II STRUCTURE AND FUNCTION OF SALT GLAND - A REVIEW

Salts from substratum are continuously transported into plant shoots via the transpiration stream. In plants growing under saline habitat salt accumulation may eventually reach a hazardous level and survival of plants may depend on reduction of salt content of the shoot. This reduction of salts in the shoots is done by salt glands.

Secretion of ions by special salt glands is the best known mechanism for regulating mineral content of plant shoot. Salt secretion is a common phenomenon in various halophytic plant genera. It is found in the following genera of terrestrial halophytes Cressa - Convolvulaceae, Frankenia - Frankeniaceae, Spartina, Chloris, Aeluropus - Gramineae, Statice, Limonium, Plumbago, Armeria - Plumbaginaceae, Glaux - Primulaceae, Tamarix, Reaumuria - Tamaricaceae. In mangrove species it is found in Acanthus - Acanthaceae, Avicennia - Avicenniaceae, Laguncularia - Combretaceae, Aegiceras - Myrsinaceae and Aegialitis - Plumbaginaceae (Mullan, 1931; Walter and Steiner, 1937; Chapman, 1944; Liphschitz and Waisel, 1974).

In all known cases, salt is secreted from well defined salt glands located on or sunken into the epidermis. Although structure of salt gland varies greatly in different plants, it is strikingly similar in plants of same genus or even within one family.

There is confusion between salt gland and hydathode. Hydathodes are recognized as structures of varying degrees of specialization which emit water and solutes to the surface of the plant. Haberlandt (1914) has divided hydathods into two functional types :

a. Passive hydathodes : Those having direct communication with the conducting system in which the secretion is a process of filteration under pressure.

b. Active hydathodes : Those not directly connected with conducting system. These are active in secretory process. Thomson (1975) has used the term salt gland for active hydathodes.

During last decade lot of literature has accumulated on structure and function of salt glands. In the begining of seventies (1971) Luttge has reviewed the glands in plants where he has discussed salt glands. Waisel (1972) while dealing with biology of halophytes has given an account of salt glands. Poljakoff-Mayber and Gale (1975) have also given a good account of salt glands. More recently, Fahn (1979) has reviewed the work on salt glands in great details.

The history of study on salt glands can be traced as far back as 1887, when Volken has reported salt glands of some desert plants.

Simplest type of salt gland is observed in Spartina (Skelding and Winterbotham, 1939) which is two celled. In Atriplex trichome like structure or bladder is present to excrete out excess salt. (Osmond et al. 1969). In Avicennia and Acanthus glands are composed of an indefinite number of cells, usually between 5 to 9 arranged in a group of 4 or more cells located on top of one stalk and 2 to 4 collecting cells. Ruhland (1915) has stated that in Static the salt gland is composed of a complex of 16 cells which contain dense cytoplasm large nucleus, and many small vacuoles. Adjacent to the gland and situated interior are four large, highly vacuolated cells. Ruhland has termed these cells as collecting cells. Except for the walls between the innermost gland cells and the collecting cells, identified by Ruhland as a transfusion zone, the gland is encapsulated by a thick cuticular layer containing small pores in its outer mantle. Skelding and Winterbotham (1939) have reported that in Spartina gland consists of only two cells, a small outer cap cell positioned on a neck-like protrusion of a large

basal cell. Both cells contain granular cytoplasm. They have reported presence of pits in walls between the basal cell and mesophyll cell and in walls connecting the basal cell with the epidermal cells. Cells of salt glands differ in many respects from the surrounding epidermal or parenchymatous cells. Salt gland cells lack a central vacuole and the number of mitochondria and other organelles is extremely high.

A. Ultrastructure

The salt glands have been observed under electron-microscope in case of Avicennia, Acanthus and Aegiceras. At the top of the gland the cuticle is traversed by many narrow pores in case of the salt glands of A. marina (Shimony et. al (1973). The cuticle is frequently separated from the walls of the secretory cells along the outer surface of the gland creating a large generally electron transparent cavity or collecting compartment between the secretory cells and the cuticle. The inner portion of the cuticle is often loosely organized along the summit of the cuticular cap and electron dense material is frequently observed in the irregular gaps in this region. The cuticular layer extends over the gland and inward along the lateral sides of the gland where it abuts with the walls of the collecting cells or

sub-basal cells. In most cases the walls of the secretory cells are not contiguous with walls of collecting and sub-basal cells. In many glands where the cell wall is absent along the lateral walls of the innermost gland cells or basal cells, an unusual interfacial structure occurs. In this region the cuticular material is more electron dense than the associated encapsulating layer and small fibrils are often observed in the cuticular matrix. In case of mangroves, the walls of the collecting cells or sub-basal cells do not appear to be cuticularized at any point in and near the transfusion zone (Poljakoff-Mayber and Gale, 1975).

Characteristically, numerous plasmodesmata occur in transfusion zone wall between the inner secretory cells and the collecting cells. The secretory cells of the gland are also interconnected by plasmodesmata.

In secretory cells of Limonimum, Tamarix etc. wall protuberances are seen. Occasional small protuberances are observed in Aegialitis, but they appear to be absent in the glands of Avicennia, Acanthus, Aegiceras. However, in Avicennia, the amorphous substance present between the cuticle and upper walls of secretory cells, which often occurs also on the inner surface of the side walls of the

secretory cells, may also play a role in the migration of the salt secreted from the protoplasts. The absence of these structures in some glands indicate that cell-wall protuberances are required for intensive short-distant transport.

As pointed out by several authors, the collecting cells are ultrastructurally very similar to the underlying mesophyll cells. They have large vacuoles often containing electron dense material, well developed chloroplasts and a peripheral cytoplasm with mitochondria and other organelles. Since the collecting cells or sub-basal cells are almost identical in appearance to mesophyll cells and are strikingly different from the secretory cells, except for location, it would seem they may have only a small role in the secretory process.

The main gland cells are characterised by a dense cytoplasm, many mitochondria, large nuclei, and small vacuoles, frequently with an electron-dense content. Variation in the ultrastructure of different cells within the gland has been reported and variation in the fine structure of the secretory cells exists between the different glands. Plastids occur in all secretory cells of the glands. They have little internal membrane development consisting mostly of small vesicles and some lamellae particularly in the peripheral

regions of the organelles. The plastids generally have a very dense stroma. In the outer secretory cells of Avicennia the plastid matrix is often completely opaque.

Osmond et. al. (1969), in case of Atriplex has reported that the gland is covered by a cuticular layer with the lateral walls of the stalk cell as completely cuticularized and/or suberized. Electron microscopic studies by Smaoui (1971) have indicated that these walls may not be entirely cuticularized and that they have an outer electron dense cuticular layer, a middle layer and an inner striated layer. Osmond et. al. (1969) and Smaoui (1971) have reported the presence of plasmodesmata in the wall between the epidermal cell and the stalk cell and in the wall between the stalk cell and the bladder cell. The stalk cell has numerous mitochondria small vacuoles and a dense cytoplasm. Osmond et. al. (1969) have stated that the stalk cell is packed with endoplasmic reticulum although this is not clearly evident in their micrographs. The stalk cells may have chloroplasts with a modestly developed internal membrane system of grana and interconnecting membranes.

In case of two celled salt gland of Spartina, Levering and Thomson (1971) have found that the external surface of the gland is covered by a thick cuticle, but the walls of the underlying basal cells are not cutinized. The cuticle is expanded outward over the cap

cell creating a cavity between the cuticular layer and the cell wall of the cap cell. Levering and Thomson (1971) have observed thin electron dense strands across the cuticle. Plasmodesmata occurre in the thick wall between the basal cell and cap cell as well as between the basal cell and the adjacent mesophyll cells. No plasmodesmata have been observed in the wall between the basal cell and the overlying epidermal cells. Wall protuberances bordered by the plasmalemma, extend into the basal cell from the wall between the basal cell and the cap cell. The nucleus of the basal cell is quite large. Plastids containing plastoglobuli, a dense stroma and a few peripheral vesicles and membranes are also present. The dominent features of the cap cell are a large nucleus and a dense cytoplasm containing mitochondria and plastids with plastoglobuli and a few internal membranes often aggregated into a hexagonal lattice.

B. Mechanism of salt excretion

Haberlandt (1894, 1918) has introduced the term hydathode for the structures secreting water in the liquid state. He has distinguished between epidermal hydathodes and epithem hydathodes, epithem being a parenchymatous tissue penetrated by intercellular spaces. From physiological point of view he has regarded epidermal hydathodes as active hydathodes. The epidemal

hydathodes secrete, together with water, ions and minerals. They are referred to as salt glands and chalk glands, depending on major constituent of the secreted solute (Metcalfe and Chalk, 1950). Thomson (1975) has introduced the term 'salt glands' for both these types.

Haberlandt (1914) has pointed out that active hydathodes or salt glands may be unicellular or multicellular as in Plumbago and Statice. The vesiculated hairs or bladders represent glands in Atriplex (Osmond, et. al. 1969). In Spartina salt gland is two celled (Skelding and Winterbotham, 1939). Five to nine celled gland is observed in Acanthus, Avicennia, Tamarix etc. In Statice gland is composed of complex of 16 cells (Ruhland, 1915) Campbell and Strong (1964) have reported that salt glands of Tamarix pentandra develop from a single protoderm cell and consist of six secretory cells. Salt gland of Acanthus also develops from single cell (Fahn, 1979).

Salt gland secretion shows variety of mineral elements. It includes cations Na, K, Ca, Mg and the anions Cl, So_4, Po_4, No_3 and HCO_3 (Thomson 1975).

Berry (1970) has presented a detailed analysis of the crystalized salt from Tamarix branches which indicated that Na, K, Mg and Ca comprised about 99% of

the total cations. Similarly NO_3, Cl, HCO_3 and SO_4 constitute 60% of the total. Small amounts of micronutrients have been also observed in the excreted fluid. Shimony and Fahn, (1968) have concluded that pectic material was present in the secretion from glands of Tamarix. Pollack and Waisel, (1970) have reported the presence of organic materials including free amino acids and proteins in the secretion of Aeluropus. Ruhland (1915), has reported an organic content of 20 - 40% in secretion of Statice.

The physiological aspect of ion secretion has been discussed by many authors (Ariz, et.al.1955; Helder, 1956; Hill, 1967 a & b; 1970 a & b; Luttge, 1969; Osmond et. al., 1969; Shachar - Hill and Hill, 1970; Waisel, 1972; Thomson, 1975; Hill and Hill, 1976). Cytoplasm, rich in ribosomes, large nuclei and the presence of numerous organelles, particularly mitochondria in the secretory cells, supports the view that the elimination of ions from the cytoplasm requires energy. This view is further substantiated by physiological observations such as differences in concentration of the secreted fluid compared to Xylem sap and root medium (Scholander et. al., 1962; Berry, 1970; Pollack and Waisel, 1970) electropotential studies (Hill, 1967 a & b; 1970 a & b) sensitivity to

temperature (Arisz et. al., 1955) and the effect of metabolic inhibitors (Atkinson et. al., 1967). According to Shimony et. al. (1973), a downhill salt gradient appears to exist in Avicennia leaves from near the Xylem to the gland and is continued through the gland itself, the secretory cells being lowest in salt content.

Arisz, 1956 and Helder (1956) have reviewed the structural and physiological studies of salt glands and concluded that due to the enclosure of the glands by a thick, impermeable cuticle apoplastic transport was not possible and only symplastic transport occured between the collecting cells and the innermost cells of the gland. This concept includes the idea that the cuticular material is functionally and morphologically analogous to the casparian strip in root endodermal cells (Luttge, 1971; Arisz, 1956; Helder, 1956). However, ultrastructural studies indicate that apoplastic transport to the glands can not be ruled out. Support comes from the facts that in Spartina the inner walls of the basal cell are free from cuticular material and are contiguous with the walls of the underlying basal cells; in Atriplex lateral walls of the stalk cells and the wall of the bladder cell do not appear entirely cuticularized.

The occurance of numerous plasmodesmata between the collecting cells and the inner or basal cells of the gland indicates symplastic transport of ions through the protoplasmic channels of the plasmodesmata which is of major importance. Another possible role for the plasmodesmata are passage ways for the movement of metabolic substrates to the glands (Poljakoff-Mayber and Gale, 1975). Presence of a large number of mitochondria in gland cells suggests that the gland cells are highly active and since they lack well developed chloroplasts, except possibly in Atriplex (Osmond et. al., 1969), they must be dependent on adjacent tissues for metabolic substrates.

Ziegler and Luttge (1967) have studied the electron microscopic localization of chloride in the leaves of Limonium. They have reported the presence of chlorides in the plasmodesmata between the inner gland cell and the collecting cells. This supports the concept that the plasmodesmata are involved in the passage of ions to the gland. Since the apoplastic route to the gland is not blocked by cuticular material and the certainty of the localization of chloride in the plasmodesmata is still unresolved the question of how ions move to the glands, whether through the apoplast, symplast or both, still not answered (Thomson, 1975).

C. Passage of the salt out of the glands

Ruhland (1915) has observed pores in the outer cuticle covering the glands of Statice. Ziegler and Luttge (1967) have reported the presence of heavy chlorides in the pore regions both under and on the outer surface of the cuticle. These observations leave little doubt that the passage of salt out of the glands, particularly of Limonium type is via cuticular pores.

A presence of large cavity, collecting cavity, between the cuticle and the underlying secretory cells suggests that secretion occurs under pressure. Similar cavities have been observed in other types of glandular trichomes and hairs (Esau, 1965). In Abutilon hair cells, Findlay and Mercer (1971) have shown that as exudation proceeds the cuticle expands above the terminal cell, forming a cavity in which the nector solution apparently gets collected. Subsequently, small droplets of nector are rapidly emitted through the cuticle and the expanded cavity collapses. They have concluded that the secretions collect in the sub-cuticular cavity and when a sufficient hydrostatic pressure develops, the fluid is released through pores which are valve like and possibly pressure sensitive. The similarity of cuticular cavity in salt glands to that of Abutilon suggests it is also a collecting compartment. Whether the salt solution is released by

a mechanism similar to that proposed by Findlay and Mercer for Abutilon is not known but it seems likely that secretion occurs under pressure.

Most hypotheses recognize that once the salts are released into the walls of the secretory cells and the subcuticular cavity, backflow into mesophyll tissue is blocked. This supports that the cuticularized zone apparently has a role similar to that proposed for the casparian strip in the endodemis of roots.

Electron microscope studies of Smaoui (1971) and Thomson (1975) have indicated that these walls are not entirely cutinized. In Atriplex the salts apparently accumulate in large vacuole of bladder cell and with subsequent rupture of the bladder cell, are released to the surface of the leaf. By this mode of secretion, the salts are not released directly to the walls and the problem of direct backflow into the tissue does not exist.

The basic question of how the salt solution is eliminated from the cytoplasm of the secretory cells into the apoplast has not yet received a final answer. As mentioned before, many vesicles and vacuoles occur in the cytoplasm of the secretory cells. In some cases multivesicular structures are observed between plasmalemma and cell wall. Thomson and Liu (1967), Shimony and Fahn (1968), Thomson, et. al. (1969) and

Shimony, et. al. (1973) have suggested that the vesicles and small vacuoles of the cytoplasm of the secretory cells are involved in salt secretion. Osmond, et. al. (1969) have suggested that in salt glands of Atriplex transport of ions from cytoplasm to the vacuole may be brought about by vesicles which fuse with the tonoplast. Thomson, et. al. (1969) have noted that such vesicles or vacuoles fuse with the plasmalemma. The concept of small compartments being involved in ion transport and accumulation has attracted the attention of many investigators.

It is of interest to mention that the processes of salt secretion of the various glands of one leaf or even small portions of it are not synchronized (Hill, 1970 a & b). Shimony, et. al. (1973) have observed in Avicennia that neighbouring glands differed considerably in acid phosphatase activity as indicated by histochemical tests.

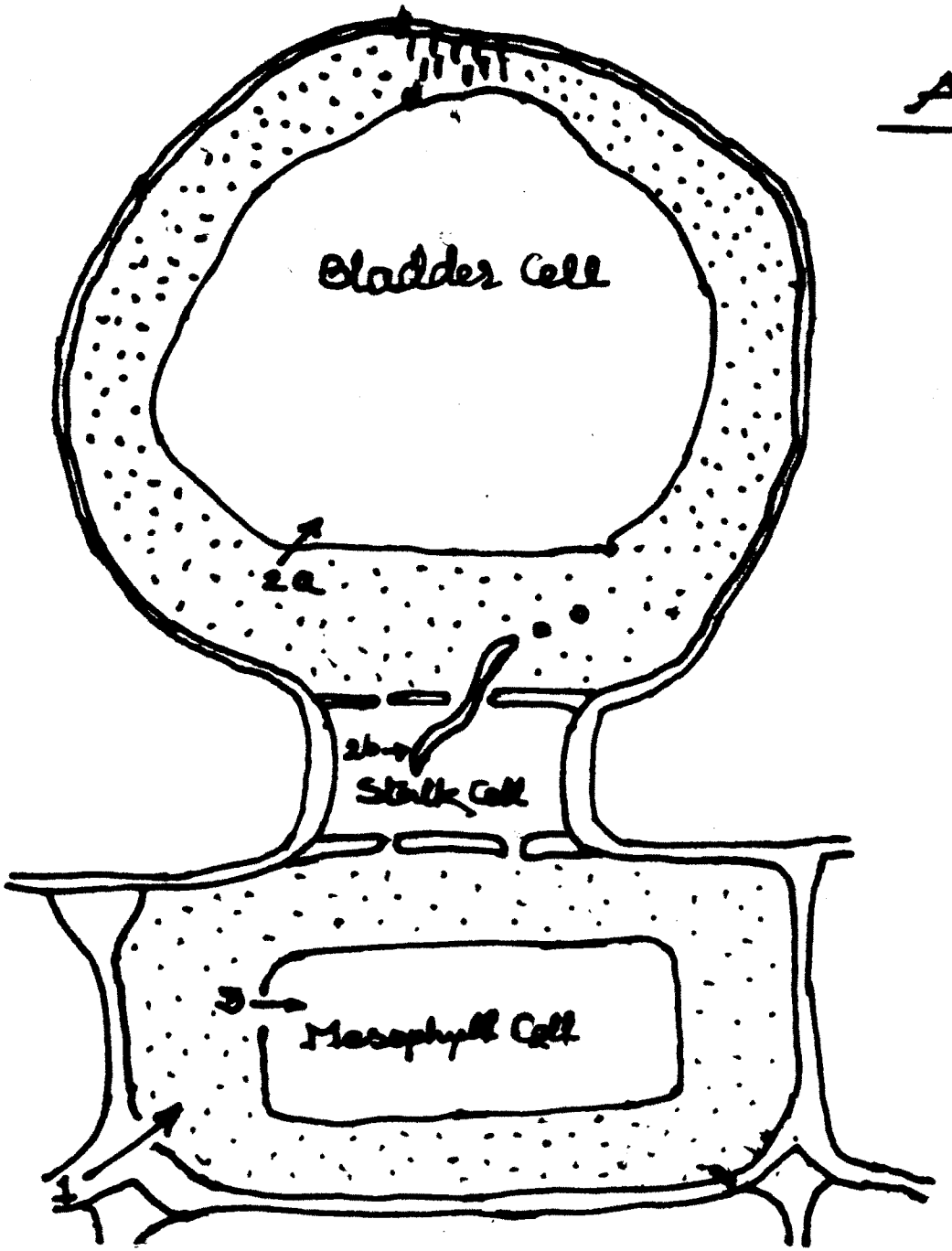
Arisz et. al. (1955) have reported that secretion was sensitive to temperature and metabolic inhibitors. Secretion stopped when oxygen was absent. Similarly KCN drastically reduced the secretion.

Several studies indicate that salt secretion follows a diurnal pattern of high activity during the day and low activity at night. However, there is

FIG. 1

Mesophyll - stalk - bladder system of *Atriplex*.

Atriplex



little evidence, for most plants, that the secretion is directly related to photosynthesis (Poljakoff - Mayber and Gale, 1975).

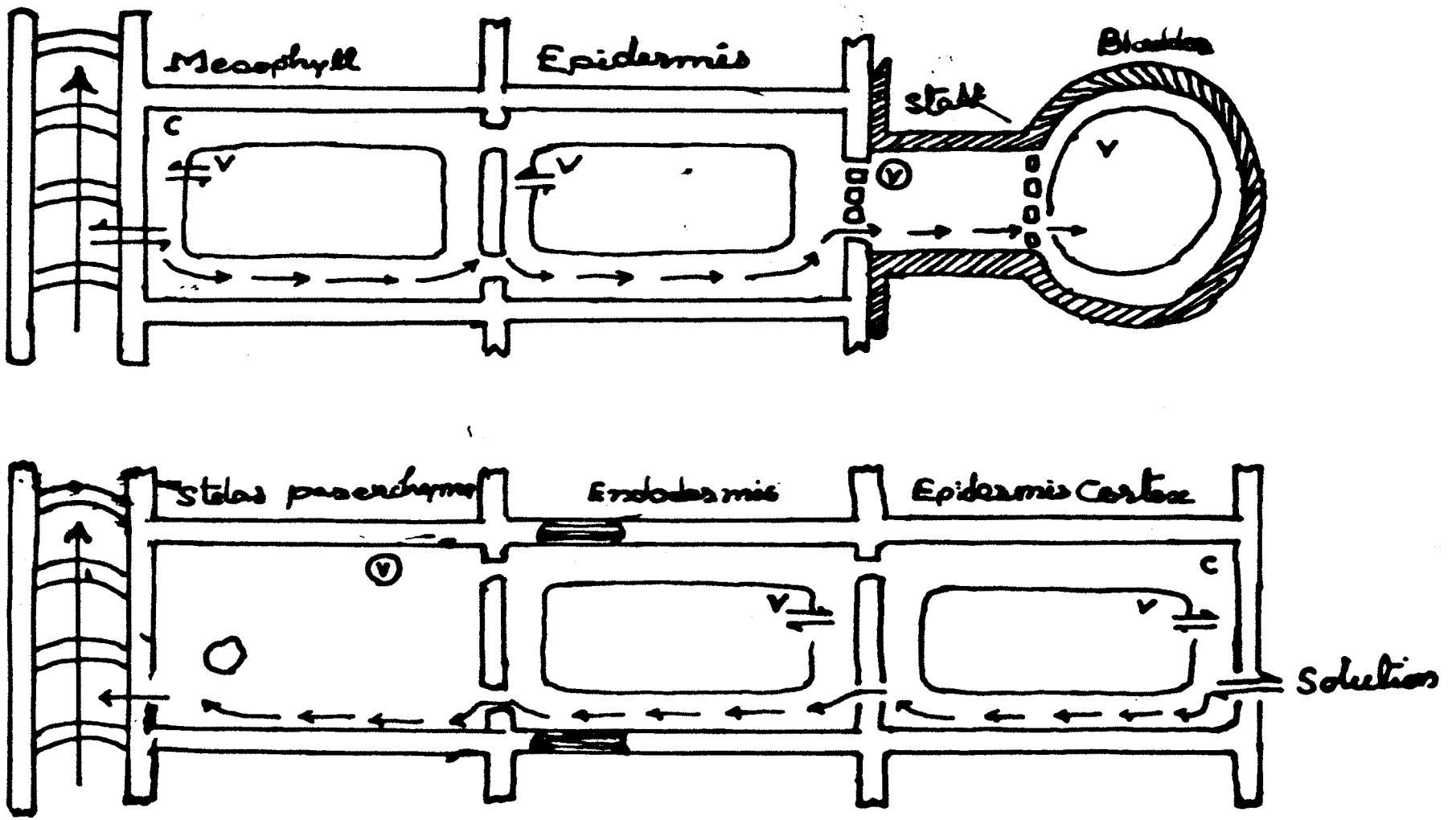
D. The mesophyll-stalk-bladder system of Atriplex.

The mesophyll-stalk-bladder system of Atriplex has been used as an example of leaf-gland systems in figs. 1 and 2. The only difference between this system and "normal" salt glands is that excretion does not export salt to the surface of leaves of Atriplex, but into the large bladder vacuole, which eventually collapses under field conditions releasing salt to the leaf exterior.

This system has been studied by using strips of Atriplex spongiosa leaves floated on labelled solution Cl excretion from the external solution into the bladder vacuole is considerably enhanced by light. Transport from the external solution into the bladder vacuole is against an electrochemical potential gradient. Energy for both, light - dependent Cl accumulation by mesophyll cells of A. spongiosa and for Cl transport into the bladder vacuoles is derived from photosynthetic energy transfer. This photosynthesis dependent Cl transport is not linked to ATP produced in photophosphorylation, but it is more closely coupled with photosynthetic electron flow. It is possible that this coupling is mediated by redox reactions involving pyridine nucleotides.

FIG. 2

Simplified structural models of a leaf - salt-gland system and of a root showing the analogy of these systems in respect to translocation of solutes.


Mesophyll
Epidermis
Bladder
stalk
C
V
V
V
V
Stelar parenchyma
Endodermis
Epidermis Cortex
V
C
V
V
Solution

However, the photosynthetic activity in the stalk and bladder cytoplasm is too small to account for the photosynthetically dependent Cl excretion into the bladder vacuoles. There are two basically different ways of linking electron transport in thylakoid membranes to pumps at other membranes. The first possibility is a purely physical mechanism. Electron flow may alter ion fluxes across the thylakoid membranes in such a way that eventually cytoplasmic ion concentrations are affected. This, in turn may change the electric membrane potentials at the plasmalemma and tonoplast as determined by the Goldman or constant field equation. These changes may be measured and related to influxes and rates of hydrogen exchange during photosynthesis.

The second possibility of linkage is a biochemical one. Photosynthetic electron flow creates reducing power in the form of reduced pyridine nucleotides ($NADPH_2$). Although pyridine nucleotides don't readily traverse chloroplast membranes, transfer of their reducing potential from the chloroplast to the cytoplasm via reduced substrates is possible in a number of redox reactions.

In A. spongiosa, linkage of ion excretion into the bladder vacuoles with photosynthetic electron transport in the chloroplasts of the leaf blade

requires rather good contact between bladders and mesophyll cells. This contact is only possible via cytoplasm because the cell wall cutinization in this system does not allow a free space method.

E. The salt-glands of Limonium

As in Atriplex, in Limonium leaves location of pumps excreting ions is not clear. The suggestion that salt is concentrated when it passes from the leaf parenchyma cells into the gland cells is not supported by results of micro-autoradiographic studies. Tracer efflux analysis of leaf discs of Limonium suggests that there are three effective compartments for Na and four compartments for Cl distribution in the tissue. These compartments are assigned to the free space, cytoplasm and vocuole. The additional compartment of Cl is unidentified but may be represented by the chloroplast or mitochondria.

From the tracer flux kinetics it becomes evident that the transit of Na and Cl ions taken up by discs of Limonium leaves and excreted by glands occurs in the cytoplasm, since half times of cytoplasmic filling are correlated with those of transit. It is assumed that glands export ions directly from the cytoplasmic space controlling ion concentration in this phase. The salt export mechanism of Limonium is subject to metabolic induction

in response to external medium concentration, and thus it is highly adaptive to varied degrees of salinity. The location of pumps in the salt gland leaf mesophyll system the four neighbouring individual glands in a microscopic observation showed significantly different kinetics of volume efflux (Luttge, 1971). This suggests that some event located in the glands themselves must play a role in the overall process of excretion.