Water Extrusion in the Trap Bladders of *Utricularia vulgaris*

II. A Possible Mechanism of Water Outflow

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In the trap bladder of *Utricularia vulgaris*, increase in sucrose concentrations in bladder lumen fluid decreased resetting rate. Addition of 350 mM sucrose to lumen fluid stopped the resetting. Therefore, water seems to move down the water potential gradient between the lumen and the arm cells of bifid trichomes, which are the site of inlet in the water pathway. Application of dinitrophenol, sodium azide, KCN, monooiodoacetic acid or pentachlorophenol in lumen fluid much reduced the water outflow. Temperature coefficient of bladder resettings was about 2. No effect of darkness on resetting rate was found. These facts show that the resetting requires energy supplied from respiration and there exists an active ion transport mechanism somewhere in the water pathway. No effect on the resetting was seen upon immersing the bladder in 700 mM sucrose solution. In the capital cells of the pavement epithelium in its outer and middle zones, which are the site of outlet in water pathway, membrane potential and resistance were lower than those in other cells. These facts indicate that bulk flow of the cell sap from the capital cells to the outside takes place by intracellular hydrostatic pressure.

Key words: Bulk flow — Trap bladder — Trap resetting — *Utricularia vulgaris* — Water and solute transport.

In the preceding paper (Sasago and Sibaoka, 1985) we concluded that inlet and outlet of water outflow from the lumen to the outside during resetting of the *Utricularia* bladders are the bifid trichomes and the pavement epithelium in its outer and middle zones, respectively, based mainly on morphological evidences. This conclusion entirely differs from the hypothesis proposed by Nold (1934) and almost agrees with that by Sydenham and Findlay (1975).

A sudden expansion of the concave bladder upon stimulus is followed by slow decreases in bladder width and internal hydrostatic pressure. This resetting process (from convex to concave bladder) is caused by continuous water outflow from the lumen to the outside. Sydenham and Findlay (1973, 1975) examined some aspects of the resetting process and proposed a model of solute and water transport across the bladder wall.

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In this study we examined the effects of various factors on the resetting process, to learn its mechanism. Moreover, measurement of oxygen consumption of the bladder, some electrophysiological measurements of bladders and bladder wall cells, and measurement of short-circuit current across the bladder wall were made.

**Materials and Methods**

The preparation of the trap bladders of *Utricularia vulgaris* L. and the basic experimental solution, or artificial pond water (APW), were described previously (Sasago and Sibaoka, 1985).

In all the experiments reported in this paper, the fluid in bladder lumen was replaced with APW before an experiment. The replacement was made with a 2-ml hypodermic syringe filled with APW. A bladder was immersed in APW and the needle of the syringe was carefully inserted into its lumen through the entrance so that the trap door was not injured. The APW in the syringe was injected into the lumen to rinse its interior and to fill it up with APW. When the APW in the lumen was substituted for a test solution, the latter was injected into the lumen in the same way. The test solution was made by adding a salt or drug in an appropriate amount to APW and its pH was adjusted at 6.5 with addition of NaOH or HCl.

To measure the cation concentrations of exudation fluid, bladder lumen was rinsed and filled with APW by the syringe twice, one day before and just before the experiment. The outer surface of the bladder was washed with deionized water and then blotted lightly. The bladders thus prepared were placed in a moist chamber. About 5.5 μl exudate was collected together from 30-40 bladders with a micropipette. The exudate gathered was analyzed by atomic absorption spectrophotometry.

The methods of measuring the changes in bladder width and rates of water outflow of bladders were described previously (Sasago and Sibaoka, 1985). In measuring the width change, a bladder was measured twice: the first was used as control (bladder inside and outside were soaked with APW) after the first expansion and the second as a specific treatment (one side was soaked with a test solution and the other with APW) after the second expansion. The differences in bladder widths at 10 and 20 min after expansion were measured (termed resetting rate) and ratios of the second measure to the first one were calculated (termed ratio of resettings). The ratio of resettings and other measurements of various quantities in this paper are expressed by the mean value and standard deviation, with the number of samples in parentheses.

Oxygen consumption of a batch of 20-30 bladders was measured with a Clark type oxygen electrode in the dark at 24°C. The bladders were placed in 5 ml APW which was buffered with 10 mM Tris-HCl at pH 7.0, and were stirred.

Standard electrophysiological methods were used to measure the potential difference (PD) between the bladder lumen or a cell interior of bladder and external solution. An ordinary glass microelectrode filled with 3 M KCl was inserted into the lumen or a cell. External reference electrode was the same type as the former. To measure membrane potential and resistance of a cell in pavement epithelium, a single