but that the weight of the creature, as it crawled over the valve, bent it downward and inward, just as a small pebble would do if pushed about on its surface. He compared the deformation of the valve, and its elastic recoil after being pushed inward, with the behaviour of a rectangular piece of flexible cardboard, bent into a semi-cylinder and supported at the sides. A slight pressure exerted upon the convex surface at either end creates a deep furrow, which straightens out immediately upon the release of the force.

And so the matter rested for the third of a century, until it was taken up again in 1911 by the entomologist Brocher, and this time with a more fruitful outcome. Brocher (1) was impressed with the fact that none of the previous investigators had observed in detail the actual course of events as an animal entered a bladder, and undertook to witness this occurrence for himself. He began by placing a copepod, injured slightly so that it could not slip away, upon the valve of the bladder. In most cases, nothing came of this procedure, but several times, as he manoeuvred the animal upon the valve with a needle, it suddenly vanished, just as Darwin's particles of blue glass had vanished, and was later found inside the bladder. Next he tried projecting a small crustacean against the valve from a pipette. Usually this endeavour was also fruitless, but upon one occasion a cladoceran, which had mounted to the top of the water column in the pipette, was engulfed by the bladder, along with the bubble of air which followed it from the tube, and to the surface of which it adhered. The entry of the air bubble indicated to Brocher that *the bladder experiences an instantaneous increase in volume at the time of ingesting its prey.* In other words, it sucked in the animal and the bubble as the expanding rubber bulb sucks water into a pipette. He further observed that the swallowing of the prey occurred only when contact had been made with the region of the valve at the base of the four long bristles, and that it never succeeded in a bladder which contained a bubble of gas, or had already captured an animal. Careful examination convinced him that the functional bladders were slightly concave on the sides, while those which would not react were somewhat convex in the same region. The change from the concave to the convex condition occurred at the time when an animal was captured, and was responsible for the change in volume. The difference between the two states is clearly indicated in his camera-lucida outlines (see also photographs by Merl (28), Figs. 1 and 2, and Fig. 2 in this text). Moreover, Brocher could sometimes observe that the bladder trembled slightly, as though it had been jarred, at the time it enlarged to suck in its prey.

Brocher discovered the reason for his numerous failures, during the earlier part of his investigation, to observe the capture of prey. Examination of bladders which had never been removed from the water showed that almost without exception they were free from gas-bubbles. However, if a plant is merely lifted from the surface, and immediately returned to the water, a large proportion of its bladders will be found to contain air-bubbles. The bladder expands as it passes through the surface film and, since the expansion is completed in the air, a bubble of gas is sucked in. Often he could discern a faint clicking sound as a bladder expanded. The plants with which he had previously experimented had been transferred through the air, and many of them had thereby exhausted their power of expanding and had become inactive. By using the proper precautions and transferring the plants from the pond in which they grew to the experimental vessel without bringing them out of water, a larger number of bladders were secured in the active condition. Upon stimulating such bladders at the base of the four bristles of

the valve, it was possible to obtain the expansion in almost every case.

Brocher demonstrated, then, that the bladder is not passive in the capture of its prey, the animal forcing its way inward past the yielding valve, but on the contrary, upon receiving the proper stimulation, it undergoes an increase in volume, which causes an inflow of water, carrying with it any small animal so unfortunate as to have released this chain of events. Brocher's paper was published, however, in a journal which did not claim the attention of many botanists, and in consequence escaped general notice.

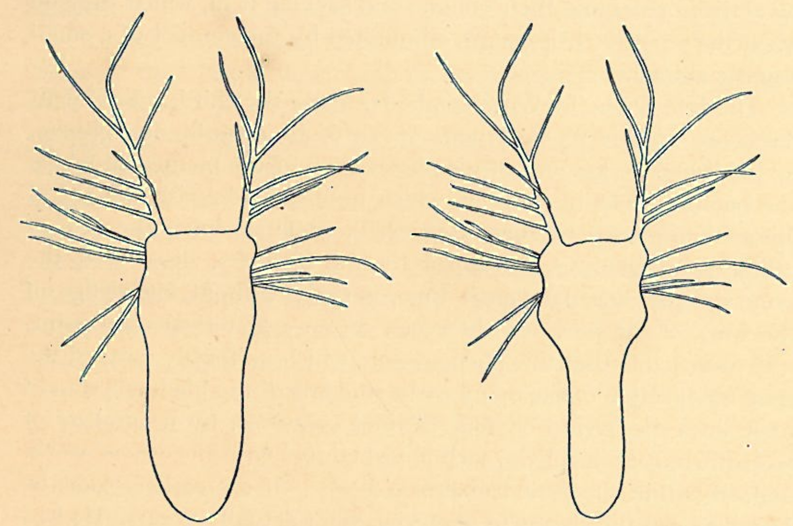


Fig. 2. The same bladder of *Utricularia vulgaris* before (right) and after (left) touching the valve with a needle. Camera-lucida sketches.

While distributing for class study specimens of an undetermined Indian species of *Utricularia*[1] with very large bladders, Ekambaram (13) noticed "light crackling sounds resembling the ticks of a watch" which emanated from the plants as he lifted them from the vessel in which they were growing. A series of observations satisfied him that the particular organs of the plant responsible for the noise were the bladders. By a comparison of the condition of the bladders before and after their removal from the water, Ekambaram discovered that a change of configuration had taken place, and concluded that the almost explosive alteration of form was accompanied by the

[1] Ekambaram states that this species was "very near *U. flexuosa*."

sounds which had originally attracted his attention. The unmolested bladders were usually "nearly empty, with walls closely adpressed against each other, so that there was very little cavity inside, and the bladder as a whole was biconcave." Many of those which had been lifted from the water were greatly distended, had convex lateral walls, and contained a bubble of air. The change from a less to a more spherical shape was accompanied by a considerable increase in volume, and this sudden distension could be induced by touching with a needle one of the bristles inserted on the valve. Ekambaram concluded that the bladders are irritable, and capture their prey by actively enlarging their volume and sucking it in, which they do when the sensitive bristles are stimulated by the contact of a small aquatic animal.

The late Dr C. L. Withycombe (34) made the third independent discovery of this phenomenon. In 1916, as a youth of eighteen, he published an account of his observation of the manner in which the bladder sucks in its prey, which he had made with the aid of only a hand-lens. In 1925, Hegner (21), while studying the fate of unicellular organisms entrapped by the bladders, discovered the same reaction for the fourth time, entirely without knowledge of the work of the previous observers. From a historical standpoint, it is remarkable that this phenomenon, which uniformly escaped the keen observation of the many early students of the bladders, should have been discovered on four distinct occasions, by naturalists of four different nationalities each unacquainted with the others' work, and all within the period of fifteen years. Of the earlier students, Darwin came the closest to observing what actually occurs. He had remarked that different bladders vary greatly in thickness and in the amount of water they contain, but failed to realise the great significance of this circumstance. However, had he been a little more diligent in following up the unexplained disappearance of his glass and boxwood particles, he must undoubtedly have been led to the true solution of the problem.

IV. The Mechanism of the Bladder

The discovery by Brocher of the actual phenomenon attending the capture of prey by the bladder represented a great advance towards the solution of "le problème de l'Utriculaire," and for the second time made this plant a special object of investigation. The next step was to study in more detail the conditions under which the dilation takes place, and to discover the forces responsible for this sudden change in configuration—to determine its mechanism. Is the sudden expansion of the walls the response of irritable tissue to a stimulus, or is it merely the result of the mechanical release of certain pre-existing strains? If the former alternative is correct, what is the nature of the reaction, and which are the motor cells? If the latter, which are the strained tissues, and how are the strains engendered? In the following discussion, we shall for brevity refer to Brocher's phenomenon as "the bladder reaction," or more briefly "the reaction," without meaning by this term to imply either that it is a manifestation of irritability, or that it is a purely mechanical change.

Already Brocher had framed an explanation of the process; he assumed that during the growth of the bladder the walls increase in area without a corresponding increase in the liquid content of the cavity. As a consequence, the bladder departs from the spherical shape, which represents the smallest ratio of surface to volume in a solid object, and the lateral walls become concave. They are strained in this position, and by virtue of their elasticity tend to spring outward, which they do if the bladder is punctured by a needle, allowing the influx of water through the perforation. But since the valve fits tightly against the collar, and the bladder is perfectly sealed, the contents of the cavity cannot normally be augmented by water from without, and therefore remain under negative pressure in respect to the medium. However, when the valve is stimulated by the contact of a solid object with the region at the base of the four bristles, it contracts, pulling away from the collar, and producing an opening which allows the influx of water. The strained lateral walls are now free to spring apart, and the inflowing current carries along with it the animal which has sprung the trap by impinging against the valve. Occasionally the valve closes before the walls of the bladder have reached their position of equilibrium, in which case a second reaction is possible. Brocher never observed the recovery of the "set" condition by a fully expanded bladder, but suggested that in a state of nature it might take place after several hours.

In Brocher's interpretation of the reaction of the bladder, its release from the set position is conditioned by the special irritability of the valve. He assumed the existence of a motor tissue not, it is true, to account for the springing asunder of the walls, which results from their elasticity, but for the opening of the valve which must precede this expansion. Ekambaram (13) also believed the valve to be irritable, but thought the perceptive faculty to be localised especially in the bristles (six in the species he worked with) rather than in the region at their base. Before definitely accepting either view, it was necessary to investigate more closely the conditions under which the reaction proceeds, and to determine if the bladder behaves in a manner similar to other objects known to be irritable, such as the leaves of *Mimosa*, *Aldrovanda* and *Dionaea*, the stamens of *Centaurea* and *Berberis*, etc. Many experiments in this connection have been carried out by Merl.

Merl (28) used for his investigations bladders of *U. vulgaris* and *U. flexuosa*, severed from the leaves and floated, ventral side up, in a watch glass where they could be observed by means of a binocular or a hand lens. He determined that such separated bladders react just as well as attached ones. The reaction may be secured by touching with a needle *either the bristles or the valve itself in the region of their insertion*. Young, immature bladders do not respond. The increase in the volume of the bladder attending the reaction may be demonstrated by touching the hairs with a capillary pipette containing mercury or a coloured solution; a drop emitted from the pipette at the proper time is sucked in, even if the end of the pipette does not pass within the aperture. Isolated bladders could be observed to give a short jerk forward through the water as they expanded, the physical reaction to the influx of water. The whole reaction usually takes place in a flash and it is impossible to observe the movement in detail, but when the rate of activity is particularly low it may require 1–2 seconds for its completion, and its components may then be followed. The four bristles on the valve approach until their apices are in contact, at the same time moving backward. The edge of the valve moves inward until a semilunar aperture between it and the collar is visible, then immediately snaps forward. Meanwhile the lateral walls have dilated. The strength of the reaction is conditioned by the intensity of the touch, and if very slight contact is made with the bristles, the walls do not always completely expand. Upon a second, stronger stimulation such bladders will dilate further, as Brocher already had observed. The fullest possible dilation of the walls does not follow the ordinary gentle contact. If, however, the valve is forcibly pushed inward, or if the wall is punctured, the bladder becomes extremely swollen in appearance, and the walls assume a much greater convexity than they do as a result of simple contact with the bristles (see Merl, Figs. 1 and 2).

Merl showed that after reacting a bladder could again recover its "set" condition, and become ready for a second discharge. He

determined the period of recovery to be 15 minutes, but a longer rest period allows a greater concavity of the lateral walls to be attained. Withycombe (35) later found that the time necessary for complete recovery was related to the temperature, and that at the optimum temperature at least 30 minutes was required by the bladder of *U. vulgaris*. Czaja (9) agreed with Merl that 15 minutes is sufficient time for recovery, although he found in his earlier work (7) that full recovery required 30 minutes. Hegner (21) found 20 minutes the minimum period required for recovery. The rate of the process undoubtedly varies with the state of the material and the external conditions. Merl secured the reaction and recovery of a single bladder 14 times in 3 days. He proved by camera-lucida sketches that no change in size accompanies even repeated recovery, but the re-set bladder has the same dimensions as before reacting. Recovery takes place even if the bladder is abnormally enlarged by the forcible opening of the valve, but naturally requires a longer time under these conditions, since more water must be removed from the interior. Punctured bladders never become set again. The presence of nutritive substances does not inhibit the simultaneous recovery of the bladder, and a discharged bladder into which a piece of raw meat had been introduced was set again after 1·5 hours. Finally, it is not necessary for the bladders to be submerged, but they may react after being kept for a day in air in a moist chamber, and they remain capable of response even when the external pressure is reduced by the exhaust pump.

Ekambaram (13) showed that a bladder could be "set" by mechanical means. He pressed out most of the water by compressing the bladder between forceps, the water escaping through a slit which appeared between the valve and the collar. Upon releasing the pressure, the cavity became filled with air which entered from the intercellular spaces of the wall. After expelling this air several times by compression, until no more entered from the intercellular spaces, the bladder remained in a set condition, and responded normally to contact with the bristles. By this manipulation the same bladder could be several times set and released. This behaviour of the bladder favours the conclusion that variations in the turgor of the cells of the wall are not responsible for bringing about the set condition, and therefore are not involved in its release.

Hegner (21) made some determinations of the relative volumes of the compressed and the dilated bladders. He chose 15 large compressed bladders, and the same number in the distended condition.

After drying the exterior with filter paper, he withdrew the contents of each set by means of a fine capillary pipette. It was found that the volume of water removed from the distended bladders was 88 per cent. greater than that withdrawn from the compressed ones. In other words, the bladder upon expanding may draw in a volume of water equalling approximately 88 per cent. of that already contained in the lumen; and during the process of resetting about 47 per cent. of the water contained in the dilated bladder is somehow expelled. In attempting to gain some information as to the amount of suction produced by the bladder in expanding, Hegner found that a dead paramoecium placed 2 mm. from the orifice was drawn in when the bladder was caused to react. A dead insect larva 2 mm. long was completely engulfed when it was pushed against the valve, as was a living one of similar length when it touched the valve. Another observation which indicates the strength of the inrushing current was made by the writer. An imprisoned ostracod, which happened to be in the anterior end of the bladder, was jerked violently inward as the bladder expanded.

Merl was unable to cause the bladders to react by any kind of mechanical stimulation other than by contact with the bristles or the region of the valve at their base. Severing the supporting leaf from the plant, or the bladder from the leaf, brought about no response, and bladders just severed at the stalk were as strongly contracted as unmolested ones. By puncturing the wall with a needle the bladder was made to dilate very strongly, but apparently the valve did not open, the water entering merely through the perforation in the wall. Later Withycombe showed that a punctured bladder expands only after the needle has been removed, but does not alter its shape so long as the instrument plugs the hole and prevents the inflow of water through it. Attempts to cause the reaction by electrical stimulation were equally unsuccessful. None of the many chemicals applied to the bladder called forth response, even if they were used in strong enough concentration, or allowed to act for sufficient time, to cause injury. In all of these particulars the behaviour of *Utricularia* is strikingly at variance with that of most organs which respond to stimulation by a motor reaction. The leaf of *Aldrovanda vesiculosa* affords the best material for the comparison of an irritable organ with the bladder of *Utricularia*, since this is the only submerged plant known to give a rapid motor response, and is also insectivorous. Czaja (10) has recently investigated this reaction, and finds that the halves of the lamina close together as

a result of electrical and chemical, as well as mechanical, stimulation (see also Brown and Sharp (2) on *Dionaea*).

Having failed in his attempts to cause the bladders to react by means other than mechanical contact with a portion of the valve, Merl next endeavoured to demonstrate their sensitivity by showing that a state of rigor could be induced. He first tried to throw them into heat- or cold-rigor. He found that bladders of *U. vulgaris* still responded after being kept for 24 hours at 45° C., and after one hour at 48·5° C. These bladders were killed by exposure to 60° C. A few bladders of *U. flexuosa* still reacted after an hour at 50° C., while a temperature of 53° C. was fatal to them. Merl concluded that the maximum temperature permitting response was practically conterminous with that supporting life. Attempts to produce rigor by cold met with the same negative results. A few bladders of *U. flexuosa* were in condition to react after 1 hour 20 minutes at 1° C., although many were injured by this temperature. Bladders of *U. vulgaris* reacted after being maintained at 0–2° C. for ½ hour. At the lower, as at the higher temperatures, the limit for reaction is apparently the same as for life. Ether, chloroform, mercuric chloride, alcohol, chloral hydrate and brucin were employed in attempts to produce rigor by chemical agents, but these substances prevented the response to mechanical stimulation only when they killed or severely injured the bladder. Several of these substances were found by Czaja to cause rigor of the leaves of *Aldrovanda*. The much more numerous quantitative experiments of Czaja (9), which will be discussed in more detail in another connection, overwhelmingly support the same conclusion, although in a few instances conflicting evidence was obtained.

The experiments of Merl make untenable any theory of the reaction which postulates sensitivity (understood of course, in the meaning of the sensitivity of *Mimosa* or *Dionaea*) on the part of any portion of the bladder essentially involved in the process. Nevertheless, Withycombe, who states that his own experimental work supports that of Merl, persisted that the valve of the bladder includes a motor tissue which contracts on stimulation. If we admit this contention, *Utricularia* is unique in this respect, for every other adequately investigated motor tissue which reacts to contact responds also to other forms of stimulation, and may be thrown into a state of rigor by various means. Merl himself came to no definite conclusions as to the mechanism of the reaction, but suggests among other things that the bladders may dilate as the result of the disturbance of a system in unstable equilibrium, which exists in the set condition. This is an idea which has been expanded and elaborated by Czaja.

Czaja (7) began by considering the elastic strains present in the walls and the valve. Cohn had already shown that if the peristome, including the collar, is removed, the bladder broadens laterally (Cohn (5), Fig. 8). If a bladder is cut in the median, vertical plane by an incision beginning at the anterior end and extending posteriorly as far as the stalk on the ventral side, and an equal distance on the dorsal, the two halves gape apart laterally. Thin cross-sections, made just behind the peristome, expand toward the sides, indicating a tendency of the lateral walls to become more convex. These strains are a result of the size and distribution of the cells of the wall. The mechanical structure of the valve is very carefully considered, but for details reference should be made to Czaja's figures and description. It must suffice here to state that as a result of its outward convexity, the more its sides are forced together by the incurved walls, the more tightly its free margin is pressed against the sill. Any inward displacement of the valve results in an elastic strain and a rapid recoil, which may be understood by referring again to Büsgen's bent cardboard model (p. 269).

It was also necessary to investigate the closure of the bladder by the valve. If the gradual approach of the lateral walls during the transition from the sprung to the set condition is brought about by changes in the shape of the wall cells caused by variations in their turgor, it should be independent of a difference in pressure between the external median and the lumen of the bladder, and should proceed whether or not the bladder is completely sealed. If the setting of the bladder is contingent on such pressure differences, an incompletely sealed bladder ought to remain permanently sprung. Czaja inserted a hair between the valve and the collar, and found that the bladder remained permanently in the dilated condition, and was incapable of reacting. After several days, the hair was removed, and the bladder became set and responded soon after, indicating that no injury had been incurred. The bladder, then, is normally hermetically sealed, and this may be demonstrated in another way by immersing it in a coloured solution (eosin, congo-red or methyl-blue), in which it may remain for days without the slightest penetration of the colouring matter into the cavity (see also Merl (28) and Withycombe (35)). However, Hegner's results are somewhat at variance with this conclusion: he found that eosin, carmine and indian ink penetrated the bladders

within 2½ hours. If a hair has been inserted beneath the valve, the dye penetrates rapidly. The very perfect seal is doubtless made possible by the rich secretion of mucilage by the glands of the collar.

Czaja next showed that the whole bladder is enclosed in a semi-permeable membrane. Prat (30) has since demonstrated that the entire plant is covered by such a membrane, and the researches of Riede (31) and others have pointed out the error of the once prevalent idea that the peripheral cells of submerged aquatics are everywhere in free communication with the medium. The osmotic pressure of the cells of the wall of a *Utricularia* bladder is equal to that of a $M/5$–$M/4$ solution of KNO_3, as may be determined by observations on the plasmolysis of halved bladders. However, glycerine or sucrose solutions of no concentration will produce normal plasmolysis of the cells of whole bladders which have been immersed in them. Placing the bladders in hypertonic solutions of these substances causes the walls to become more and more concave, until finally, with sufficient concentration, the opposite walls are brought into contact, and the lumen disappears. When this occurs a light disc is evident in the centre of a bladder viewed from the side by transmitted light, and a dark, median, vertical line may be seen from the front (see Czaja (7), Figs. 7 and 8). With concentrations of glycerine up to 30 per cent. the tension in the cavity increases, but with higher concentrations the bladder totally collapses, and not until this occurs do the cells of the wall become plasmolysed. The membrane surrounding the bladder prevents the access of the solute directly to the protoplasts; as the latter lose water through the external membrane in response to the existing concentration gradient, they make good their loss by absorbing the normal liquid from the cavity, and until this is exhausted plasmolysis cannot occur. When placed in a concentrated solution of a substance to which the membrane is permeable, the bladder becomes greatly distended, and appears to be dead. In such cases, the external membrane is completely destroyed, and it is impossible to maintain in the lumen the negative pressure necessary to hold the lateral walls in the "set" position. Merl showed that if a 5 per cent. solution of glycerine is introduced into a bladder the wall cells plasmolyse, but the excess osmotic pressure within causes the whole bladder to imbibe water from the medium, and it becomes greatly inflated. Not only does the valve completely shut off free communication between the external and internal fluids, but interchange through the walls is regulated by a selectively permeable membrane.

When the bladder is placed in a hypertonic solution, the inflection of the walls causes the valve to be wedged strongly against the collar. If the over-tension produced within the bladder is only slight, the valve may be forcibly opened with a needle, but if this is allowed to proceed too far, it is impossible to budge the valve, and efforts to do so result only in destroying it. The equilibrium between internal and external forces becomes more stable as the tension increases above the normal.

Finally, it may be demonstrated that the quadrifid hairs of the interior absorb substances from the cavity. Darwin long ago supposed that their function was that of absorption, and they have been found to take up dyes such as methylene blue with great readiness. That they are actually responsible for the removal of the large volume of water necessary to set the bladder still requires fuller experimental proof. The four bristles on the valve act as a lever mechanism. The displacement of one of them causes a reaction much more readily when the bristle is bent downward than when it is bent sideward to an equal degree. They may be regarded as structures adapted to effect a considerable deformation of the free margin of the valve by means of a slight applied force, an assumption to which their structure lends support (see Merl (28), Fig. 3). The downward movement of the bristles breaks the contact between the valve and the collar. The antennae and the hairs along the sides of the peristome are not essential to the reaction, which proceeds normally when they are cut away.

We may now form a picture of the entire process and, starting with a bladder which has just expanded, trace its recovery and its subsequent reaction. After the first reaction the valve returns to its contact with the collar, and the mucilage secreted by the glands seals the seam more or less perfectly. The quadrifid hairs begin to absorb the liquid from the lumen, either forcing it eventually into the vascular system, or else to the exterior through the two-celled glands of the external wall. Since the semipermeable membrane covering the bladder allows the penetration of water slowly or hardly at all, and the valve seals up the aperture, water cannot enter fast enough to counter-balance the loss occasioned by the activity of the quadrifids, and the lumen slowly loses its contents. As a result, the lateral walls are forced inward, which may be effected either by the combined external hydrostatic and atmospheric pressure, by the tension of the water within, or by both acting together. In this new position the walls are under strain, caused by the deformation of their component turgid cells, and perhaps in part by the compression of the air bubbles in the intercellular spaces, and the pressure within the bladder becomes negative in respect to the medium. Displaced inward by the compressed walls, the bowed valve is pressed more strongly against the collar, the better to resist the growing pressure from without, and between these two forces it is in unstable equilibrium. If now a small copepod collides with one of the four bristles, the latter in virtue of its leverage deforms the rim of the valve, breaking the contact between it and the collar, and allowing free communication between the external and internal fluids. Free to expand, the lateral walls snap apart, sucking in a current of water through the aperture, and along with the stream the devoted crustacean. Should the animal seek to escape by the door which afforded such ready entry, he will find it tightly barred. The whole process occurs in the wink of an eye, and one who has witnessed it does not wonder that so many of the earlier naturalists failed to observe what actually does occur.

If the above explanation of the process is the true one, several consequences ought logically to follow, and these remain to be considered. Suppose that a bladder is not disturbed by an animal for a period much longer than that required for its complete setting, will it fire automatically from over-tension, or will the several processes involved in its setting reach a steady-rate? Merl investigated this question, but because of experimental difficulties could form no definite conclusion. In default of direct observation on normally conditioned bladders, we must rely upon the results obtained by immersing bladders in a hypertonic solution of a substance to which the membrane is impermeable. Such experiments show that after the tension has exceeded a certain limit, the system becomes more stable as the tension increases. In pond water there is probably always some infiltration of water through the walls, and this would naturally increase as the pressure gradient becomes sharper. At the same time the rate of absorption by the quadrifids must be reduced because of the greater resistance opposed to their action, and the two processes should approach each other in intensity and establish a steady-rate. The tension in the lumen can hardly, under natural conditions, exceed in numerical value the osmotic pressure of the cell-sap of the quadrifids. The presence of air-bubbles in bladders which are functional is in no way incompatible with Czaja's theory, since by the expansion and rarefaction of these the internal pressure may be reduced very considerably. It is not known to what extent this must be reduced in order that a reaction may occur. Withycombe was able to detect an increase in volume of gas bubbles contained in the lumen during the setting of the bladder, but unfortunately he gives no measurements.

The frequent discharge of bladders while passing from water into air is probably caused by the pull exerted on the bristles of the valve by the contracting surface film, which succeeds in deforming the valve.

A more serious difficulty lies in the infinitesimal force of impact apparently sufficient to produce the reaction. In Hegner's (21) experiments, paramoecia were caught with such frequency that it appears highly probable that the trap can be sprung by a minute protozoan impinging against some portion of the valve, presumably the bristles, although this was not actually observed. Ekambaram (13), by means of an ingenious device, tried to evaluate the force necessary to push inward the valve of a transversely halved bladder of his undetermined species. He records only two measurements, which, however, agree closely, and found the required weight to be 250 and 280 mg., respectively. He believes that many of the animals caught by the bladder could not exert a force of this magnitude. Had the valves used in his experiments been part of a set bladder, a portion at least of this force would have been supplied by the excess pressure of the medium, and a smaller weight would have sufficed to spring the trap. Withycombe observed that the edge of the valve fits into a groove in the collar, and believed the only possible movement which could free it to be an upward one resulting from a contraction of the valve cells. He was of the opinion that the principal motor tissue is situated at the base of the four bristles, and contracts only as a result of the stimulation of these appendages, drawing the valve out of the furrow and allowing it to be forced inward by the excess pressure of the medium. Ekambaram (14), in 1924, still persisted in believing that these hairs are sensory perceptors, and the valve a motor tissue which reacts through changes in turgescence.

The idea that the bristles are sensory arose from their resemblance to the sensory hairs of *Dionaea* and has been persistent since the time of Darwin, who rejected it. The fact that attempts to produce rigor have been apparently unsuccessful does not really invalidate the view that sensorimotor phenomena are normally responsible for the reaction, in the manner held by Withycombe. The essential part of the reaction is the dilation of the bladder, and it is altogether conceivable that, while this expansion normally is released by a contraction of the valve following slight stimulation of the perceptor bristles, the application to the valve, made insensitive by any means, of a pressure strong enough to tear it away from the collar and allow its inward movement, would produce the same end result. A box-trap, such as a boy sets to catch rabbits, which responds to the slightest touch on the trigger, may also be sprung by a stronger force applied directly to the sliding door. Apparently Merl and Czaja failed to appreciate the necessity to distinguish between these two possible modes of securing the reaction. On the other hand, the failure to secure response by any form of stimulation other than the mechanical weighs strongly against the assumption of irritability, since, as remarked above, this would be the unique instance of such strictly limited sensitivity, and at present it seems safest not to accept the view which favours it. It is certain that the activity of the bladder is not contingent on its irritability, and if it is ever demonstrated that it does possess a motor-tissue, this will probably come to be considered as an added refinement creating greater delicacy and increasing the number and kinds of prey captured, rather than an essential part of the mechanism.

V. The Membrane of the Bladder

The very extensive special investigations of Czaja (8, 9) on the nature of the membrane enclosing the bladder can unfortunately receive only the briefest mention in an article with the scope of the present, and for fuller details the reader must be referred to the original memoirs. Since the properties of the membrane are of such importance to the proper functioning of the bladder, and to the interpretation of experiments attempting to produce rigor, a few of Czaja's main conclusions are repeated here.

The experimental procedure employed was simple. Fresh, active bladders of *U. vulgaris* or *U. neglecta* were transferred in the discharged condition to the desired solution, all water adhering to the exterior having been previously removed with filter-paper, to avoid dilution. The bladders were allowed to remain in the experimental solution for 15 minutes, which normally is sufficient for them to regain the set condition, and after this period their activity was tested by touching the valve with a needle. It was found that when bladders were placed in graded concentrations of a solution of a single substance, the force which it was necessary to apply to the valve to secure a reaction increased with the concentration, until finally the bladder could not be caused to react even by applying a strong pressure. The highest concentration at which the dilation of the bladder could be secured by exerting a "strong pressure" on the valve was taken as the concentration limiting to the activity of the bladder. It was recognised that the criterion employed was in a measure subjective, but the use of a considerable number of bladders in each experiment somewhat modified the likelihood of an error in interpretation. After probing the activity of a bladder, it was returned to distilled water and its subsequent fate observed after 24 hours.

The number of substances tested in this manner was particularly large, comprising inorganic neutral salts, mineral acids and bases, organic acids, alcohols, narcotics, aldehydes, alkaloids, amides, etc. In general, these chemicals agreed in causing loss of function and irreversible injury when applied above a certain concentration. Opposed to these in their effects were a number of "indifferent" substances, including glycerine, sucrose, glucose, mannite, asparagin, glycocoll and the like. Chemicals of the first class, in concentrations below the limiting, caused no injury to the bladders, or if injurious effects were evident they were in general of a reversible nature. Above the limiting concentration, the bladders became completely unresponsive, and after the period of 24 hours allowed for their recovery were greatly distended and evidently dead or dying. A pronounced exception to this behaviour was found in mercuric chloride, which caused permanent injury in concentrations below the limiting, and the hydroxides of sodium and potassium, which did not cause irreversible injury in concentrations well above the limiting. The indifferent substances caused loss of function only when their concentration was so great that the over-tension produced in the bladder made the release of the valve impracticable, in the manner indicated in the previous section. Returning the bladder to distilled water was followed by complete recovery, unless it had been collapsed by an extremely high osmotic pressure. The appropriate micro-chemical tests failed to give evidence of the penetration into the bladder of any substance of either class from solutions more dilute than the limiting concentration, although above this concentration penetration was detected.

Czaja supposed that the bladder is covered with a cuticula in which is dispersed a hydrophil gel. The adsorption of ions by the disperse phase causes it to become more continuous, at the same time diminishing its permeability to water, and with a sufficient concentration of the electrolyte it forms an almost perfect barrier

to the penetration of aqueous molecules. The degree of tension attained by the bladder is the resultant of two antagonistic processes, the removal of the water from the lumen by the quadrifids, and its penetration through the walls from the medium to the interior. Since the value of the second rate is reduced by subjecting the membrane to the action of an electrolyte, the tension is built up more quickly and reaches a greater maximum than is possible in pond water. The over-tension makes it necessary to exert a greater force to release the valve (see p. 280) and thereby decreases the apparent sensitivity of the bladder. The effect is at least roughly quantitative, and with increasing concentrations a greater and greater force is necessary to secure a reaction. If the concentration is below the limiting, only the superficial layers of the membrane are affected, and the normal permeability may be regained by placing the bladder in pure water, where the active ions diffuse out of the gel. The coagulation of the gel stops the further penetration of the electrolyte causing it, so that the latter cannot reach and injure the protoplasm of the wall cells. Excessive concentrations, instead of producing a fine superficial condensation, throw the whole colloid into a coarse, porous coagulum, which is permeable both to water and to the chemical in question. Since the penetration of water can proceed with small obstruction, it is impossible for the quadrifids to build up the tension necessary for the reaction, and the access of the chemical injures or destroys the protoplasm. The changes caused by concentrations above the limiting are for this reason in general irreversible. The injurious non-electrolytes have a similar action on the membrane, but by virtue of different properties.

Indifferent substances do not change the condition of the membrane, which is *ab initio* impermeable to them, and their action is purely osmotic, and is reversible.

Placing bladders collected from natural waters into distilled water caused them to become more sensitive; they reacted to slighter pressure. This is explained by supposing that the small amounts of electrolyte adsorbed by the gel from the original medium diffuse out into the distilled water, which results in greater dispersion, and consequently in increased permeability of the membrane. The tension of the bladder cannot become so strong as previously, the valve is not held so tightly and may be released by a weaker contact. Bladders which were allowed to remain for a long time in distilled water showed plasmolysis by much lower concentrations of KNO_3 than were necessary to plasmolyse fresh bladders, an indication of the increased permeability of the former. Thus, fresh bladders showed plasmolysis after 15 minutes in 3 *M* KNO_3 but not in 2 *M*. After 27 days in distilled water plasmolysis was obtained after the same interval of treatment by employing *M*/2 KNO_3, and intermediate periods produced corresponding reductions in the necessary concentration. On the other hand, that an electrolyte decreases the permeability of the membrane was demonstrated by placing in 3 *M* glycerine solution bladders previously treated for 15 minutes with *M*/1 to *M*/16 KNO_3. The glycerine was unable to withdraw water from the lumina of such bladders by diosmosis, they showed no over-tension and could be sprung after 1 hour 15 minutes in the glycerine, while the walls of the control became very concave, and the bladders could not be made to react.

VI. The Number and Kind of Prey; Digestion; Absorption; Importance of the Carnivorous Habit to the Plant

A comparison of the accounts of all observers, in whatever country, indicates that by far the most common prey of the aquatic bladderworts are small entomostracan crustacea, principally of the Copepoda (*Cyclops*), Ostracoda (*Cypris*) and Cladocera (*Daphnia*). Of the Malacostraca, the fresh-water amphipod *Gammarus pulex* has been reported to occur sparingly in the bladders (Garbini (15), quoted by Brumpt (3)). Protozoa, including rhizopods, ciliates and flagellates, are often captured in considerable numbers, and the occurrence of green flagellates has been recorded. The aquatic larvae of insects, which are often large and conspicuous, have been seen in the bladders by many naturalists, and must be recognised as common victims. Larvae probably of the mosquito were found by Mrs Treat and by Darwin, in 1875, and since that time they have repeatedly been observed within the bladders. Nematodes and rotifers were found within the bladders of *U. neglecta* by Garbini, and of *U. vulgaris* by Hegner. Moseley (29) describes the capture of newly-hatched roach by *U. vulgaris*, observed at Oxford by G. E. Simms. These fish are too large to be wholly engulfed, but were held in the bladder usually by the head, although often by the tail or the still-attached yolk-sac, the rest of the body protruding through the orifice. A few examples were caught with the head in one bladder and the tail in another, the body forming a bridge between the two. Young tadpoles are also occasional victims of the bladders (Gräbner (19), Fig. 135). There is probably no species of aquatic animal sufficiently small to be ingested which is not occasionally a sacrifice to the carnivorous habit of the plant, and, as is to be inferred from the method in which capture is effected, no selection of organisms on the part of the bladder has been recorded. The relative abundance of different species among the prey is probably determined almost wholly by the size, habits, activity and density in the medium of the various organisms. In addition to animal booty, small algae, such as diatoms, blue-greens and desmids, are often observed within the bladder.

The consideration of the quantity in which the prey are captured is of importance in forming an estimate of the value of this form of heterotrophic nutrition to the plant. Statistical observations made upon plants taken from natural habitats are of most value in this connection, but the frequently made determinations of the rate of entry of small animals into empty bladders placed in well-populated water are also of great interest. Garbini (15) investigated the contents of 610 bladders of *U. neglecta*. Of these 62 were empty, 44 contained unrecognisable debris, while the remaining 504 contained various organisms, to the number of 2084, or an average of about 4 to the bladder. Four species alone accounted for 1550 individuals, or three-quarters of the entire catch; these were *Stilonychia mytilus* Ehrbg. (a protozoan) 195, *Chydorus sphaericus* D. F. Müller (a cladoceran) 872, *Monomatta longiseta* Bartsch (a rotifer) 185, and *Cyclops signatus* Koch 298. It is probably a general rule that when a large number of animals is trapped, one or a few species will constitute the great majority, while the remaining species captured will have only a scattering representation. Hegner gives figures indicating the total number of organisms falling prey to a single large plant. He estimated from a partial count that a branch consisting of a main stem 110 cm. long, and bearing 4 side branches with a combined length of 110 cm. supported approximately 13,860 bladders. In 10 bladders selected at random, the number of *Entomostraca* which had been captured ranged from 6 to 22, with an average of 12 per bladder. On a conservative estimate, the bladders of this portion of the plant contained about 150,000 *Entomostraca*, in addition to numerous animals of other classes. Since the victims eventually break down and disintegrate, while new ones are constantly captured by the same bladders, the total number of organisms trapped by a single favourably situated plant during a summer must be enormous. In other material, Hegner found 512 euglenas in a single bladder, and 10 bladders examined contained an average of 215 of these flagellates each.

Of observations regarding the rapidity with which animals are captured, those of Büsgen (4) and Brumpt (3) are of the most interest. The former placed a spray of *U. vulgaris*, bearing empty bladders, into a vessel of water teeming with the cladoceran *Chydorus*. Within 1·5 hours, the bladders examined had captured on the average 3 animals each, whence Büsgen estimated that the entire specimen, 15 cm. long, and with 15 developed leaves, must have engulfed no less than 270 of these animals. A single bladder had accounted for 12 individuals, or an average of one every 8 minutes. Brumpt's experiments demonstrate the completeness with which the fauna of a given volume of water may be utilised by the bladders. He placed 100 larvae of *Anopheles maculipennis*, 1·5 mm. long, into a vessel containing 2 young branches of *U. vulgaris*, and in less than 3 hours almost half were taken. When the experiment was repeated, using *Culex apicalis* instead of *Anopheles*, 50 per cent. were caught within the first hour. Hegner (21) found that 30 per cent. of the bladders immersed in *Paramoecium* cultures succeeded in capturing one or more individuals within an hour.

As perhaps best exemplified by the observations of Simms (Moseley (29)) mentioned above, the bladders may often capture animals considerably larger than themselves. The mosquito larvae caught in the bladders may be almost 1 cm. in length, in which case they are coiled up within the lumen, and a larva 7·3 mm. long was found in a bladder 3 mm. in length. Mrs Treat observed that the entry into the bladder of such a large larva required in one case between 3 and 4 hours. Darwin also noticed larvae which were half within, and half outside the bladder. According to Brumpt, the larvae which came under his observation were usually held with either one or more of the posterior segments or the entire body except the head within the bladder, but the head always protruding, indicating that they had been drawn in with the posterior end foremost. The posterior end, which bears the anal brushes, is very actively moved while the animal swims, and for this reason probably often beats against the valve and releases it. Ostracods 0·6 mm. long and 0·4 mm. broad easily enter the large bladders of *U. vulgaris americana*.

Do the bladders of *Utricularia*, like the pitchers of *Sarracenia* and *Cephalotus*, possess any provision to lure on the prey they are to capture? Several adaptations for the attraction of aquatic animals have been pointed out by various naturalists, some of which are, to say the least, fanciful. Büsgen observed that small aquatic animals are attracted by vegetable mucilage, and believes that the slime secreted by the numerous glands surrounding the aperture serves to attract the prey. Mrs Treat observed larvae feeding on the long hairs at the orifice. Von Luetzelburg demonstrated the presence of sugar in the collar and the lower margin of the valve, and believed that this, along with the slime, attracts small organisms. Darwin surmised that the spot of light reflected from the convex surface of the valve might serve as a lure.

Several, but not all, of the purely terrestrial insectivorous plants secrete proteolytic enzymes which effect the digestion of their prey. This seems clear in the cases of *Dionaea*, *Drosera*, *Drosophyllum*, *Nepenthes* and *Pinguicula*. On the other hand, *Sarracenia* and *Cephalotus* lack the faculty of producing such secretions, and certain genera, such as *Genlisea*, *Polypomphyolyx* and *Aldrovanda*, still await investigation. Darwin could discover no digestion of the small cubes of roast meat, albumen and cartilage which he inserted into the bladders of *Utricularia*, and concluded that no proteolytic enzyme was present there. Later Büsgen (4) and Goebel (18) announced their confirmation of Darwin's conclusions.

The period necessary to bring about the death of an imprisoned animal ought to afford some suggestion of the intensity of the action of possible digestive enzymes, or of the presence of any poisons. Mrs Treat observed that larvae remained alive within the bladders for 24–36 hours. Cohn found that captured animals occasionally lived for 6 days, and records that the larva of a fly, after swimming around in the bladder for 3 days, finally made good its escape by eating a hole through the wall. Büsgen believed that the bladders are protected from such injury, inflicted either from within or without, by the presence of tannin in the cells, which renders them distasteful to animals. He observed that a piece of tissue previously treated with alcohol or hot water, to remove the tannin, is readily attacked by cyprids, while a fresh piece is avoided. He found that animals captured in the bladders often died within 24 hours, while others, which had become motionless after this period of captivity, revived when removed to fresh water. From this behaviour he inferred that suffocation was perhaps responsible for the death of the victim. This mode of death has been suggested by other authors, and the question of asphyxiation within the bladders requires further investigation, especially in the light of Hegner's results. However, all of these fragmentary observations show that death does not always rapidly supervene, but the animals may live a considerable time in such captivity. The more careful experiments of von Luetzelburg (26) indicated that the contents of the bladders exert some deleterious effect upon the captured organisms, even when removed from the lumen, but he did not distinguish between the fluids of the cavity and those present in the cells of the wall. By grinding whole bladders in a mortar and extracting with glycerine, he secured a solution in which larvae and small crustacea showed signs of approaching death after 11 hours. In the control (glycerine water) they behaved much as in their normal medium. Placed in this fluid, flies swam on their sides after 7 hours, but recovered upon removal.

Most of the scanty observations available seem designed to determine the longest period of imprisonment an animal can survive, rather than the shortest interval necessary to destroy it. Long periods of persistence may indicate merely an old or otherwise languishing bladder. The careful researches of Hegner (21) on the fate of captured protista are free from this criticism. Hegner found that euglenas not only remained alive indefinitely within the bladders, but actually multiplied there. *Phacus longicaudus*, another green flagellate, also lives and may multiply within the bladders; captured specimens of *Heteronema acus*, although not killed during confinement, were not observed to increase in numbers. On the other hand, captured paramoecia died in an average time of 75 minutes after their entry, and their disintegration usually followed rapidly. If, instead of being captured in the normal manner, the paramoecia were inoculated into a bladder by a pipette, their period of activity, although it still varied greatly, was generally considerably lengthened, and in certain cases reached 17 days, but in others death supervened after 25 minutes. If the bladder had been previously irrigated by sucking out the contents with a fine pipette, animals captured or inoculated into it generally died within 2 hours, although here again great variation was evident. In old, dead bladders, or in bladders killed by heat, paramoecia did not die within a short time, and often eventually made their escape. When placed in liquid withdrawn from the bladder cavity, paramoecia were not killed even after 48 hours, and the result was the same whether the bladders which supplied the liquid had previously killed paramoecia, or had not been infected by them. *Stentor polymorphus*, *Colpidium colpoda* and *Stylonychia pustulata* were all killed when impounded in the bladders, but *Centropyxis aculeata*, a shelled rhizopod, might live a week, and apparently died only from starvation. These experiments raise many interesting questions, the answers to which must await future investigation. Why should the green flagellates *Euglena*, *Phacus* and *Heteronema*, and the rhizopod *Centropyxis* remain unharmed within the bladders, while the colourless infusoria *Paramoecium*, *Stentor* and *Stylonychia* are soon killed? Why should paramoecia die after a short period within the bladder, but remain alive for 48 hours and more in the same liquid when removed from the bladder? Do these results indicate that the substance which causes death is not always present in effective concentration in the lumen, but is secreted there when a captured animal gives the proper stimulus? Is suffocation a factor in bringing on the death of the prey?

Von Luetzelburg reinvestigated the problem of the occurrence of digestive enzymes within the bladder, with results contrary to those of Darwin, Büsgen and Goebel. He placed small, angular particles of egg albumen and cheese in an extract made as described above (p. 290) and after three days the edges had lost their sharpness, and showed signs of corrosion not evident in the control. Neither when these proteins were used, nor with raw or cooked flesh, fibrin, milk, etc., could he secure a positive biuret reaction of the solution after 8 hours. Although no disinfectant was used, no odour of indol or skatol became evident in the solutions, and even after 27 days under conditions favourable for bacterial growth, no evolution of bacteria or moulds occurred. Drops of this extract liquefied gelatin within 4 days. The behaviour of a pure extract, made without the addition of glycerine or other substance, was next investigated. Cubes of albumen and cheese subjected to its action gave signs of corrosion as in the glycerine extract, and oil globules gave evidence of saponification after 8 hours. Yet all of the common tests failed to give a clear indication of the presence of soluble protein derivatives in the fluid. Finally von Luetzelburg was able to demonstrate the digestion of casein in the following manner: to 30 c.c. of bladder extract he added 50 c.c. of a suspension of casein containing 1 per cent. of Na_2CO_3. After neutralisation, the addition of 1 per cent. of ethyl alcohol caused the precipitation of the protein. The bladder extract was allowed to act upon a similar, unprecipitated mixture, and after 13 hours the addition of alcohol no longer caused a precipitate, indicating that the casein, which had originally formed the precipitate, had been digested. In this experiment, which was repeated 10 times with the same result, several drops of ether or chloroform were added to the solution to suppress bacteria. The tryptic enzyme present in the sap must be either very inactive or very dilute, considering the long period required for it to produce results even in such great concentration of the bladder fluid.

The conspicuous absence of the odours resulting from putrefactive decay in solutions containing protein substances, and the failure of bacteria or moulds to thrive in them under conditions apparently proper to their development, was rather surprising, and required further investigation. The lumina of young, still-fasting bladders seemed wholly free of bacteria, and showed no evolution of them when filled with sterile culture medium introduced by a pipette, under aseptic precautions. The surface of bladders was sterilised by treatment with $HgCl_2$ 1 : 1000, water and alcohol, and the fluid then pressed out of the cavity and allowed to drop on a gelatine plate, using all precautions to prevent contamination. Here only a very meagre and sickly culture of bacteria resulted from the inoculation. Von Luetzelburg concluded that bacteria do not normally produce the decomposition of captured animals, their presence is merely incidental, and their growth is checked by some deleterious substance produced by the bladder. Only injured or old bladders contain many bacteria and protozoa. Goebel (18) had previously demonstrated that the fluid in the pitchers of *Cephalotus* contains some substance which prevents the putrefaction of the captured insects, and had already suggested, as a result of some fragmentary experiments, that the bladders of *Utricularia* might be found to possess a similar peculiarity. Von Luetzelburg was able to demonstrate conclusively the presence of benzoic acid within the bladders, and this is the agent which prevents the evolution of the bacteria responsible for putrefaction. He obtained crystals of this acid, determined its melting point and crystalline form, and studied its physiological action on moulds and bacteria. Benzoic acid is present also in the leaves of the related *Pinguicula vulgaris*. The presence of such a disinfectant in the bladders is important, since indol and skatol are toxic to them even in great dilution.

Although unable to demonstrate the action of proteases upon the ingested food, Darwin undertook to show that the substances derived from what he believed to be the bacterial decay of captured animals were absorbed and utilised by the plant. He observed that in completely empty bladders the protoplasm in the arms of the quadrifid hairs is clear and transparent, except for a small, more highly refractive body which he took to be a "modified nucleus," but which Goebel (18) demonstrated to be a small crystal of calcium oxalate. After the bladders have made a capture, the appearance of the quadrifids changes. The protoplasm now becomes yellowish and often shrunken, and contains numerous, highly refractive, yellowish

granules. This granulation is similar to that which he had observed in the tentacles of *Drosera* and the glands of *Dionaea* upon the absorption of food, and naturally he concluded that it indicated the absorption of nitrogenous substances in this case also. He found that he could induce a similar condition of the quadrifids by placing halved bladders in solutions of NH_4NO_3, $(NH_4)_2CO_3$, urea, meat extract and putrid infusions of raw meat, but gum arabic and sugar solutions were inactive. Similar but more pronounced alterations of the protoplasm occurred in the glands surrounding the orifice of the bladder and finally led to the death of the cells. This observation, coupled with what he thought to be the irreversible nature of the granulation of the protoplasm, raised the question of whether the phenomena which he observed might not indicate injury to the protoplasm rather than the absorption of food. The inquiry was taken up at a much later date by von Luetzelburg, who showed that a very similar appearance in the protoplasm could be induced by such non-nutritious chemicals as $ZnSO_4$, $PbCO_3$, $MnSO_4$, $Ca(NO_3)_2$ and $HgCl_2$, and by extremely dilute solutions of indol or skatol, and therefore granulation is not conclusive evidence of the absorption of food. He further showed that such phenomena as Darwin had recorded result from the presence of an excess of readily digestible food, but that they could be reversed if the food is removed as soon as the granulation of the protoplasm becomes evident, and the bladder washed with sterile water. If the substance is allowed to remain in the bladder for 1 or 2 days longer, the granulation is not reversible, and leads unconditionally to the death of the hairs and eventually of the entire bladder.

Goebel (18) showed that the granules present in the bladders which he studied were not proteins, but globules of oil, probably the lecithin which oozes from the bodies of the dead crustaceans. He assumes that this is a source of the fat stored in the winter bud in the autumn.

Certain changes which occur in the bladder, and in the leaf of which it is a part, upon feeding the former with nutrient solutions, furnish excellent evidence that the proffered food is absorbed and promotes the growth of the plant. If asparagin, albumen or flesh-extract is introduced into a bladder by means of a fine pipette, an increased production of chlorophyll is manifest throughout the whole bladder, and all portions of the antennae, even those normally colourless, become intensely green. The whole bladder experiences an increase in size which in *U. minor* may amount in 4 days to one-third of the original dimensions. In *U. vulgaris* giant bladders, 6·2 mm. long, were produced by this artificial feeding. In addition, the antennae become greatly swollen, especially at their bases, and the whole bladder assumes a monstrous appearance (see von Luetzelburg (26), Figs. 7 and 8, p. 169). A great number of adventive shoots arise from leaves bearing bladders fed in this manner and in one instance 19 shoots sprang from a single leaf. However, Czaja (7) found that when bladder-bearing leaves were kept for a long time in distilled water, the bladders died and the leaves gave rise to adventive shoots, so that the production of such growths cannot in itself be taken as a criterion of favourable nutrition. In addition "double-bladders," or rather two single bladders arising from the same stalk, spring from such artificially fed leaves, and the leaf apex may be stimulated to produce a bladder. These results occur even when the solutions used ultimately bring about the death of the bladder, provided that the food is not supplied in excessive concentration.

The best method of securing information concerning the value or necessity of an animal diet to a carnivorous plant is to grow two parallel series of cultures, one of which is provided with flesh, the other deprived of it, and to compare the vegetative and reproductive activity, as expressed in growth and the production of seed, of the two lots. This approach to the problem has been used with cogent results in the case of *Drosera rotundifolia* by Büsgen, Francis Darwin, and Kellerman and von Raumer (see Goebel (18), p. 207), proving convincingly the value of a carnivorous diet. The results of similar experiments with *U. vulgaris*, carried out by Büsgen (4), in which vegetative growth alone was measured, are less convincing only because of the very small number of plants used with success. Büsgen started his cultures with the apical portions of shoots which he cut off just above the youngest leaf-bearing bladders which had captured prey. All cultures were supplied with water from the same source, which contained numerous animals on the one hand, and had been strained to remove the larger organisms on the other. Most of the experiments inaugurated had to be discontinued because the plants throve so badly, from unfavourable weather or other reasons. The results of the series which gave the clearest results are presented here:

	Length July 4 cm.	Length July 30 cm.	Growth in 26 days cm.	
Plant *a*, fed in greenhouse	14·5	47·0	32·5	Mean 21·8 cm.
Plant *b*, fed in greenhouse	9·0	20·0	11·0	
Plant *α*, unfed in greenhouse	13·0	28·5	15·5	Mean 10·5 cm.
Plant *β*, unfed in greenhouse	11·0	16·5	5·5	

The fed plants also produced more leaves than the unfed. Other shoots were kept in vessels placed in a manured hot-bed. Here the fed plants grew 60 cm., as opposed to the growth increment of 30·4 cm. made by the unfed plants. The average increment in length of the fed plants was twice that of the unfed, in both situations. In another series the fed plants grew well, while most of the unfed soon stopped growing and produced winter buds, so that no direct comparison could be made between the two cultures. However, the unseasonable production of turions is itself an indication of conditions unfavourable to growth (Glück (16), Goebel (18)). Goebel ((18), p. 206) "saw years ago, in De Bary's laboratory at Strassburg, unfed plants which in size lagged immensely behind those which were fed, but otherwise under the same conditions."

In conclusion, the criticism of Langeron (25) that "en réalité, nous ne savons rien sur la fonction des vésicules des utriculaires," seems to the present writer to be hardly justified. Langeron adduces in support of his contention cases in which plants were grown from winter bud to winter bud in water containing little plankton. The presence of the turion with its large supply of stored food renders the plant for a time independent of outside sources of nutriment, and introduces a complicating factor hard to evaluate; the production of the second winter bud is likely to ensue the faster the more unfavourable the external conditions become. Undoubtedly overfeeding causes the injury or death of the bladder, but similar unfavourable results follow the capture by *Drosera* of very large insects, because they are too bulky to become thoroughly impregnated with formic acid and digestive enzymes before putrefactive decay sets in. Small animals, captured a few at a time and slowly killed and digested, supply an abundance of nitrogenous food, and the result of their ingestion is not so harmful. The bladders do not normally remain functional throughout the vegetative season, and it is probably true that the capture of animals somewhat shortens their period of activity, if only because of the accumulation of unassimilable debris, although no direct experiments on this point, other than those of von Luetzelburg and of Darwin on artificial feeding, are known to the writer. However, even in the latter case, before dying the bladders pass on to the leaf valuable food substances which cause more rapid growth. Other organs, which absorb substances essential to the welfare of a plant, or manufacture its food, suffer a similar fate; the root-hair is at best a very transient structure, and the leaf of an evergreen or a tropical plant, over a long period of its activity, accumulates substances which interfere with its functions and cause its death. They are not for this reason considered as without advantage to the plant. Excess feeding under artificial conditions causes abnormal and monstrous growths, but these have not been reported as occurring under natural conditions, where the supply of food is more uniform and in a form less rapidly available. The fact that other species, not carnivorous in habit, can thrive side by side with a carnivorous species affords no good evidence that animal food is not advantageous or even essential to the latter. The two species may merely have solved in different ways the problem of surviving in the (to most plants) unfavourable environment of a bog or marsh, the one proclaiming itself by an obvious structural modification, the other hidden in occult physiological changes. It is also possible that *Utricularia* is a facultative rather than an obligate carnivore, and the fact that it may be placed in an environment in which it can thrive without animal food affords no proof that in another habitat the organic diet is not indispensable[1].

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[1] Our discussion in the present paper has been devoted exclusively to the aquatic species of *Utricularia*. The terrestrial and epiphytic species differ so conspicuously both in their conditions of life and in the structure of the bladders that it is hardly safe to apply to them any of the conclusions reached from a study of the aquatic species. Practically no work has been done on their physiology, and the observations on the prey captured by them are not nearly so satisfactory as in the case of the more familiar floating species (see Schimper (32)).

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