THE RAPID MOVEMENT OF THE BLADDER OF *UTRICULARIA* SP.

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Abstract

The changes which occur in the internal pressure, volume, membrane potential difference (p.d.), and membrane resistance during the firing of bladders of *Utricularia* sp. are described.

Firing of a bladder leads to an increase in the internal hydrostatic pressure from about −17 kPa to about −5 kPa and an increase in luminal volume of more than 40%. The opening and closing sequence of the trapdoor occurs within 10–15 ms after mechanical stimulation of one of the sensitive hairs located on the trapdoor.

When a bladder is fired, characteristic changes occur in ψ_{io} (the p.d. between the lumen of the bladder and the outside solution), ψ_{vo} (the p.d. between the interior of a wall cell and the outside solution), ψ_{iv} (the p.d. between the lumen and the interior of a wall cell), and R_{io} (the resistance between the lumen and the outside solution). The results indicate that the changes in ψ_{io} and ψ_{iv} are not simply a result of a short-circuiting of these p.d.s when the trapdoor opens but, like ψ_{vo}, result from actual changes in the respective membrane p.d.s.

Application of the Nernst equilibrium equation indicates that in a set bladder sodium and potassium ions are actively transported into the lumen from the outside solution and that chloride ions are actively transported in the reverse direction. Furthermore, sodium and potassium ions must be actively transported from the cells of the bladder wall into the lumen and probably chloride ions are actively transported in the opposite direction.

Although it has generally been considered that the firing process is a purely mechanical one there is some evidence for the existence of an excitatory step. Various aspects of this process are discussed.

I. INTRODUCTION

The bladders attached to underwater stems of aquatic species of *Utricularia* capture small animals by a unique and highly specialized method. The bladders, oval in shape, 1–5 mm long, and hollow, have a hole at one end which is covered by a trapdoor attached along a semi-circular line to the periphery of the hole. The free bottom edge of the trapdoor rests firmly in contact with rows of specialized cells. Attached near the free edge of the trapdoor, and projecting outwards from it, are a number of two-celled hairs. A small mechanical deformation of any one of these sensitive hairs (by a passing insect, for instance) causes the bladder to fire. The trapdoor opens inwards and there is a rapid flow of water into the bladder; the volume of the bladder increases, and finally the trapdoor shuts. After this rapid movement the bladder resets comparatively slowly to its original or set condition. Any small

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animal caught by the bladder remains inside, eventually dies, and is digested and absorbed by the bladder.

Early work on the capture of prey by *Utricularia* bladders has been reviewed by Skutch (1928). Lloyd (1935, 1942) has also reviewed the early literature, and written in detail of the structure of *Utricularia*, and the mechanism of action of the bladders.

Nold (1934) has investigated various aspects of the physiology of *U. vulgaris*, but mainly the resetting process, and found that the bladder, in resetting, loses approximately 40% of its weight. He also found that the electric potential of the lumen with respect to the outside was about 40 mV, but the electrodes he used were large and unsatisfactory by modern standards. Diannelidis (1948) also showed that the inside of the bladder of *U. vulgaris* was electrically positive with respect to the external medium. Diannelidis and Umrath (1953) measured a potential difference (p.d.) of 104 mV between the interior and exterior of the bladder of *U. vulgaris*, and a p.d. of −57 mV between the vacuole of a wall cell and the external medium. By using a large externally applied current impulse they were able to fire the bladder, in most cases producing a transient change in the internal potential.

This paper describes measurements of volume, internal pressure, membrane p.d., and membrane resistance in bladders of *Utricularia* sp., and changes in these quantities when the bladder is fired.

II. Materials and Methods

(*a*) *Materials*

Utricularia sp. was collected from Wilpena Pound, S.A. The species is unidentified because no flowers have been seen. It was stored in tanks in the laboratory in its native river water or in a culture medium comprising $NaHCO_3$, 0·5 mM; NaCl, 1 mM; $NaNO_3$, 0·2 mM; KH_2PO_4, 0·017 mM; K_2SO_4, 0·05 mM; $MgSO_4$, 0·1 mM. The bladders remained suitable for experimental use for 6–8 weeks. The basic experimental solution was Flinders pond water (FPW) comprising NaCl, 2·0 mM; KCl, 0·2 mM; $CaCl_2$, 0·05 mM. The bladders selected for use in experiments were 1·5–3 mm long.

(*b*) *Volume of the Bladder*

The volume of a bladder in both its set and fired states was measured with a tracer dilution method. Bladders, separated from the plant and soaked in FPW for several hours to ensure that all were fully set, were transferred to FPW containing radioactive chloride. Each bladder, viewed under a microscope, was fired by mechanical stimulation (with a fine glass probe) of one of the sensitive hairs attached to the trapdoor, removed from the radioactive solution, washed in inactive FPW for 300 s to remove any excess radioactivity adhering to the walls, blotted lightly, and placed on a dry planchet. A 1-μl sample of the luminal solution was collected and placed on another planchet containing 0·5 ml FPW. A solution containing 0·3 ml FPW and 0·2 ml of drying solution (10% isopropyl alcohol, 90% 1 mM NaOH, 0·4% glucose) was placed on the planchet containing the remainder of the bladder. Samples (1 μl) of ^{36}Cl FPW were collected to determine its specific activity. All radioactive samples were counted on a Nuclear–Chicago gas-flow counter.

If s_o and s_i (c.p.s. μl^{-1}) are the specific activities of the external radioactive solution and the luminal solution respectively after the bladder has been fired, V the volume of the set bladder, $V+\delta V$ the volume of the fired bladder, and Y the total radioactivity entering the bladder when fired, then

$$\delta V = Y/s_o$$

and

$$\delta V/(V + \delta V) = s_i/s_o.$$

Hence

$$V = Y(s_o - s_i)/s_i s_o.$$

(*c*) *Pressure Inside the Bladder*

Single bladders were mounted in FPW in a Perspex chamber as shown in Figure 1 and observed with a microscope at magnifications of up to about 300. The bladder was fixed in a vertical plane to enable the mechanical stimulation of a sensitive hair, and the subsequent movement of the side walls of the bladder during firing and resetting, to be followed under the microscope.

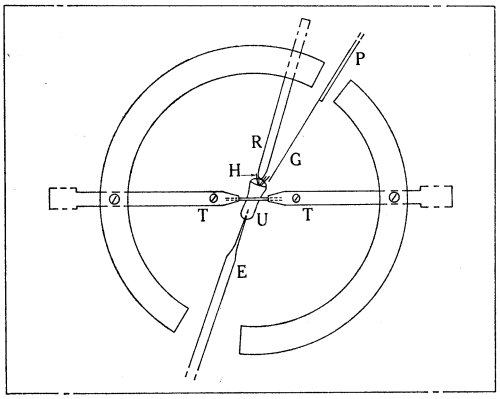

Fig. 1.—The experimental chamber in which single bladders of *Utricularia* sp. were mounted in FPW. The bladder *U* was held by its stem between two Perspex tweezers *T*. A Perspex rod *R* was used to prevent forward movement of the bladder during the insertion of a pressure-measuring capillary or microelectrode *E*. A fine glass probe *G*, attached to a 20-mm strip of piezoelectric material *P*, was positioned near one of the sensitive hairs *H*.

A method similar to that of Green and Stanton (1967) was used to measure the hydrostatic pressure in the lumen of the bladder. A glass capillary tube, 0·2 mm internal diameter, 0·8 mm external diameter, drawn out to 20 μm external diameter at one end, was filled with distilled water leaving a small air bubble a few millimetres from the tip, and sealed at the larger end with Araldite. The capillary was mounted in a Narashige micromanipulator and inserted into the lumen through the end of the bladder distant from the trapdoor, where least movement occurs during firing and resetting. The length of the air bubble, before and after insertion of the capillary, was measured, from which the internal pressure was calculated.

(*d*) *Concentration of Ions in Luminal Fluid*

Bladders, cut from the plant, were transferred to FPW for several hours. Each bladder was then removed from the solution, blotted lightly, and placed on a Perspex slab smeared with silicone grease. A 1-μl microcap was inserted through the mouth of each set bladder into the lumen and a 1-μl sample of the luminal solution collected. For sufficiently accurate determination of the concentrations of potassium and sodium by flame photometry, it was necessary to pool the samples from about 10 bladders. Six separate pooled samples (i.e. from a total of 60 bladders) were analysed.

The concentration of chloride in the lumen was determined by an electrometric titration method, similar to that of Ramsey *et al.* (1955). This method was satisfactory for samples of luminal fluid from single bladders.

The concentrations of sodium, potassium, and chloride in the river water in which *Utricularia* grows were determined using the methods described above.

(*e*) *Electrical Measurements*

Standard electrophysiological methods were used to measure ψ_{lo} (the electrical p.d. between the lumen of the bladder and the external solution), ψ_{vo} (p.d. between the interior of a single cell in the bladder wall and the external solution), ψ_{iv} (p.d. between the lumen and the interior of a wall cell), and R_{lo} (electrical resistance between the lumen and the external solution).

Glass microelectrodes, tip diameter 1–2 μm, filled with 3M KCl, were inserted into the lumen or into the interior of a wall cell. The electrodes could be inserted without firing the bladder. The external reference electrode was a 3M KCl-filled microelectrode of tip diameter 10 μm. The inserted electrodes were located at the thin edge of the bladder, distant from the trapdoor, where least move-

ment occurred during firing and resetting. The p.d.s ψ_{io}, ψ_{vo}, and ψ_{iv} were recorded in the set bladder, during firing and for periods of up to 3·6 ks during resetting, and displayed on quick-response chart recorders and a Tektronix 564B four-channel storage oscilloscope.

The resistance R_{io} was determined by passing pulses of constant current I through a microelectrode inserted into the lumen to a silver–silver chloride electrode in the external solution, and measuring the resultant change $\delta\psi_{io}$ in ψ_{io}. Then

$$R_{io} = \delta\psi_{io}(A/I),$$

where A is the surface area of the bladder. A could be determined with a microscope if at the end of the experiment a fine longitudinal incision into the bladder was made and it was then spread flat on a microscope slide.

The bladder was fired by a mechanical displacement of the end of one of the sensitive hairs by 10–15 μm, for 10 ms, towards the centre of the trapdoor. The displacement was produced by a fine glass rod touching the end of the hair, and attached to the end of a 20-mm-long strip of piezoelectric material that bent when an appropriate electric p.d. was applied to it.

In an experiment aimed at measuring conductance changes, and indirectly the length of time for which the trapdoor remained open when the bladder fired, ψ_{io} was held at its resting level by a voltage-clamp circuit similar to that used by Findlay (1964) with single cells of *Chara australis*. The current flow in the clamp circuit when the bladder was fired gave a measure of the conductance between lumen and outside. A large current flow should occur during the time the trapdoor is open. Mean values are given in the text together with the standard error of the mean, and number of samples in parentheses.

III. Results

(a) *Volume and Internal Pressure*

(i) *Volume*

The volumes of 12 bladders from one batch of material were measured before and immediately after firing by the tracer dilution technique. The mean volume of set bladders (V) was $1·77 \pm 0·15$ μl, the mean volume of fired bladders $V+\delta V$ was $2·54 \pm 0·22$ μl, and the mean increase of the set volume $\delta V/V$ was $43·5 \pm 2·1$%.

When a bladder was fired external solution entered the lumen through the trapdoor and the lateral walls moved outward so that the width of the bladder through its centre almost doubled. During the resetting phase solution was gradually expelled from the lumen and the lateral walls moved inward. The rate of resetting of different bladders was variable and depended considerably upon the time of storage. The more matured bladders reset at a slower rate. Figure 2 shows a graph of the width of a bladder as a function of time after firing.

All bladders were fully reset within 9–12 ks after firing, but could usually be fired 2·4 ks after a previous firing. A single bladder could be fired up to eight times at intervals of 2·4 ks without appreciably affecting the relationship between change in bladder width and time shown in Figure 2.

(ii) *Internal Pressure*

The mean luminal hydrostatic pressure in fully set bladders from one batch of material was -17 ± 1 (5) kPa with respect to the external solution. After each bladder was fired, the internal hydrostatic pressure approached that of the outside but after 30 s (the resolution time of the apparatus) was still negative by between 5 and 10 kPa. Figure 3 shows the luminal pressure as a function of time over a period of about 6 ks during which time the bladder was fired three times. The pressure-measuring capillary

was inserted into the lumen of the bladder at zero time, immediately after the bladder was fired. As fluid was transported from the bladder the internal pressure became more negative until eventually it reached a steady level approximately 1·2 ks after firing. When the bladder was again fired there was a sudden reduction in the magnitude of the pressure as water moved into the lumen. After 6 ks the bladder was again fired and the pressure-measuring capillary then withdrawn.

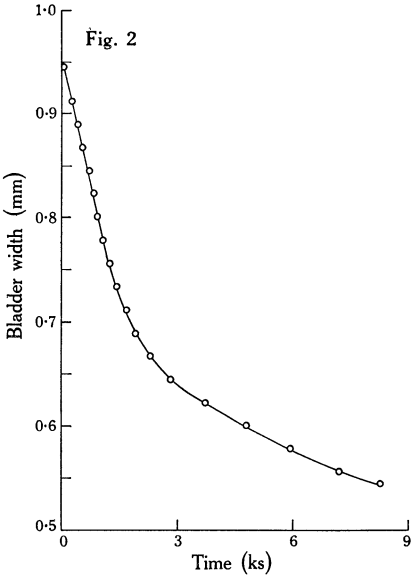

Fig. 2.—Change in width of a bladder as a function of time after firing.

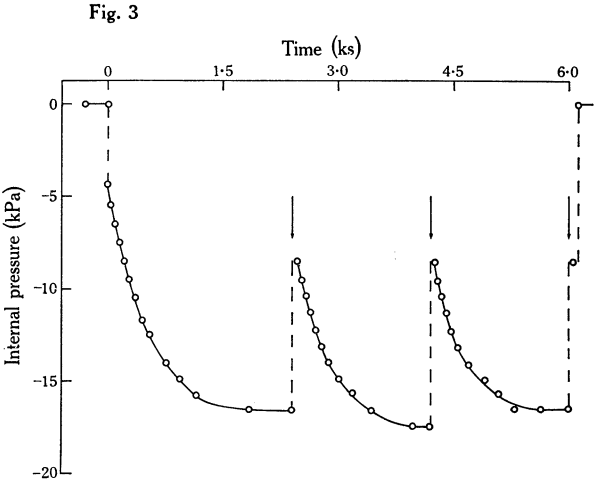

Fig. 3.—Change in luminal pressure as a function of time. The arrows indicate when the bladder was fired.

(*b*) *Concentration of Ions in Luminal Fluid*

The concentrations of potassium, sodium, and chloride in the luminal fluid of bladders which had been in FPW for at least 86 ks are tabulated below, together with the ionic concentrations in FPW:

	Ionic concentrations (mM)		
	Na	K	Cl
Luminal fluid	10·9±0·5 (6)	7·8±0·2 (6)	8·2±0·7 (10)
FPW	2·0	0·2	2·3

(*c*) *Electrical Measurements*

(i) *Resting Potential Differences*

Microelectrodes carefully inserted into the lumen of set bladders, and into the interiors of cells in the bladder wall, recorded fairly steady potentials with respect to the outside reference electrode for times of at least 1·8 ks. In bladders bathed in FPW, the p.d. recorded by a microelectrode gradually inserted into the outer and then into the inner cell layer of the wall of a bladder remained unchanged at about −170 mV

until the tip penetrated into the lumen. The p.d. then suddenly changed to about +135 mV. If the electrode was inserted further into the lumen so that the tip eventually penetrated a cell of the inner layer of the opposite wall the p.d. suddenly returned to −170 mV where it remained until changing to near zero when the tip passed through the outer cell layer and into the external medium.

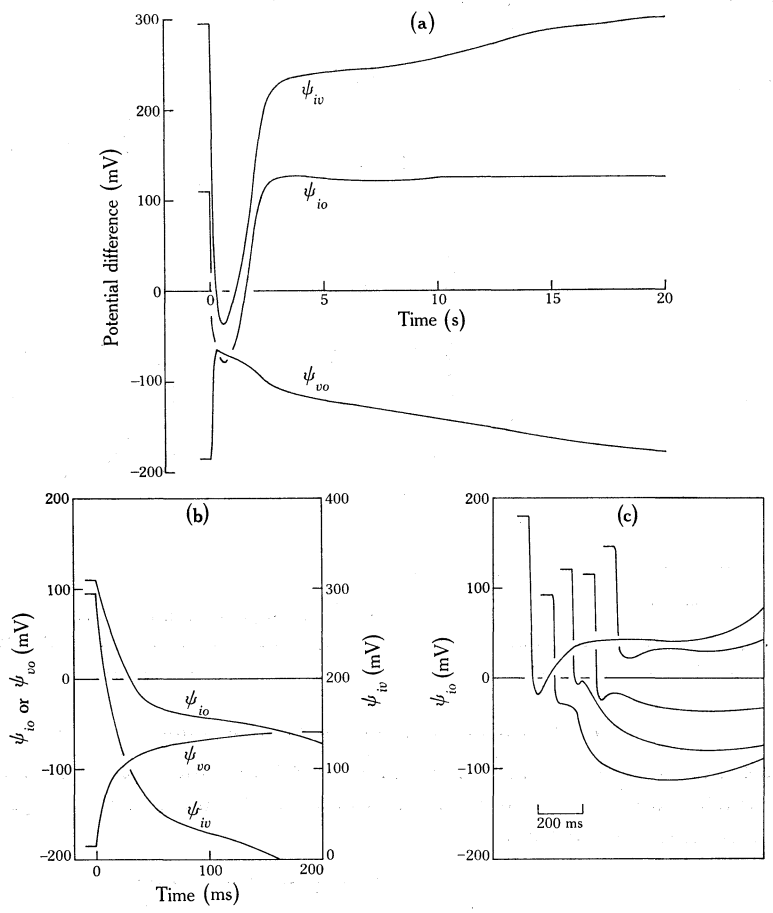

Fig. 4.—(*a*) Changes in the p.d.s ψ_{io}, ψ_{vo}, and ψ_{iv} occurring in the same bladder within 20 s after firing. Zero time is at the start of the mechanical stimulus. (*b*) Changes in the p.d.s ψ_{io}, ψ_{vo}, and ψ_{iv} occurring in the same bladder as shown in (*a*) within 200 ms after firing. (*c*) A series of changes in the p.d. ψ_{io} occurring in five different bladders within 1 s after the first firing. Each curve has been displaced in time.

Simultaneous measurements of ψ_{io}, ψ_{vo}, and ψ_{iv} were made on 31 bladders with FPW as external solution. The results, including measurements of ψ_{vo} alone in a further seven bladders, are recorded in the following tabulation:

	ψ_{io}	ψ_{vo}	ψ_{iv}
P.d. (mV)	133±5 (31)	−167±4 (38)	299±7 (31)

(ii) *Effects on ψ_{io} of Changes in Sodium and Potassium Concentrations and pH in the External Solution*

An increase in $[K_o]$ from 0·2 to 2·0 mM, with an accompanying change in $[Na_o]$ from 2·0 to 0·2 mM in order to keep $[K_o+Na_o]$ constant and thus eliminate changes in ψ_{vo} due to a Donnan system in the cell walls (Hope and Walker 1961), caused a depolarization of 15–20 mV in ψ_{vo}, but no change in ψ_{iv}.

Changes in the pH of the external solution from 4·0 up to 9·0 produced practically no change in ψ_{io}.

(iii) *Transient Potential Differences*

When a bladder is fired by a mechanical stimulus to one of its sensitive hairs, transient changes in ψ_{io}, ψ_{vo}, and ψ_{iv} occur. Figure 4(*a*) shows changes in these p.d.s within one bladder within 20 s after firing, and Figure 4(*b*) the changes within 200 ms. These p.d.s were recorded in a bladder which had not been fired for 12 ks previously. Figure 4(*c*) shows changes in ψ_{io} following the first firing in five different bladders within 1 s after firing.

Changes in ψ_{io}, ψ_{vo}, and ψ_{iv} following the first firing for at least 86 ks were recorded in 12 bladders. The resting levels and the peak values are tabulated below:

	ψ_{io}	ψ_{vo}	ψ_{iv}
Resting p.d. (mV)	131±12	−165±9	296±16
Peak p.d. (mV)	−24±15	−67±8	76±18

A bladder can usually be fired at least eight times, and often more, before the resetting process becomes incomplete or fails altogether. Figure 5 shows a series of changes of ψ_{io} following successive firings of one bladder at intervals of about 2·4 ks.

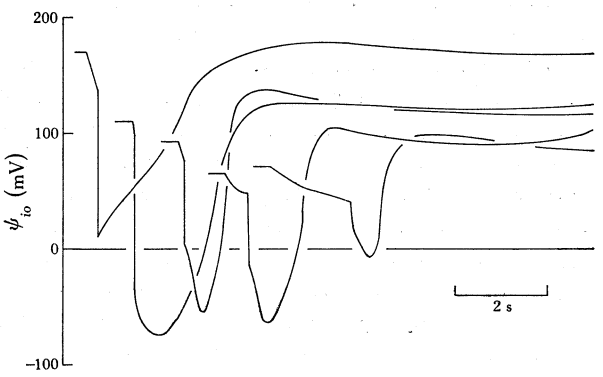

Fig. 5.—A series of changes in the p.d. ψ_{io} following successive firings of one bladder at intervals of about 2·4 ks. Each consecutive curve has been displaced in time.

Before the first firing in the series the bladder had been set for at least 86 ks. During the experiment ψ_{io} gradually drifted to a less positive value. The last two transient responses in the series both show a small slow depolarization of potential before the rapid depolarization. This slow depolarization was closely associated with a slow inward movement of the centre of the trapdoor prior to the rapid opening.

Old bladders or bladders which had been treated with 1 mM sodium azide in the external solution or with low temperature could not be fired by the normal mechanical

displacement of a sensitive hair. To fire these bladders it was often necessary to actually force the trapdoor inwards with a probe.

(iv) *Resting and Transient Resistances*

In eight bladders from one batch of material which were set and had not been fired for several hours, the electrical resistance between the lumen and the external FPW was $11 \cdot 8 \pm 0 \cdot 6\ \Omega\ m^2$. When a bladder was fired the resistance dropped to a value less than 25% of the resting value. Figure 6 shows a typical change in R_{io} after a bladder had been fired. The shortest time after firing at which an accurate measurement of resistance was possible with a current pulse lasting 1 s was about 3 s.

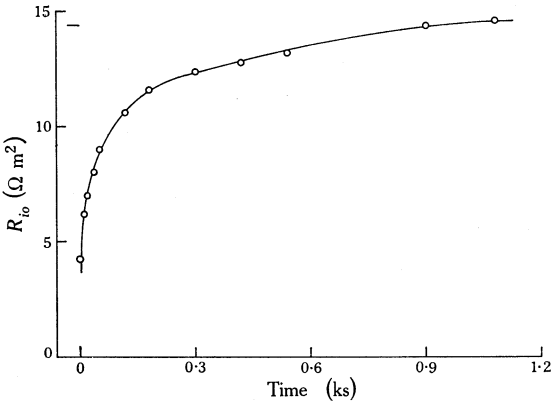

Fig. 6.—A typical change in the resistance R_{io} as a function of time after firing. The resting resistance of the set bladder is shown at the top left of the curve.

Changes in the electrical conductance between the lumen and the outside solution could also be determined by measuring the current necessary to hold ψ_{io} constant (with a voltage clamp) when the bladder was fired. Figure 7(*a*) shows the clamp current, I_c, when ψ_{io} was held near its resting level. As the control in the feedback circuit controlling ψ_{io} was limited by the resistance of the current electrode, some excursion in ψ_{io} occurred, but it was small compared with the changes in ψ_{io} without clamping. Figure 7(*b*) shows the clamp current, and Figure 7(*c*) the conductance g_{io} between lumen and outside, both in the first 170 ms after the start of the stimulating pulse. The conductance, as a function of time, was calculated from the equation $g_{io} = I_c/\Delta\psi_{io}$ where $\Delta\psi_{io}$ is the difference between the curves of ψ_{io} versus time when ψ_{io} is clamped and when it is not. In 10 bladders it was found that the value of g_{io} was substantially higher than its resting level for 10–15 ms after the stimulus, and then decreased to less than twice its resting value in about 30 ms. This result implies that the trapdoor opened and closed within 30 ms.

(*d*) *Lack of Response of Bladder to Electrical Stimuli*

Two types of electrical stimuli were used in attempts to fire bladders of *Utricularia* sp. The first type of stimulus consisted of a pulse of current between two plate electrodes in the external solution, with the bladder between them. Current pulses of up to 20 μA would not fire the bladder. In the second type of stimulus, current pulses of up to 3 s duration were passed between lumen and outside solution through a

microelectrode in the lumen. Changes in ψ_{io} from its resting value of about 130 mV to +380 mV and to −120 mV did not fire the bladder.

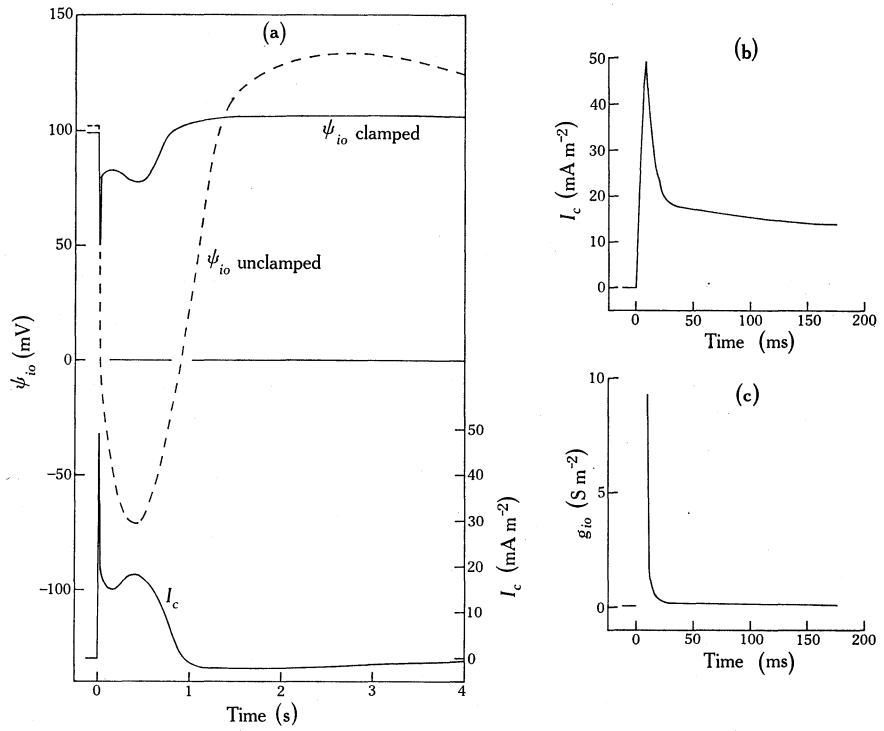

Fig. 7.—(*a*) Membrane current I_c as a function of time during a voltage clamp of ψ_{io} at its resting level when a bladder was fired. The clamped p.d. (solid line) is shown compared with the normal change in ψ_{io} without clamping (dotted line). (*b*) The membrane current during voltage clamping of ψ_{io} in the first 170 ms after the start of the stimulating pulse. (*c*) The conductance between lumen and outside (g_{io}) in the first 170 ms after the start of the stimulating pulse. The earliest time at which an accurate conductance measurement could be made was 10 ms after the start of the stimulating pulse. The resting conductance is shown at the bottom left of the curve.

IV. Discussion

It has generally been considered (Lloyd 1942) that the hydrostatic pressure in the lumen of *Utricularia* bladders in their set condition is at a lower value than that in the external solution. Water in the lumen of set bladders will be under tension as the walls, because of their structure and the turgidity of their cells, tend at all times to assume a convex form and therefore a state of less stress. We have shown by direct measurement that the hydrostatic pressure in the lumen of a set bladder is lower than that outside. The presence of this negative internal pressure poses interesting problems about the processes necessary to establish it, and maintain it for as long as the bladder is set. When a bladder is fired the internal pressure increases to a less negative value and there is a simultaneous outward movement of the lateral walls leading to a rapid flow of water into the lumen and a relatively large increase in the luminal volume. Figure 2 indicates that an equivalent volume of water will be transported from the lumen to the

outside when the bladder again sets. We have examined the role of solute and water transport in the setting of the bladder and the results will be described in a later paper.

The electrochemical equilibrium potentials for potassium, sodium, and chloride in the lumen of a set bladder, calculated from the Nernst equation, e.g.

$$\psi_K = (RT/zF) \ln([K_o]/[K_i]),$$

are $\psi_K = -92$ mV, $\psi_{Na} = -43$ mV, and $\psi_{Cl} = +32$ mV. The observed value of ψ_{io} is +133 mV. If it is assumed that in a bladder which is fully set the fluxes of the ions between lumen and outside are in a steady state, then one must conclude that all three ions are actively transported, K^+ and Na^+ inwards, and Cl^- outwards. Furthermore, ψ_{io} could not be a multi-ionic diffusion potential of K^+, Na^+, and Cl^- because its magnitude exceeds the magnitudes of ψ_K, ψ_{Na}, and ψ_{Cl}. It is also unlikely that ψ_{io} is a hydrogen ion diffusion potential, because for $\psi_{io} = 133$ mV the luminal pH would need to be at least two units less than the external pH, i.e. about 3, a rather low value. In any case, ψ_{io} does not depend on external pH in the range 4–9, unlike cells of the Characeae (Kitasato 1968; Adrianov *et al.* 1968).

An interesting feature of the electrical measurements is the large p.d., about −300 mV, between the interior of a cell in the bladder wall and the lumen. Passive equilibrium of K^+, Na^+, and Cl^- between these phases would require in the vacuole $[K] = 1{\cdot}2 \times 10^3$M, $[Na] = 1{\cdot}6 \times 10^3$M, and $[Cl] = 5{\cdot}5 \times 10^{-8}$M. Clearly, there must be active transport of K^+ and Na^+ from the cells of the wall to the lumen, and probably of Cl^- in the reverse direction.

When a bladder is fired characteristic changes occur in the p.d.s ψ_{io}, ψ_{iv}, and ψ_{vo}, and in the resistance R_{io}. The depolarization phase of ψ_{io}, as shown in Figure 4(*c*), consists effectively of two separate stages with quite different time courses. During the time the trapdoor is open the luminal and outside solutions will be in direct contact, and ψ_{io} will be effectively short-circuited and approximately equal to the overall junction potential between the two solutions. This diffusion potential would be no more than a few millivolts. However, the peak negative value of ψ_{io}, as shown in Figure 4(*c*), can be up to −100 mV 0·5 s after the bladder has fired, when the trapdoor has again closed and when the conductance between lumen and outside is low. These results imply that the transient change in ψ_{io} cannot simply be the result of a short-circuiting of this p.d. by the opening of the trapdoor, and must therefore result from an actual change in membrane p.d. between the lumen and the outside. In fact, both ψ_{iv} and ψ_{vo} change. Figure 4(*b*) shows that ψ_{vo} rapidly depolarized to −65 mV and then returned in about 25 s to its resting level of −185 mV. The change in ψ_{vo} would be unlikely to arise as a consequence of the sudden movement of the bladder which is complete within 30 ms of stimulation and must therefore result from a change in membrane p.d. between the interior of a wall cell and the outside. At the same time ψ_{iv} underwent a large negative transient change lasting about 3 s between +295 and −35 mV. This change, like that of ψ_{io}, cannot be explained simply by a short-circuiting of ψ_{iv}, and must be due to a change in the membrane p.d. between the lumen and the vacuole of a wall cell. After repetitive firing, for instance, another stage becomes apparent in the depolarization phase of ψ_{io}. This slow change in ψ_{io}, preceding the rapid transient (Fig. 5), could result from an increase in electrical conductance of the trapdoor, or a slight leakage at the edge of the trapdoor, either of which would partially short-circuit ψ_{io}.

Lloyd (1942) photographed the movement of the trapdoor in *U. vulgaris*, and found that the trapdoor opened in about 6 ms, and was closed again within 24 ms. Our value (determined from electrical measurements) of 10–15 ms for the opening and closing sequence is thus close to that of Lloyd. The firing of the *Utricularia* bladder is considerably faster than the movement of other plant organs except for the movement of the column in some species of *Stylidium* (Findlay, unpublished data).

There has been considerable discussion in the past (Merl 1922; Withycombe 1923; Lloyd 1942) of the question of whether the bladder of *Utricularia* spp. operates simply as a mechanical trap, set off when the trapdoor is levered open slightly by the movement of one of the sensitive hairs, or whether it operates through an excitatory process. Such a process might be one in which the initial mechanical stimulus is transduced to an electrical one at the base of the sensitive hair, which in turn causes action potentials to be propagated out into the cells of the trapdoor, resulting in a loss of solute, and consequently turgor, from these cells. This loss of turgor would cause the trapdoor to cave in under the inwardly directed hydrostatic pressure gradient, open, and allow fluid into the lumen. In a system such as this, where electrical changes occur at various steps in the process, appropriate externally applied electrical stimuli should be able to excite the system. An analogous excitatory system operates in *Dionaea*, Venus's fly-trap (Jacobson 1965).

Merl (1922) and Lloyd (1942) concluded that the bladder operates simply as a mechanical trap, because it could only be fired by mechanical means. Diannelidis and Umrath (1953), however, have fired bladders of *U. vulgaris* with pulses of electric current delivered from an induction coil connected to electrodes in the external solution, on either side of the bladder. The magnitude of the current pulses was not given.

Contrary to the findings of Diannelidis and Umrath (1953), we have been unable to stimulate bladders by electrical means. However, we have some evidence that does suggest the existence of an excitatory step in the firing process. The behaviour of a bladder when, with repetitive firing, the trapdoor collapsed partially inwards before opening [Section III(*c*)(iii)] indicates that a loss of turgor in the cells of the trapdoor occurred as a result of the stimulation. It is difficult to see how a small displacement of the tip of a sensitive hair could directly cause a loss of turgor in cells of the trapdoor. The accompanying electrical changes during repetitive stimulation as shown in Figure 5 are consistent with a number of excitatory processes provided certain assumptions are made. For instance, in a process in which the amount of solute, and consequently turgor, lost by cells of the trapdoor decreased with repetitive stimulation, the loss of turgor by a few cells in the vicinity of the sensitive hair after the first stimulus, and in a short time, would be sufficient to fire the bladder, but following each stimulus loss of turgor would need to occur in an increasingly greater number of cells before decreasing the rigidity of the trapdoor sufficiently to allow it to open and fire the bladder. If there were a finite velocity of propagation of action potentials from cell to cell in the trapdoor the time interval between stimulus and firing of the bladder would increase with successive stimuli. In old bladders and in those that had been treated with sodium azide or low temperature the lack of response to the normal intensity of mechanical stimulation suggests a loss of excitability in the cells of the trapdoor. Other processes which can be envisaged to result in loss of excitation include a decrease in the rate at which action potentials propagate from cell to cell in

the trapdoor, an increase in the rise time of each action potential, a change in the threshold of each action potential, or a decrease in membrane permeability to a particular ion or ions which causes each action potential.

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