ION ABSORPTION IN *ATRIPLEX* LEAF TISSUE

II.* SECRETION OF IONS TO EPIDERMAL BLADDERS

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Summary

The epidermal bladders of several *Atriplex* species contain high concentrations of ions. Chloride was secreted from the solution or the lamina to the bladders, against a concentration gradient. Transfer of ^{36}Cl to the bladders was strongly light stimulated, but uptake to the lamina was much less sensitive.

Electrical potential measurements showed that the vacuole of the bladder cell was highly electronegative with respect to the bathing solution. Switching from dark to light and vice-versa resulted in transient changes in potential. In some instances the potential settled to a level which was more negative in the light than in the dark. These observations suggest that uptake of chloride into the bladders is an active process.

Autoradiographs of intact and sectioned bladders after exposure to $K_2{}^{35}SO_4$ and $K^{36}Cl$ showed that radioactivity was concentrated in the stalk cell and peripheral cytoplasm of the large vacuolated bladder cell. Electron microscopy showed that the stalk cell and peripheral cytoplasm of the bladder cell contained chloroplasts, numerous mitochondria, much endoplasmic reticulum, and many small vesicles. The stalk cell has the submicroscopic characteristics of a salt gland and, as it is connected to the bladder cell and the epidermal cells by plasmodesmata, may secrete ions from the leaf symplasm to the bladder cell.

I. Introduction

Salt encrustation of the surface of saltbush leaves has been recognized for many years. When describing the doubtful value of the western plains of New South Wales, Bartly (1892) saw "only the saltbush . . . its leaves covered with glittering saline particles". Charley (1959) showed that the salt leached by rain from leaves of *Atriplex* growing in the field may amount to about half the total salt content of these plants. Salt secretion may be important to the salt balance of individual plants and possibly to the whole community (Charley 1959). Black (1954) described the leaf anatomy of two *Atriplex* species in detail. The bladder-like hairs of the epidermis, which he termed vesicles, comprise a large (100–200 μ diam.) vacuolated cell, the bladder cell, resting on a stalk cell which is initially short but which may elongate. Under saline conditions the bladder cell contains much salt, as can be seen

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by the formation of a cloud of silver chloride when these cells are ruptured under a drop of silver nitrate. The highly reflective salt crust of leaves found in the field evidently forms when these waxy bladder cells collapse and dry.

Leaf salt-secretion systems in other plants are characterized by the transfer of ions in solution of high concentration against an electrochemical potential gradient via morphologically discrete glands (Arisz *et al.* 1955; Scholander *et al.* 1962; Atkinson *et al.* 1967; Hill 1967*a*, 1967*b*). The experiments described here present evidence for the transfer of ions from the leaf lamina to the epidermal bladders in a similar, but in several respects novel, system. The distribution of ions in these leaves and some features of the submicroscopic structure of the bladders have been described briefly elsewhere (Osmond 1963; Robertson 1968).

II. Materials and Methods

(*a*) *Materials*

Atriplex seedlings were grown in sand, soil, and water cultures. Sand cultures were irrigated with the solutions used for water cultures. Water culture solutions used in the experiments of Figure 1 and Table 1 contained, in m-equiv/l, potassium 6, calcium 5, magnesium 2, nitrate 10, phosphate 1, sulphate 2, and Fe EDTA and micronutrients (Osmond 1966). NaCl was added as required. Plants used in isotope transfer and electrical potential measurements were grown in a similar culture solution, with 5 mM NaCl.

(*b*) *Distribution and Transfer of Ions*

The only reliable method for estimating ion content of bladders was to separate these from the lamina. This was done in two ways. The ion concentration values of Table 1 and Figure 1 were obtained by gently scraping both surfaces of turgid leaves with a razor blade and washing the leaf free of debris with deionized water. The ion content of lamina and washings were then estimated using methods described earlier (Osmond 1966). This method gave values of 21% for the fresh weight of bladders as a proportion of the total fresh weight of 1–2-cm leaves.

In isotope transfer experiments the upper surface of small leaves was brushed free of bladders. Two 3 by 12-mm slices were cut from each leaf and collected in 0·5 mM $CaSO_4$. These slices were transferred to KCl solutions of various concentrations labelled with ^{36}Cl (Australian Atomic Energy Commission, Lucas Heights, N.S.W.), and containing 0·4 mM $CaSO_4$ (final specific activity $1{\cdot}65 \times 10^{-5}$ disintegrations/min/μmole chloride). Samples of four slices were removed at intervals and ions were exchanged from lamina free-space in unlabelled solution (5°C) changed after 10 and 30 min. Experiments were done in a constant-temperature, shaking water-bath at 25°C and the light source used was a 400 W Philips mercury vapour lamp yielding 25 mW/cm^2 (400–700 mμ). Slices were then frozen over dry-ice in a closed chamber and the frozen bladders were brushed from the lower surface onto a planchet. This technique removed all bladders which, from the lower surface, comprised 20% of total leaf fresh weight. Microscopic examination showed that both methods removed the entire bladder, i.e. the large bladder cell and the stalk cell. Bladders and lamina were spread on planchets with a drop of propanol and fixed with a little 0·1% gelatin before being dried at 70°C, then counted under a gas-flow counter. Radioactivity in bladders or lamina was expressed as disintegrations/min/mg fresh weight after calibration of the flow counter by reference to [^{36}Cl]chlorobenzene in a scintillation counter.

(*c*) *Autoradiography*

Autoradiographs were prepared after leaf slices and segments were exposed to labelled solutions and free-space ions had been exchanged as described above. The specific activities of

ions used were: 0·1 mM $K_2{}^{35}SO_4$, 400 μCi/μmole; and 90 mM $K^{36}Cl$, 40 μCi/μmole. Three methods of autoradiography were employed. In the first of these, the surface-stripping technique of Läuchli and Lüttge (1968) was used. Small paradermal sections of *Chenopodium album* leaves were exposed to 0·1 mM $K_2{}^{35}SO_4$ for 7 hr at 25°C. These were then covered with moist stripping film and exposed for 20 days in a deep-freeze before development. The high density of bladders on *Atriplex* leaves prevented good contact between bladder and film which is essential in this method. The second method involved the use of sectioned material which was coated with stripping film on a microscope slide (Branton and Jacobson 1962). *A. spongiosa* leaf slices which had absorbed 0·1 mM $K_2{}^{35}SO_4$ for 4 hr at 25°C were dipped in isopentane–8% methylcyclohexane cooled to a slightly viscous solution in liquid nitrogen. These slices were vacuum freeze-dried overnight or dried by freeze substitution of water in anhydrous ether at −25°C. The freeze-dried slices were embedded in paraffin under vacuum. The freeze-substituted sections were transferred to benzene, benzene–paraffin mixtures, and finally embedded in pure paraffin (Lüttge and Weigl 1965). Sections were exposed to film in a deep-freeze for 3 weeks. The third technique consisted of removing the bladders of young leaves with Sellotape. Young leaves were placed in 90 mM $K^{36}Cl$ for 10 hr at 25°C in the light. The leaves were then placed on a strip of Sellotape which was gently pushed against both surfaces and removed with a large number of adhering bladders and their stalk cells. This was immediately stuck to a slide covered with stripping film and exposed in a deep-freeze for 2 months. The Sellotape was removed before development. Kodak AR-10 stripping film was used in all experiments.

Quantitative measurements of the silver grain density in different areas of these autoradiographs were made in 6·8 μ diameter fields with transmitted light (440 mμ) using a Leitz microdensitometer (MPE). Background density on unexposed areas of film was taken as 100% transmission and extinction calculated from percentage transmission measurements of developed areas. Extinction so measured is proportional to the radioactivity present (Läuchli and Lüttge 1968). Each value given is the mean of 10–20 separate measurements.

(*d*) *Measurement of Electrical Potentials*

Leaf slices were floated on a well-stirred bathing solution (5 mM KCl, 0·5 mM $CaSO_4$). A glass microelectrode, filled with 0·3M KCl, was inserted into a bladder and the electrical potential measured with respect to the bathing solution (Scott, Gulline, and Pallaghy 1968). Insertion of the electrode in air was accomplished more readily than when the whole tissue was submerged and this method preserved the natural relationship between the bladder and other cells. Microscope lamp illumination with a heat filter was used in all light-response experiments.

(*e*) *Preparation of Electron Micrographs*

Slices of young leaves from plants grown in water culture solution as in Section II(*a*) plus 5 mM NaCl (low salt) and 250 mM NaCl (high salt) were fixed for 2 hr in 0·5% OsO_4 buffered with veronal acetate (pH 7·2). Sucrose to 0·2M was added to the fixative. The leaf slices were dehydrated in a graded acetone series (25%, 50%, 75%, 3 changes in 100%) and embedded in Araldite. The Araldite was polymerized at 60°C for 2 days.

Fixation of the highly vacuolated bladder cell presents special difficulties and in other experiments glutaraldehyde fixation was used. Young leaves taken from soil-grown plants, which had been treated daily with 50 mM NaCl for 1 week, were fixed in 2% glutaraldehyde–0·1M phosphate (pH 7·1) for 17 hr at 4°C. The material was rinsed in 0·1M phosphate–0·1M sucrose for 1 hr and transferred to the same solution, containing 1% OsO_4, for 90 min at room temperature. It was then dehydrated in an ethanol series, washed in propylene oxide, and embedded in Araldite.

Thin sections were cut with a Si-Ro-Flex or LKB Ultramicrotome using glass knives and mounted on 200-mesh specimen grids coated with a carbon-stabilized Parlodion–amyl acetate film. Some sections were stained with lead citrate (Reynolds 1963). Sections were observed using a Siemens Elmiskop 1A or an AEI model 6 electron microscope.

III. Results and Discussion

(*a*) *Distribution of Ions*

The distribution and concentration of ions, estimated on a water content basis, in young *A. spongiosa* leaves (1–2 cm length) is shown in Table 1. These data were obtained from leaves which had formed during 2 weeks' growth on water cultures containing 5, 50, and 250 mM NaCl and each value is the mean of four replicates of at least 20 leaves each. The ion concentration in bladders and lamina increased with external concentration and bladders always contained a higher concentration of sodium and chloride than the external solution. In experiments with *A. inflata*, *A. vesicaria*, and *A. nummularia*, a similar distribution of ions was found. The ion concentration of bladders and lamina increased with age and, because bladders of old leaves contained less water, estimated concentrations of sodium and chloride reached 2·7 and 1·8M respectively. The K : Na ratio of bladders always reflected that in the leaf rather than the external solution but the bladder usually contained a higher proportion of sodium.

Table 1

Ionic distribution and ion concentration in bladder and lamina of young *A. spongiosa* leaves removed from plants grown in saline culture solutions

External Ion Concn.	Tissue	Ion Distribution (m-equiv/g total dry wt.)			Ion Concentration (M)	
		Na^+	K^+	Cl^-	Na^+	Cl^-
5 mM NaCl	Bladders	0·73	0·52	0·10	0·30	0·04
	Lamina	0·68	0·76	0	0·08	0
50 mM NaCl	Bladders	1·52	0·35	0·85	0·63	0·35
	Lamina	1·26	0·31	0·36	0·15	0·04
250 mM NaCl	Bladders	2·44	0·25	1·72	1·02	0·72
	Lamina	2·37	0·30	1·08	0·28	0·13

In annual species such as *A. spongiosa* and *A. inflata*, the chloride concentration in the lamina increased markedly with increased external levels (Fig. 1). This was accompanied by a 40% increase in lamina thickness due to hypertrophy of mesophyll cells. Perennials such as *A. vesicaria* and *A. nummularia* contained higher lamina chloride concentrations, but this did not increase with external concentration. In both cases the chloride concentration in bladders increased markedly with increased external concentration. These data suggest that the secretion of ions to the bladder may be important in maintaining low mesophyll salt status, particularly in rapidly growing species.

(*b*) *Transfer of Ions*

The uptake of labelled chloride by lamina and bladders was studied in the light and dark. Figure 2 shows the time-course of ^{36}Cl uptake from 5 mM $K^{36}Cl$ solution at

25°C. Uptake in the lamina in the light was linear for 70 hr and for the first 20 hr was identical to uptake in the dark. Lamina uptake in the dark subsequently declined. These data show that light was unlikely to be an important factor for ion uptake in leaf slices in the short-term experiments described earlier (Osmond 1968).

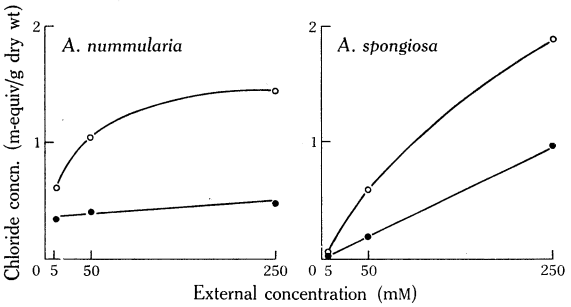

Fig. 1.—Concentration of chloride in bladders (○) and lamina (●) of leaves from two *Atriplex* species grown in culture solutions containing NaCl.

For the bladders, a very different time-course was found (Fig. 2). Very little label was transferred in the first few hours. Between 15 and 40 hr after the start of the experiment, light stimulated the rate of chloride uptake to the bladders about 8–10-fold, but thereafter the rate declined. In the dark, transfer to the bladders continued at a slow, more or less steady rate throughout.

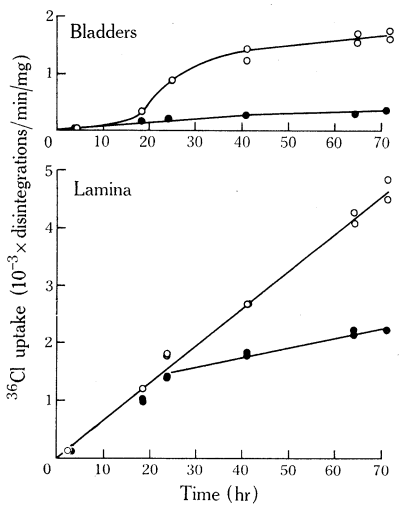

Fig. 2.—Time course of ^{36}Cl uptake to lamina and bladders of *A. spongiosa* leaf slices in 5 mM K^{36}Cl at 25°C in the light (○) and dark (●).

Figure 3 shows the result of another experiment in which low (0.25 mM) and high (5 mM) KCl concentrations were used. The specific activity is identical in both experiments so transfer rates are comparable. At these two concentrations, which represent system 1 and system 2 levels of Epstein (1966) and Osmond and Laties (1968), the pattern of ion transfer to the bladders was substantially similar to that shown in Figure 2. There was a pronounced lag in chloride uptake to cells of the lamina at the low concentrations, similar to that found in beet disks (Osmond and Laties 1968). The leaves used to provide material for the experiments in Figure 3

were much younger than those used for the experiment of Figure 2. In these and other experiments, light stimulated uptake to the bladder much more effectively in young leaf tissue.

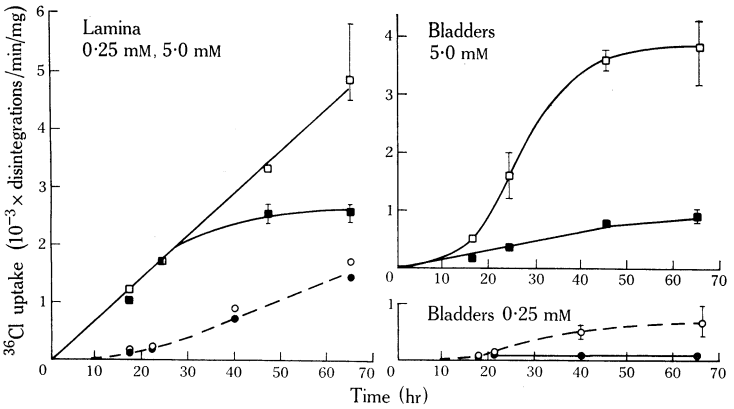

Fig. 3.—Time course of ^{36}Cl uptake in lamina and bladders of *A. spongiosa leaf* slices in 5 mM K^{36}Cl in light (□) and dark (■) and in 0·25 mM K^{36}Cl in light (○) and dark (●). Vertical bars indicate standard deviation of the mean.

The specific activity of chloride in the lamina as a whole may be estimated very approximately from the data of Table 2 and Figures 2 and 3. After 42 hr in the light the lamina specific activity approached that of the external solution. Assuming

TABLE 2

CHLORIDE CONCENTRATION IN THE LAMINA AND BLADDERS OF *A. SPONGIOSA* LEAF SLICES

Slices were treated in 5 mM KCl + 0·5 mM $CaSO_4$ in the light or dark at 25°C. The calculated Nernst potential for passive chloride distribution (E_{Cl}) under these conditions and the observed mean resting potential (E) are also shown. Chloride concentration (Cl_i) expressed as μ-equiv. chloride/g fresh wt.; E expressed as mV ± standard deviation, the number of experiments being shown in parentheses

Tissue	Pretreatment	Cl_i	Cl_o/Cl_i	E_{Cl}	E
Lamina*	Initial	10·2	0·49	+18	—
Bladders†	Initial	26·2	0·19	+43	−89 ± 23 (14)
Lamina	42 hr light	15·0	0·33	+26	—
Lamina	42 hr dark	8·5	0·59	+14	—
Bladders	42 hr light	33·8	0·15	+49	−105 ± 3 (10)
Bladders	42 hr dark	18·3	0·27	+33	−83 ± 13 (4)

* Chloride concentration is the mean from three samples of four standard slices.

† Chloride concentration of bladders measured from a single sample prepared from 60 standard slices.

this, the light-stimulated flux of ^{36}Cl to the bladder is of the order of 1 μ-equiv/g/hr in 5 mM KCl. Variation between batches of material precludes more exact calculation

at present, but this flux is of the same order as that measured for chloride influx in *A. spongiosa* mesophyll cells and in bean roots (Osmond 1968; Scott, Gulline, and Pallaghy 1968).

(*c*) *Electrical Potential Measurements*

When slices of young *A. spongiosa* leaves were mounted in a flowing solution of 5 mM KCl and 0·5 mM $CaSO_4$, and microelectrodes were inserted in the vacuole of the bladder cell, tracings of electrical potential difference between bladder vacuole and solution similar to those shown in Figure 4 were found. The tracing in Figure 4(*a*) shows three important features. Firstly, the potential obtained was always electronegative (−60 to −110 mV) with respect to the external solution. Secondly, when the tissue was switched from light to dark or dark to light, immediate transient changes in the potential were observed. A switch from dark to light resulted in a transient increase to a less negative potential followed by a more negative potential.

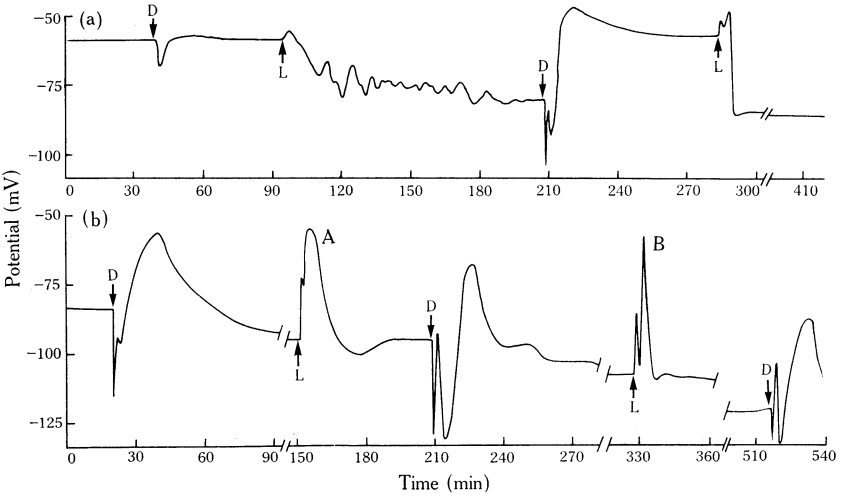

Fig. 4.—Tracings of electropotential between vacuole of bladder cell of *A. spongiosa* leaves and external solution. *L*, Light on. *D*, Light off.

Switching from light to dark produced an immediate transient more negative potential followed by a less negative potential. The subsequent slow transient of smaller amplitude was more negative in the light than in the dark for at least 1 hr after the rapid transient. Finally, cells occasionally showed a permanent alteration in potential after a change in illumination, the potential being more negative in the light.

Figure 4(*b*) shows a second type of potential response found in some cells. In these the resting potential became more negative throughout the experiment and no significant differences between light and dark were observed. The form of the rapid transient was unchanged, however. Figure 4(*b*) also shows an interesting effect of light intensity, which was manipulated by power supply to the microscope lamp, on the length of the transient response. Light transient *A* was obtained with the lamp supply set at 5 A and light transient *B*, of much shorter duration, with 6 A.

Diphasic transients in response to light, shown in Figure 4, have been observed between single green cells of moss leaves and their bathing solution (Lüttge and Pallaghy, unpublished data). Thus the complex transient is not necessarily a product of the multicellular system between the electrode inserted in a bladder and the solution bathing the leaf slice. Mean values for the electrical potential between bladders and the bathing solution are shown in Table 2, together with the chloride concentration found in bladders and lamina during an experiment of the type shown in Figures 2 and 3. Also shown in this table are the calculated values for the potential between bladder and solution which would be expected if chloride were passively distributed in this tissue, according to the Nernst equation (Dainty 1962). Strictly, the use of the Nernst equation is valid only if there is near flux equality. Table 2 shows that there is net flux into the bladder in the light and outwards in the dark. However, the fact that there is a net influx of chloride against an electrical gradient in the light shows at once that chloride movement to the bladder is unlikely to be a passive process. These data, together with the demonstration that chloride is transported against a chemical concentration gradient by light-stimulated processes, implicate an active chloride pump between the bathing solution and the vacuole of the bladder.

Preliminary experiments show that the electrical potential between the vacuole of mesophyll cells and the bathing solution is similar in sign and magnitude to that found for bladders. For passive distribution of chloride between mesophyll cells and the bladders, the only requirement is that bladders be about 15 mV more positive than mesophyll cells. Such a difference would be difficult to detect experimentally because of the large variation between the measured potential in individual bladders. It is impossible to further define the site of active anion uptake at this stage.

(*d*) *Autoradiography and Electron Microscopy*

Microautoradiographs of sections and intact bladders confirmed the transfer of label to this tissue and provided some indication of the distribution of label between the bladder and the stalk cell. Figure 5 shows a small segment of an *A. spongiosa* leaf with several bladders, photographed from above. The magnification of this photograph is exactly half that of Figures 6–10 and it thus serves as a guide to size. Figure 6 shows an autoradiograph of a *Chenopodium* bladder after $^{35}SO_4^{2-}$ uptake for 7 hr with the bladder still in position. Quantitative measurements of the distribu-

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radioactivity is more concentrated in the folds of the collapsed bladder (arrow). Radioactivity was also concentrated in the cytoplasm of a stomatal guard cell (*S*). ×540.

Fig. 7.—Transmitted light photograph of autoradiograph prepared from *A. spongiosa* bladder and stalk cell removed by the Sellotape technique. Radioactivity is concentrated in the stalk cell and cytoplasm of bladder (arrow). ×570.

Fig. 8.—Incident light photograph of bladder shown in Figure 7. The highly reflective areas (*W*) are waxy particles from the bladder and the more diffuse areas (arrow) correspond to the silver grains appearing black in Figure 7. ×570.

Figs. 9 and 10.—Autoradiographs of successive sections through an epidermal structure of *A. spongiosa* leaves. This corresponds in shape and size to a bladder and shows the concentration of radioactivity in stalk cell and peripheral cytoplasm of the bladder. Both ×570.

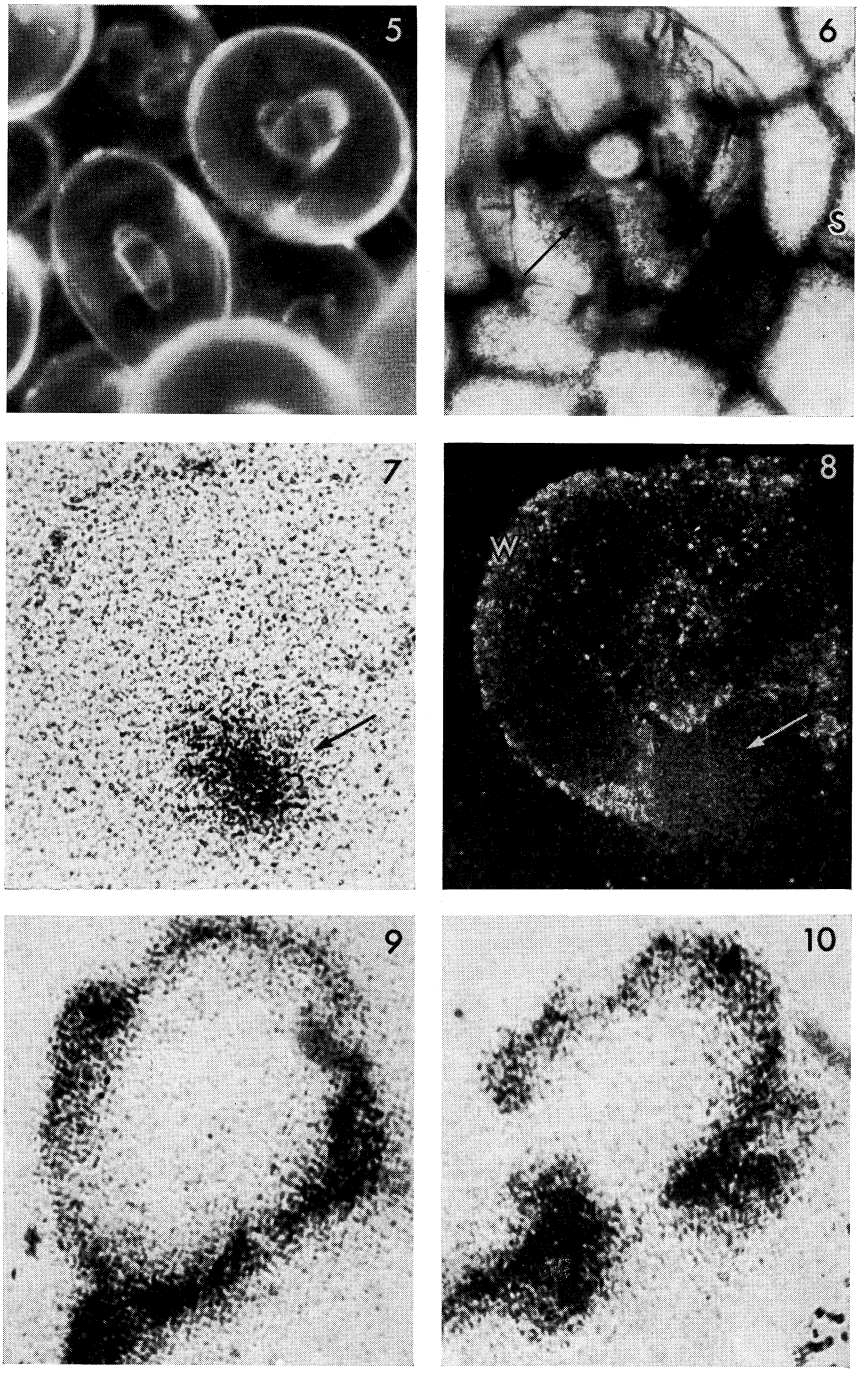
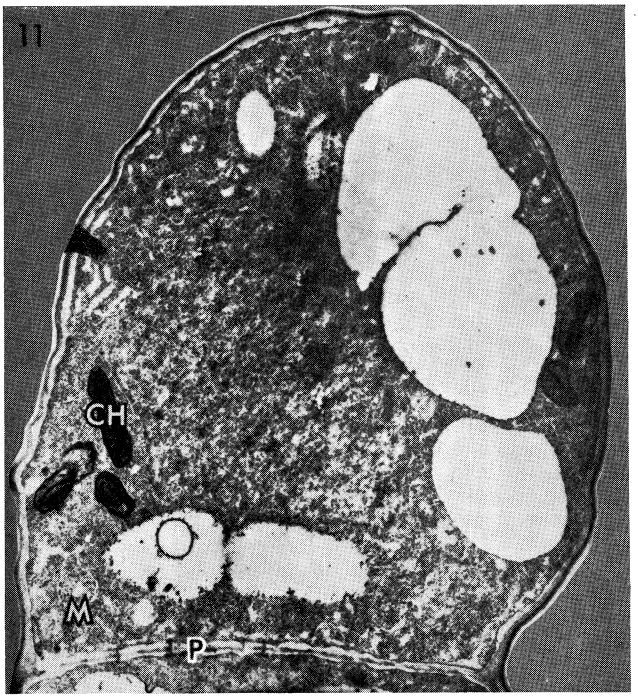


Fig. 5.—Surface view of bladders of young *A. spongiosa* leaf. ×270.

Fig. 6.—Transmitted light photograph of collapsed *Chenopodium* bladder and corresponding autoradiograph prepared by the surface-stripping technique. Silver grain density shows that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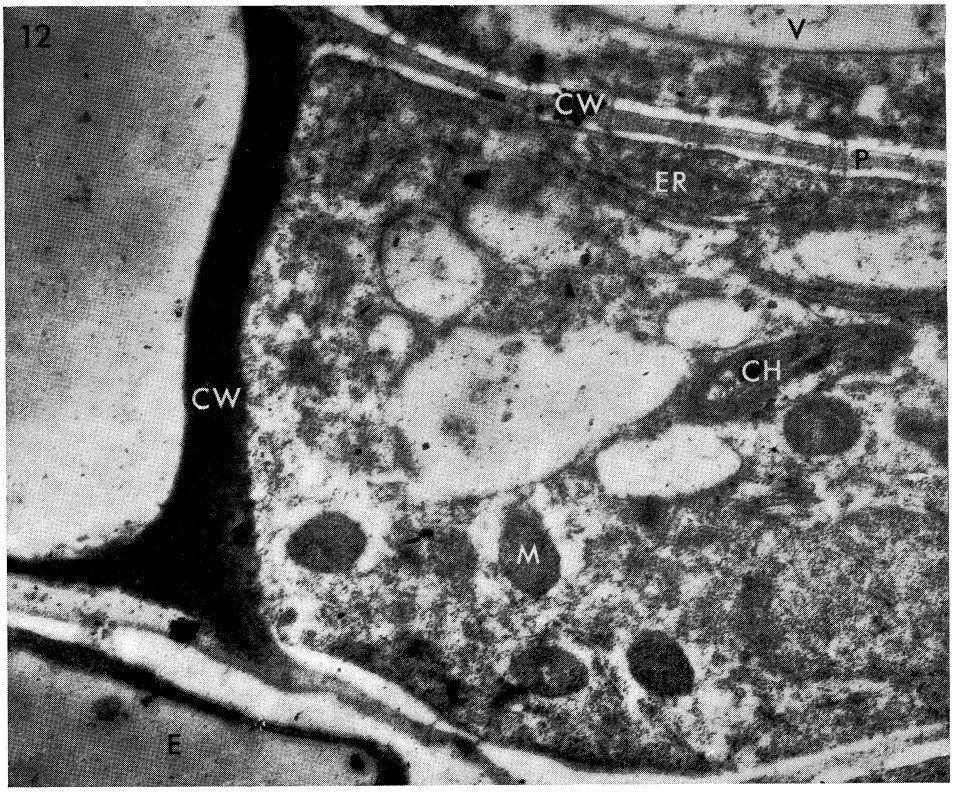
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tion of label in such pictures, prepared by the surface-stripping technique, are given in the following tabulation (arbitrary units, duplicate experiments):

Epidermal Cells (vacuole)	Stomates	Folded Areas of Bladder	Bladder
0·01	0·49	1·44	0·40
0·13	0·67	1·77	0·40

These data show that label in the region of the bladder is not due to contamination from epidermal cells. The 3–4 times intensification of the image due to folding of the bladder (arrow, Fig. 6) suggests that label may be concentrated in a thin cytoplasmic lining. This is consistent with the higher concentration of label in the densely cytoplasmic stomatal guard cells adjacent to the bladder (*S*, Fig. 6).

When bladders were removed from *A. spongiosa* leaves with Sellotape and fixed to film after 10 hr in 90 mM $K^{36}Cl$, a clearly defined image was obtained. Figure 7 shows the circular areas of radioactivity corresponding in dimensions to the bladders, and smaller, more concentrated areas of label within these. The concentrated area (arrow) has dimensions similar to those of the stalk cell of young bladders. Figure 8 is an incident light photograph of the same field as Figure 7. The larger and more or less irregularly shaped bright particles in this photograph (*W*) are wax from the bladder surface which has adhered to the film. These particles show the outline of the collapsed bladder, and comparison with Figure 7 shows that the large circular area of radioactivity corresponds to the bladder. The small and regular bright areas of Figure 8 represent reflecting silver grains which appear black in the transmitted light of Figure 7. The distribution of radioactivity in the different areas of the bladder and the label surrounding the bladders was as follows (arbitrary units, four experiments):

Stalk cell	0·64, 0·62, 0·66, 1·05
Bladder	0·17, 0·22, 0·18, 0·24
Between bladders	0·01, 0·02, 0·07, 0·06

The concentration of radioactivity in the stalk cell suggests that it may play an important role in the secretion of ions to the bladder proper. The label between the bladders was significantly higher than background and presumably leaked from the bladders during preparation.

Autoradiographs prepared from sectioned material gave less satisfactory results. Leaf slices were supplied with 0.1 mM $^{35}SO_4^{2-}$ for 4 hr. Usually the bladders were lost from sections during preparation. However, some sections showed radio-

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Fig. 11.—Electron micrograph of a developing bladder cell. Note the chloroplasts (*CH*) and mitochondria (*M*) in the cytoplasm. Several cytoplasmic vacuoles are seen in the bladder cell, and plasmodesmata (*P*) connecting the bladder cell to the stalk cell can be seen in the wall between the two cells. × 8,000.

Fig. 12.—Section through a stalk cell and bladder cell on a leaf from *A. spongiosa* plants grown on low-salt water culture. The stalk cell, as well as containing mitochondria (*M*), chloroplast (*CH*), and endoplasmic reticulum (*ER*), has an external cell wall (*CW*) which stains darkly, as does the cuticle over the epidermal cell (*E*). Some plasmolysis has occurred along the cell wall between the bladder cell and the stalk cell, thus making the plasmodesmata (*P*) clearly visible. The cytoplasm of the bladder cell and the large central vacuole (*V*) are also shown. × 20,000.

activity in partially intact bladders, and Figures 9 and 10 show two successive sections through an epidermal structure presumed to be a bladder. Radioactivity is concentrated in a thin circular band of cytoplasm and in the stalk cell, supporting the deductions from autoradiographs prepared by different methods.

Figures 11–17 show electron micrographs of the stalk cell–bladder complex. Figure 11, prepared from a very young leaf by the glutaraldehyde method, shows a developing bladder cell and a small portion of the stalk cell. At this stage the bladder cell contains several distinct vacuoles together with chloroplasts, mitochondria, a nucleus, and some endoplasmic reticulum. The cell wall between the bladder cell and stalk cell is penetrated by numerous plasmodesmata. Figure 12 shows portion of an older stalk cell and the lower part of the bladder cell which now contains a single large vacuole (see Fig. 13). Again the connecting plasmodesmata are obvious, as is the densely staining material of the side walls of the stalk cell. The stalk cell is relatively small and is packed with small vacuoles, mitochondria, and endoplasmic reticulum. Although this cell was connected to the epidermal cell underneath by plasmodesmata, similar connections between other leaf cells were not so apparent. Substantially similar organization was observed in sections of *A. inflata* and *A. vesicaria* leaves.

When plants were grown in high-salt water cultures some changes in ultrastructure were observed. Figures 13–15 show the organization of the stalk cell from leaves of high-salt plants. The low magnification of Figure 13 shows the typical appearance of the two-cell complex with prominent chloroplasts and mitochondria in the bladder cell cytoplasm. In Figures 14 and 15 an extensive development of endoplasmic reticulum and small vesicles in the stalk cell is evident. Figures 16 and 17 show that this proliferation of membranes and vesicles is particularly marked in the cytoplasm of the bladder cell, and golgi bodies are clearly defined. These structures were not found when leaves of salt-treated plants (soil-grown) were fixed by the glutaraldehyde technique. Consequently, much further critical and quantitative work is required to assess the role of the proliferation of endoplasmic reticulum, golgi bodies, and small vesicles in relation to increased salt uptake to the bladder vacuole. The concentration of radioactivity in stalk cell and bladder cell cytoplasm (Figs. 5–10) suggests an association of these structures with the ion transfer process.

IV. Conclusions

These experiments confirm the transfer of significant quantities of salt to the bladders of *Atriplex* leaves (Charley 1959) but do not, at present, relate this to overall salt balance in leaf tissues. Young leaves appear to secrete chloride to the bladder more readily than old leaves and young leaves are the first to sustain obvious damage

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Fig. 14.—Section through epidermal cell (*E*), stalk cell (*S*), and bladder cell (*BL*). Plasmodesmata connect the epidermal cell to the stalk cell with its mitochondria (*M*) and cytoplasmic vesicles (*CV*) and the stalk cell to the bladder cell. *V*, vacuole. ×2,100.

Fig. 15.—Higher magnification micrograph of the stalk cell. The endoplasmic reticulum (*ER*) appears to be well developed and may contribute to the formation of cytoplasmic vesicles (*CV*) in the stalk cell. There are many more extremely small vesicles in the cytoplasm of the bladder cell which may be formed as a result of golgi body (*G*) activity. Other symbols as defined previously. ×8,000.

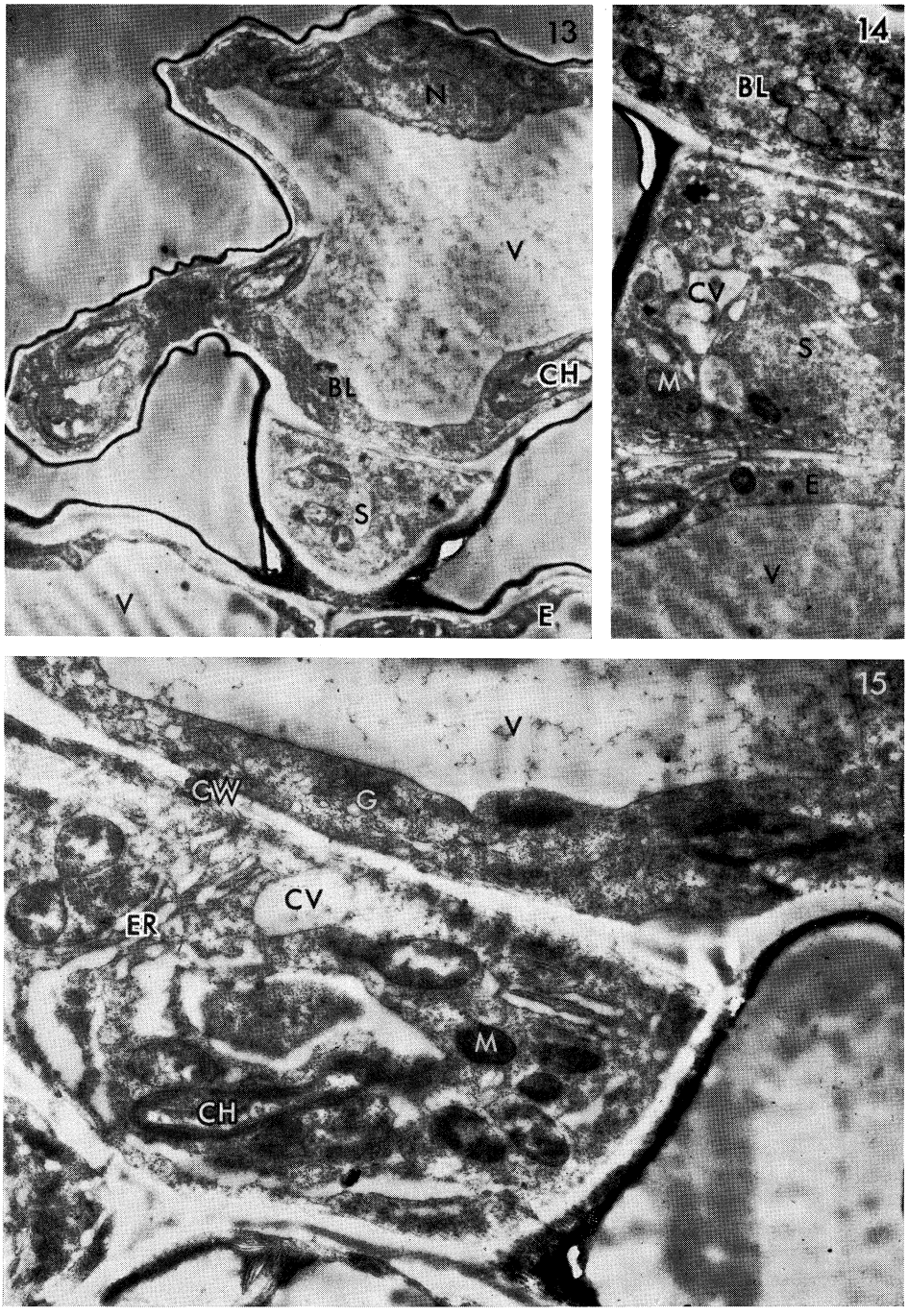

Fig. 13.—Low-power electron micrograph of an entire *A. spongiosa* bladder showing epidermal cells (*E*), stalk cell (*S*), and bladder cell (*BL*). The large central vacuole (*V*) and thin layer of cytoplasm containing numerous chloroplasts (*CH*), mitochondria, and a nucleus (*N*) are separated from the epidermal cells by the small, densely cytoplasmic stalk cell. ×2,100.

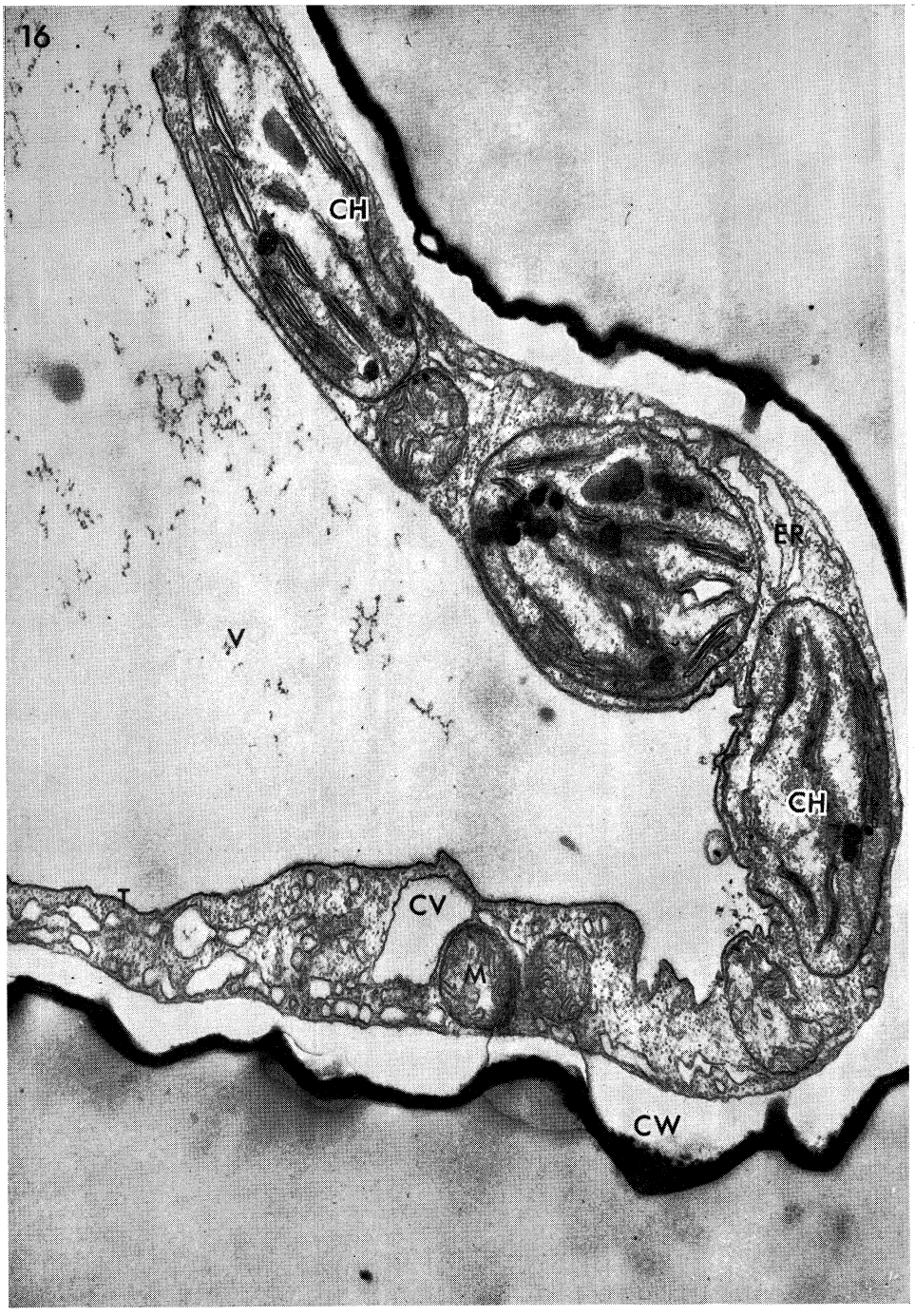

Fig. 16.—Electron micrograph showing the vacuole (*V*), cytoplasm, and cell wall (*CW*) of a mature bladder cell of *A. spongiosa* from high-salt water culture. Mitochondria (*M*), chloroplasts (*CH*) endoplasmic reticulum (*ER*), and cytoplasmic vesicles (*CV*) clearly seen. *T*, tonoplast. ×22,500.

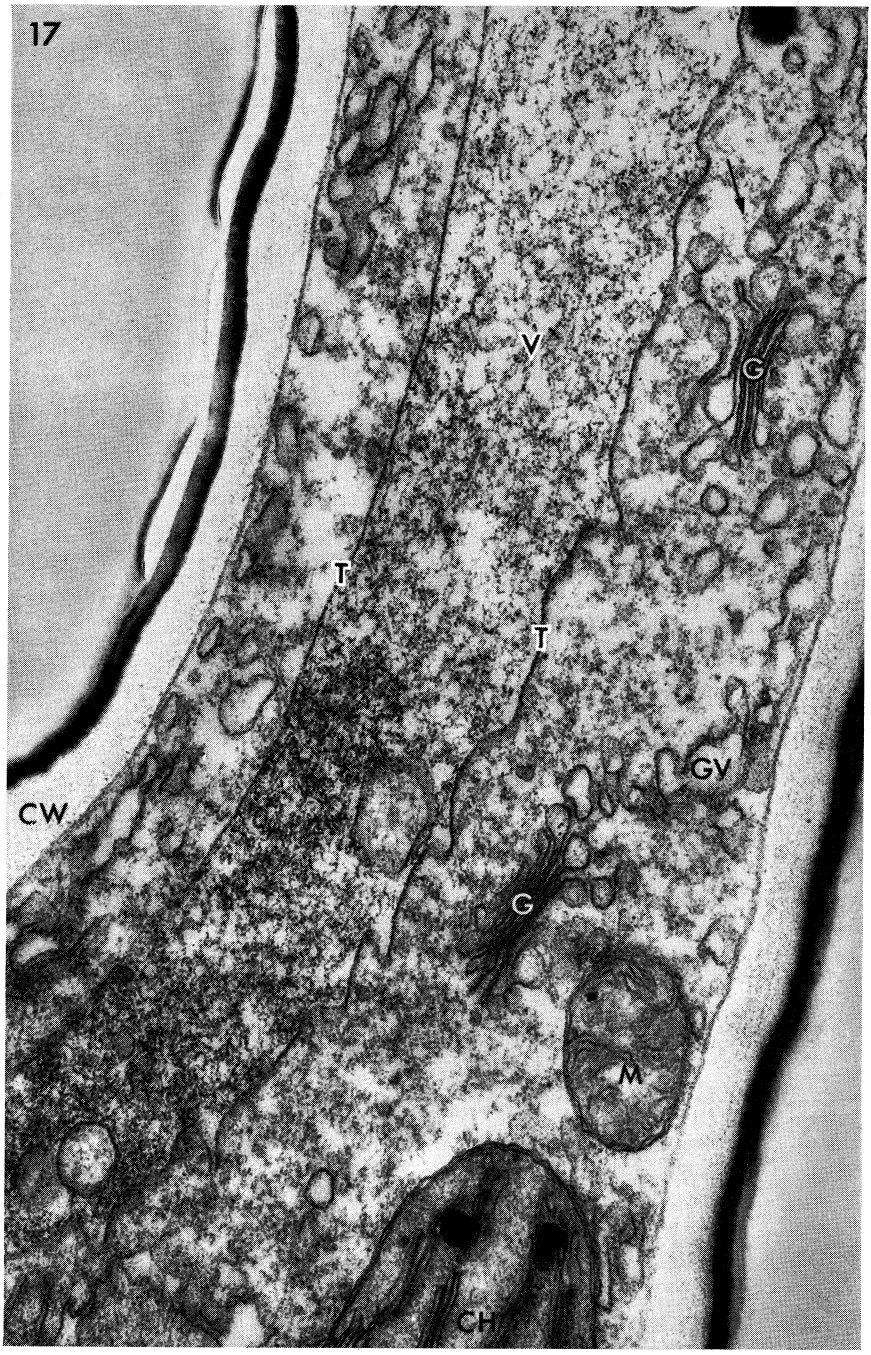

Fig. 17.—Portion of the cytoplasm and vacuole (*V*) from a mature bladder cell of *A. spongiosa* from high-salt water culture. The bladder cell has been compressed during preparation but the fixation of the cytoplasm with golgi bodies (*G*), golgi vesicles (*GV*), chloroplasts (*CH*), and tonoplast (*T*) is good. The arrow indicates the possible fusion of a golgi vesicle with endoplasmic reticulum. ×40,000.

under extremely saline conditions (Osmond 1963). The characteristic gland-like structure of the stalk cell is found only in young leaves, and the stalk cell elongates and may divide in older leaves (Black 1954; West, unpublished data). By the time leaves are large enough to be manipulated in tracer flux experiments it is possible that only a small proportion of the bladders are fully functional. Consequently the chloride flux rates measured may be much underestimated. The leaves of *A. spongiosa*, an annual species, produce only one crop of bladders when grown in water culture. Perennials such as *A. nummularia* produce many crops of bladders per leaf when growing under field conditions (Black 1954) and may thus continue to secrete salt from the lamina. These and other factors must be taken into account in assessing the real significance of the secretion process. In particular, experiments with high levels of chloride and with different species would be useful.

The experiments do show that chloride is actively transferred to the vacuole of bladders from the solution bathing mesophyll cells. The evidence may be summarized as follows:

(1) Chloride is transferred to bladders against a concentration gradient.

(2) As the electrical potential of the bladder vacuole is about 85 ± 20 mV more negative than the bathing solution the transfer of chloride must be active.

(3) The transfer of chloride and the electrical potential are very sensitive to light, suggesting that photosynthesis may be a source of energy for the active uptake.

Similar criteria have been used to describe the active secretion of salt from *Limonium* leaves (Arisz *et al.* 1955; Hill 1967*a*, 1967*b*). There are at least three sites at which the active step in the transfer of chloride to the bladder may occur in the multicellular system. These are shown diagrammatically in Figure 18. Active transfer of chloride across the plasma membrane of mesophyll cells (site 1), followed by diffusive movement through the symplasm to the bladder vacuole, is consistent with the above data. However, in other tissues, plasma-membrane-mediated active uptake is found only at very low concentrations (Lüttge and Laties 1967; Osmond and Laties 1968). Diffusive entry of chloride from 5 mM KCl into mesophyll cytoplasm is likely. Active chloride uptake to the vacuole of mesophyll and bladder cells from the symplasm would also be consistent with the data presented above. In mesophyll cells this has been indicated as a property of the tonoplast (site 3, Fig. 18), and similar events may occur at the bladder cell tonoplast (site 2*a*, Fig. 18).

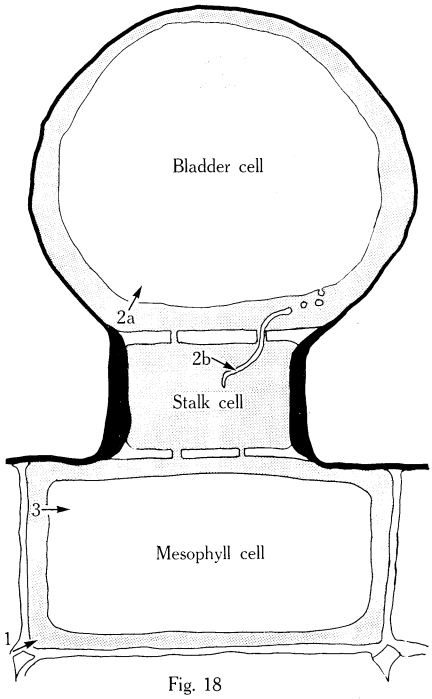

Fig. 18

However, the electron microscopic and autoradiographic data suggest an alternative system. The complex structure of stalk cell and bladder cytoplasm and the concentration of radioactivity in these areas are probably associated with ion transfer to the bladder vacuole. Thus site 2*b*, Figure 18, shows an active transfer of chloride to the membrane system of stalk cell cytoplasm. These membranes appear to pass from stalk cell to bladder cytoplasm in some sections (Fig. 12) and may give rise to the many small vesicles found in the bladder cytoplasm (Figs. 16 and 17). Fusion of these vesicles with the bladder tonoplast could effect active chloride transport from cytoplasm to vacuole. This sequence of events is similar to that proposed by Matile and Moor (1968) for the movement of proteins from cytoplasm to vacuole in maize root tip cells, and is not new to discussion of ion transport (Lüttge and Krapf 1968; Robertson 1968). The endoplasmic reticulum has been implicated in ion transfer to the vacuole of beet cells (Jackman and Van Steveninck 1967) and small vesicles have been reported commonly in gland cells (Ziegler and Lüttge 1966; Atkinson *et al.* 1967; Thompson and Liu 1967).

The submicroscopic structure of the stalk cell of the *Atriplex* bladder is strikingly similar to that of the cells in multicellular salt glands of *Limonium* (Ziegler and Lüttge 1966), *Aegialitis* (Atkinson *et al.* 1967), and *Tamarix* (Thompson and Liu 1967; Shimony and Fahn 1968). Gland cells are characteristically connected to adjacent cells by numerous plasmodesmata and otherwise isolated by heavily cutinized walls. An obvious analogy may be drawn with the Casperian strip of the root endodermis. In both instances this organization evidently precludes the diffusive passage of ions via the free-space. The stalk cell of the *Atriplex* bladder may draw ions from the leaf symplasm and pass them to the bladder vacuole. The reality of this analogy is being examined.

The light stimulation of active chloride uptake to the bladder and mesophyll cells may be dependent on chloroplasts in mesophyll, stalk cell, and bladder cytoplasm. As bladder uptake is much more light sensitive than mesophyll uptake, the two events may be independent. The 10-fold stimulation of uptake to the bladder in the light is comparable with the light effect on ion fluxes in giant algal cells and greatly exceeds light effects previously reported in higher plant mesophyll cells (Rains 1968). Salt secretion from *Limonium* leaves is light stimulated but the glands contain no chloroplasts (Arisz *et al.* 1955; Ziegler and Lüttge 1966). There is no direct effect of light on salt secretion in *Aegialitis* (Atkinson *et al.* 1967). Further work is in hand to determine if this stimulation of chloride flux is mediated by photosynthetic energy production or a direct effect of light on membrane characteristics.

So far, the experiments reported do not show the unusually rapid salt fluxes reported in some other glands (Atkinson *et al.* 1967). The results suggest that *Atriplex* leaves secrete salts to the bladder at rates similar to vacuolar uptake in mesophyll cells. By transporting salt to the epidermal bladder rather than to the vacuole of mesophyll cells, salinity stress in the photosynthesizing tissues may be avoided to some extent. Accumulation of much chloride in the lamina is accompanied by hypertrophy of mesophyll cells and increased leaf thickness (Black 1958). The stalk cell of the bladder thus represents a simple and less specialized gland compared with that found in other species. This comparatively simple system is very suitable

for further experiment, and may be a useful system in which to study aspects of symplastic transport and light-dependent ion uptake.

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