"STUDIES ON ION TRANSPORT IN LIMONIUM"

by

Adrian Hill

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SYNOPSIS

The excretion of ions by the glands on the upper surface of Limonium vulgare , Mill. (syn. Statice limonium) was studied by means of a short-circuit technique. The 'active' transport of sodium and chloride ions in an outward direction was demonstrated, the difference between these two ion transports being correlated with the short-circuit current. The inward transport of potassium ions was also demonstrated, together with the outward transport of rubidium, caesium, bromide and iodide ions. Evidence is produced that the alkali metal ion and halide ion transports are not independent, but are linked in a non-stoichiometrical manner.

From measurements of the impedance of the leaf, the transglandular resistance has been calculated, and comparison with the ionic conductance of the glandular membranes shows that a serious discrepancy exists, as has been noted for many other biological tissues.

The ion transport and the electrical properties are discussed in the light of modern theories and work on other materials.

CONTENTS

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INTRODUCTION

UPTAKE STUDIES

(i) The study of ion transport in plant cells is in its infancy, whilst the position with regard to animal cells is relatively much more advanced; many of the techniques in this field originate in work on animal tissues. Plant cells are frequently more complicated than animal cells in that they contain a large central vacuole bounded by an inner cytoplasmic surface, and numerous chloroplasts which may occupy a considerable amount of the cytoplasmic space. However, as the whole they are less specialised in their mode of existence, and this particular specialisation of animal cells and tissues has made the investigation of their phenomena much easier.

An initial task is to distinguish the differences in activity of an ion between the cell, as a part of the cell, and the environment. This has not usually meant only the measurement of environmental concentrations. The activities have often been assumed to be equal to the concentrations, and this assumption has in fact been strongly criticised.[1]

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An initial task is to determine the difference in activity of an ion between the cell, or a part of the cell, and the environment. This in fact has usually meant only the measurement of intracellular concentrations. The activities have then been assumed to be equal to the concentrations, and this proceedure has in fact been strongly criticised.[1]

Ion distributions in plants are often changing slowly with time, even when they appear to be steady, due

to maturation and ageing of the cells. As the cell properties alter, so do the ionic distributions. There are also examples of ion distributions which alter very quickly, due to fast reversible changes in membrane properties: this phenomenon is generally referred to as excitability and has been found in several plants, higher and lower, to be a highly characteristic property. 3,91,45.

How is the general ion distribution to be explained? To begin with, we must decide whether the system, from a thermodynamical aspect, is in a steady-state or in true equilibrium. The general criterion for deciding this, is whether or not the ion in question is in flux equilibrium. Where there is no net flux the ion distribution is assumed to constitute an equilibrium; where there is a net flux of constant magnitude, a steady state is indicated. Both cases are characterised by parameters which are time - independent. When this important point is decided a simple mathematical model can be set up, in the light of which the measurements may be interpreted. If there is no adequate fit, then it is generally assumed that the ion distribution, in part at least, is the result of metabolic interaction. That this assumption is correct, may be tested by a variety of metabolic studies. The peculiar and individual ion distributions found in living cells are

therefore regarded as being due in part to interactions with metabolic processes, inside the temporarily stable matrix which the cell presents. The cellular situation, where it concerns ion movement is described by general membrane theory.[4,5,6] The membrane appears to be a fundamental structure which regulates the passage of ions, molecules, and even electrons into cells or cell compartments.

The sites of the metabolic interaction of 'ion pumps' as they are often called, is in the majority of cases still a matter for debate. A membrane located mechanism is probably the major candidate for this role but there are those who consider the interaction to be a bulk property of the cytoplasm.[7,8] The biological membrane is universally regarded however, as a permeability barrier which regulates the passive fluxes of ions and molecules, and which undoubtedly has selective properties,[10,11] giving rise to an electrical capacitance and displaying a fine-structure of apparently great constancy.[9,12]

The driving force on a ion is the gradient of electrochemical potential $\bar{\mu}$, and to determine this the potential gradient over the membrane must be measured, as well as the difference in concentrations. A considerable amount of work on the problem of ion transport in plant cells has been done without consideration of

the factor of potential differences. In such cases the metabolic transport of any specific ion cannot be considered proved, but the indirect evidence can be very strong. Most of this work has been concerned with studying the uptake or secretion of ions by whole tissues, and an analysis of the uptake patterns by Michaelis-Menten kinetics, here applied to ion-carrier combination. Much of the work has also been done in conjunction with metabolic studies, on the assumption that treatments such as low temperatures and general inhibitors will usually slow down metabolically-linked transport, often to negligible values. Where activity gradients or uptake rates are decreased by stopping the provision of energy, either from photosynthesis or from respiration, such transport can be provisionally assumed to be metabolic; exceptions to this general rule occur when the ion participates in chemical reactions inside the cell, as probably occurs for example, during the uptake of phosphate[13], sulphate[14], and iodide[15]. In these cases it is necessary to show that the ion exists in the free state inside the cell, and the uptake observed has not been due to the ion being bound or metabolised. Where the two ions of a salt are both accumulated against an activity gradient, then one of them at least, must be metabolicly transported.

The application of Michaelis-Menten kinetics to the familiar asymptotic uptake curves has been valuable in revealing the various competitions between ions for uptake sites, by the method of reciprocal plots.[16.] It has also been possible to obtain values for K_m a Michaelis constant for particular cases of uptake.[17.] This whole approach has been criticised on the grounds that changes in potential difference as well as changes in concentration gradients will occur during uptake, and these will alter the electrochemical potential gradients on which passive ion fluxes ultimately depend, leading also to experimental curves which are asymptotic.[18.] This criticism, together with the difficulty of interpreting the results of experiments with mixed salt solutions,[19,20,81.] must make for extreme caution in interpretation. It is true to say that the great body of work on the problem of metabolic ion uptake requires to be underpinned by a study of the ion fluxes under conditions where the electrochemical potential gradients can be reasonably well ascertained in the respective tissues. A start in this direction is the measurement of intracellular potentials as well as intracellular concentrations. Some of the major results of this approach with respect to a number of important ions are summarised in Table I, where cases are listed in which metabolic transport seems quite well established.

TABLE I.

UPTAKE OF IONS BY PLANT TISSUES AND YEAST CELLS

Note: the transport of an ion 'through' a plant tissue must involve the uptake or secretion of that ion by certain cells of the tissue. In the absence of bulk transport such as xylem flow or cyclosis, it is therefore regarded as a cellular transport process.

TABLE I

Ion Uptake - Selected References.

Tissue	Transport	Ref.
Yeast cell	Cations inward	Conway & Duggan,[67,68,69]
	Mg^{2+}, Mn^{2+} inward	Rothstein, Hayes Jennings and Hooper[70]
	Sodium inward	Conway and Moore[66]
	Na/K exchange	Conway, Ryan and Carton[74]
Ulva (frond)	Na/K exchange	Scott and Hayward[71]
Fucus (frond)	Iodide inwards	Klemperer[72]
Porphyra (frond)	Na/K exchange	Eppley[76,77,78]
Hormosira (bladders)	Na/K exchange	Berquist[79]
Hordeum (Barley roots)	Alkali metal ions inward	Epstein and Hagen[20]
	Alkaline earth ions inward	Epstein & Leggett[81]
	Orthophosphate species inwards	Hagen & Hopkins[82]
	Halide ions inwards	Boszormenyi & Cseh[19]
Beta (Red Beet root tissue)	Potassium inwards	Sutcliffe[84] Briggs[85]
	Na/K exchange	Briggs, Hope & Pitman[86]
Daucus (Carrot root tissue)	Na/K exchange	Sutcliffe & Counter[26]
Solanum (Potato tuber tissue)	Chloride inwards	Laties[25]

TABLE I (Cont'd)

Tissue	Transport	Reference
Limonium (leaf glands)	Chloride outwards	Arisz, Camphius Heikens and Tooren[87]
Potomogeton (leaf)	Calcium through	Lowenhaupt[80]
Valisneria (leaf)	Chloride through	Arisz[24]
Nicotiana (leaves)	Rubidium inwards	Jyung and Wittwer[83]

ION FLUXES AND ELECTRICAL POTENTIALS

(ii) When a system is at equilibrium there is no gradient of electrochemical potential for any mobile ion present, and so we may simply write:

$$\bar{\mu}_o = \bar{\mu}_i$$

where the subscript refers to two compartments. As the electrochemical potential is given by the relationship:

$$\bar{\mu} = \bar{\mu}^o + RT\log_e A + zFE, \quad (22.)$$

we are led immediately to the expression

$$\Delta E = E_i - E_o = \frac{RT}{ZF} \log_e \frac{A_o}{A_i}$$

This equation is known as the Nernst equation, and is widely used in physiology for determining whether an ion is passively distributed between two compartments. The experimental proceedure is as follows: the difference of concentration between the two compartments is determined together with the prevailing temperature. The expression $\frac{RT}{ZF} \log_e \frac{A_o}{A_i}$ is then calculated, and this represents the potential difference between the two compartments under which the ion would be in true equilibrium. A microelectrode probe is pushed into the cellular compartment, and the potential difference measured between the probe and a similar electrode outside.

It is conventional to refer all other potentials to that of the external electrode as reference regarding it as an arbitrary zero. As the reference electrode is almost invariably located in the external bathing solution, we have:

$$E_o = 0 \quad \text{and} \quad \Delta E = E_m$$

where E_m is now the potential of the cellular compartment. If the calculated 'equilibrium potential' E_o is equal to the measured potential E_m, then the ion can be regarded as being passively distributed between the compartments. Where the equilibrium potential E_o is more positive than the measured potential E_m of a cellular space there must be some ion pump moving the ion, if an anion, into the space, or if a cation, out of the space, and vice-versa. This method opens up two areas of enquiry which have only been indirectly touched upon by conventional uptake studies; namely, the presence of different pumps at different membranes[24,25,26], and the different permeabilities to ions of the various cell membranes. With microelectrodes it is possible to penetrate separately the two major cell compartments, the cytoplasm and the vacuole, and measure their individual potentials; it is also possible to measure the concentrations of ions in these compartments by a variety of methods (see for instance MacRobbie[35]).

Most of the early work was done on giant algal cells and coenocytes, and has been reviewed by Blinks.[29*] Vacuolar perfusion with artifical sea-water (or pond water) in which the symmetrical system

Sea water / protoplasm/ sea water

was set up,[30] or bathing with natural or artifical sap i.e.,

Sap / protoplasm/ sap

gave rise to steady potentials, which were measured by the perfusion tubes themselves acting as salt bridges to external electrodes. Such experiments clearly indicated a basic asymmetry of the cytoplasmic region towards ions, and this was considered to be a property of its surfaces, i.e. the tonoplast and the plasmalemma. It was also observed that perfusion of the vacuole of Halicystis with solutions of different cation composition made little difference to the potential, indicating that the tonoplast is poorly selective towards cations and has generally a low permeability. This contrasts sharply with the plasmalemma which in some algae behaves almost as

* Note:- Blinks[29] in his review quotes the vacuolar potentials of many species, using the vacuole as the reference phase. His polarities are therefore the reverse of those quoted by modern authors.

a potassium electrode,[31] changing its potential by almost 58 mv. per tenfold change in potassium ion concentration. None of the early workers interpreted the ion asymmetries or potentials as being due to ion-pumps, but they did show that they were not due to redox potentials.[32]

MacRobbie and Dainty[27] studied the influx and efflux of sodium, potassium and chloride ions in Nitellopsis obtusa, the brackish-water characean, and showed that the cell behaved as a three compartment system, each compartment having a different rate of isotopic exchange. A similar study for potassium ions was made by Diamond and Solomon[28] working with Nitella axilaris. In both studies the authors equated the three compartments with the cell wall, a cytoplasmic non-free space, and the vacuole. MacRobbie and Dainty showed that the cell was in flux equilibrium (the intracellular concentrations were steady) for all ions across both cellular membranes and so the Nernst equation could be profitably used. After measuring the vacuolar ion concentrations they calculated the following ion equilibrium potentials:

$$E_{Na} = -15 \text{ mv}, \quad E_{K} = -130 \text{ mv}, \quad E_{Cl} = +45 \text{ mv}.$$

The measured potential of the vacuole was found to be approximately - 120 mv, and so it was postulated that between the vacuole and the external solution an inwardly directed chloride pump and an outwardly directed sodium

pump were operative. The small value of 10 mv. for $(E_m - E_k)$ suggested that potassium may be inwardly pumped. It was not possible to measure the size of the cytoplasmic non-free space, and so from the flux studies no value of the absolute activities of the ion in this space could be obtained. The Na/K ratio could be measured however, and this was no different in the cytoplasmic space from that in the vacuole. Thus the sodium pump was assigned to the plasmalemma: the (Na + K)/Cl ratio was much lower in the vacuole (0.8) than in the cytoplasmic space (74.0) and so the chloride pump was tentatively assigned to the tonoplast. MacRobbie and Dainty also made the observation that if the potassium ion is passively distributed across the tonoplast, and if there is also flux equilibrium across this membrane, then the potential of the vacuole must be almost zero with respect to the cytoplasm, for there is no gradient of potassium activity over the tonoplast.

[33]

Hope and Walker have given data for the ionic relations of Chara australis which also show that the vacuolar potassium is in electrochemical equilibrium with the bathing solution. After measuring the potential of the vacuole, some 120 mv. negative, they calculated the equilibrium vacuolar concentrations of Na, K, and Cl ions from the relationship:

$$C_i = C_o \cdot e^{\frac{-zFE}{RT}}$$

and compared them with the actual concentrations.

C_i (measured) mN. $C_k = 64$ $C_{na} = 57$ $C_{cl} = 100 - 150$

C_i (calculated)mN. $C_k = 49\text{-}56$ $C_{na} = 490\text{-}560$ $C_{cl} = 0.002$

They decided that an inwardly directed chloride pump must be operative and tentatively assigned a sodium extrusion pump t the plasmalemma. The justification for this was based on the fact that as Walker[34] had shown that the potential in this species lies all across the plasmalemma, a value of $C_{na} = 500$mN is too high for the cytoplasmic sodium concentration if sodium is passively distributed across this membrane. Hope and Walker also quote low values for vacuolar calcium, which they found to be out of electrochemical equilibrium. Presumably this is pumped out of the cell too.

Nitella translucens has been studied in considerable detail by MacRobbie[35], who also postulated a sodium extrusion pump at the plasmalemma, and an inwardly directed chloride pump at the tonoplast, on similar grounds to those in Nitellopsis. MacRobbie measured the concentrations of Na and K in the cytoplasm directly, by the technique of Kamiya[36]; the cytoplasm separates into two phases, one a stationary layer containing all the chloroplasts, the other an inner layer adjacent to the vacuole which shows vigorous streaming. The total cation

concentration (Na + K) was higher in the stationary layer and the K/Na ratio also significantly higher. Na/K ratios were similar between sap and flowing cytoplasm.

Sap:	K = 78	Na = 60	Cl = 151	K/Na = 1.3
Flowing cytoplasm:	K =117			K/Na = 1.3
Chloroplast layer:	K =340	Na =120		K/Na = 2.9

(concentrations in mM.)

Calculation of Nernst equilibrium potentials for sodium potassium and chloride ion indicated the potassium is pumped into the cell; the active fluxes of both sodium and potassium ions were inhibited by ouabain, which is known to inhibit linked sodium/potassium transport in many animal cells.[37]

E_K = -168 mv E_{Na} = -103 mv E_{Cl} = + 120 mv.

MacRobbie quotes - 140 mv. for the vacuolar potential. The ratio of the active fluxes, ϕ_{Na}/ϕ_K has been calculated to be 1·1 - 1·4, and this probably represents the action of a sodium potassium linked pump as found in many animal cells.[38] The higher values in the chloroplast layer of the sodium/potassium concentration ratio also indicated that the chloroplasts may themselves represent a non-free space in the cytoplasm.

MacRobbie continued her study of Nitella translucens with measurements of the chloride ion concentration in

the chloroplast layer of the cytoplasm,[39] and concluded that a vacuolar chloride pump is not sufficient to account for the high concentration. Thus there must be a chloride pump somewhere in the cytoplasmic phase; the high cytoplasmic chloride concentration may have been due to the chloroplasts or the mitochondria, accumulating chloride over their own membranes. These cellular organelles were not analysed separately, and so it is impossible to place the chloride pump unequivocally at the plasmalemma. MacRobbie also measured the fluxes of potassium and chloride into the vacuole (M_2), and into the cytoplasm, (M_1), and showed that M_1^{cl} was proportional to M_2^{cl} and to M_2^{K}. No such relation could be shown between M_1^{K} and M_2^{K}. This extraordinary fact remains to be explained, as it does seem to indicate some sort of linkage between the chloride fluxes at the two membranes.

Spanswick and Williams demonstrated by a similar technique that there is indeed a chloride pump presumably at the plasmalemma, but not at the tonoplast.[40] They measured the vacuolar potential with respect to the cytoplasm, and found it some 18 mv. positive, in agreement with a prediction of MacRobbie,[35] and application of the Nernst equation to the tonoplast indicated that potassium and chloride are passively distributed, but that sodium is pumped into the vacuole. Earlier work on algal cells

had supported the prediction of MacRobbie and Dainty[27] for Nitellopsis, that the potential of the vacuole would be zero with respect to the cytoplasm, but it also appears that the vacuole is approximately 5 - 20 mv. positive to the cytoplasm in Chara australis.[34, 41] It is very interesting to note that in an earlier paper of Osterhout,[48] the vacuolar potential fell from an average of 14 - 15 mv., to a constant level of 4 - 5 mv. after several days, during a vacuolar perfusion experiment; this is equivalent to a drop of + 10 mv. in the vacuolar potential. It is well known that cytoplasm will creep and seal over electrode tips during such experiments, and it would be interesting to know whether the potential of 4 - 5 mv. represents a cytoplasmic potential due to sealing of the tonoplast.

Thus a difference of interpretation exists concerning the transport properties of the tonoplast, and an important point to clear up is certainly that of the true sodium concentration in the flowing cytoplasm. Maybe it represents an age or seasonal difference between the two Nitella sources.[40]

For the red alga Rhodymenia palmata, MacRobbie and Dainty gave the equilibrium potentials

E_{na} = - 110 mv. E_K = + 75 mv. E_{cl} = 0 mv.

and the vacuolar potential measured in the same laboratory

was close to - 65 mv. in most experiments.[42] This again requires an inward chloride and potassium pump, and an outward sodium pump to explain the departure from electrochemical equilibrium. This seaweed has two types of cells in the frond, and the isotope exchange curve for the intracellular space is correspondingly non-uniform. Intracellular concentrations were evaluated as the ratio between tissue ion concentration and intracellular water.

During their study of short-circuit currents in Halicystis ovalis, Blount and Levedahl[43] determined the potentials of the cytoplasm and the vacuole in this spherical alga, and could detect no difference between the two; their calculations of the equilibrium potentials for sodium, potassium and chloride ions indicate that sodium is extruded from the cell and chloride is accumulated. Potassium is passively distributed across the cytoplasm. Blount and Levedahl concluded that the sodium extrusion pump was situated at the plasmalemma, whilst the chloride pump was at the tonoplast, by a rather tenuous argument based on Donnan potentials and by analogy to the situation in Nitellopsis. A similar application of the Nernst equation to ion distribution in Chara globularis[45] by Gaffey and Mullins indicated that potassium is also in

electrochemical equilibrium in this cell, whilst chloride is pumped inwards and sodium outwards. The potential difference across the cytoplasm seems to lie almost entirely across the plasmalemma, and the vacuolar and cytoplasmic K concentrations seem to be equal.

Halicystis ovalis:

E_k = - 84 mv. E_{na} = + 17 mv. E_{cl} = - 1 mv.

E_m = - 80 mv. (vacuolar potential)

Chara globularis:

E_k = - 184 mv. E_{na} = - 155 mv. E_{cl} = + 202 mv.

E_m = - 181 mv. (Vacuolar potential)

(Gaffey and Mullins.)[45]

Another Halicystis spp., H. osterhoutii has been studied from the aspect of internal ion concentrations by Blinks and Jacques,[46] who compared the ion concentrations with those of the natural habitat of the plant; Bermuda seawater. From their data it is possible to calculate the ion equilibrium potentials of sodium, potassium, chloride, calcium and magnesium. In this cell potassium seems to be pumped out of the cell, an unusual finding. Sodium is extruded together with calcium, as was also reported by Hope and Walker[33] in Chara australis. Chloride is accumulated, whilst magnesium appears to be in equilibrium. E_m is quoted by Blinks[29] as - 65 mv.

E_{na} = - 4 mv. E_k + 16 mv. E_{mg} = - 72 mv. E_{ca} = + 5 mv.

E_{Cl} = + 2 mv.
(calculated from Blinks and Jaques)[46]

There are a number of interesting algae with positive vacuolar potentials, Valonia macrophysa, Valonia ventricosa, Ernodesmis verticilliata and Chamaedonis annulate.[29] Valonia macrophysa has been the subject of considerable study by Osterhout.[47] From his data for internal ion concentrations it is also possible to calculate the equilibrium ion potentials for several ions. Potassium is strongly accumulated, whilst sodium is weakly extruded by the cell. Magnesium seems to be extruded as only a trace is chemically detectable in the sap. Calcium is here accumulated; most surprising of all, chloride is in equilibrium between the Bermuda sea water and the sap.

E_{Na} = + 42 mv. E_K = - 95 mv. E_{Ca} = - 5 mv. E_{Cl} = + 10 mv.
(calculated from Osterhout).[47]

E_m = + 10 mv. (potential from Osterhout et al.)[48]

The only other studies on ion transport potentials in plant cells have been made on angiosperms. Etherton and Higinbotham measured the potential of the vacuole and cytoplasm of roots, epicotyls, and coleoptiles of Avena, Pisum, and Zea.[49] They found no potential difference across the tonoplast in all cells studied; a fact reported by many workers on algae,[27,34,43,45] and after applying the Nernst

equation they concluded that potassium was in approximate electrochemical equilibrium with the bathing solution.

Etherton[50] studied in detail the effects of changing the external solution on the internal ion concentrations of Avena coleoptiles and roots, and Pisum stems and roots. The intracellular ratios of sodium/potassium are quite low in all the experiments, indicating that a sodium extrusion pump and possibly also a potassium accumulation pump are operative. Etherton measured the intracellular potential, presumably that of cells in flux equilibrium, and calculated the theoretical internal concentrations $C_o e^{-zF/RT}$ for sodium and potassium. He concluded that

(i) at low concentrations of external sodium and potassium, sodium is in equilibrium whilst potassium is accumulated.

(ii) at medium concentrations sodium is extruded whilst potassium is in equilibrium,

and

(iii) at high external concentrations both ions are actively extruded.

Changing the external solution seemed to have little effect on the membrane potential. Calculation of equilibrium potential values from Etherton's data (TABLE 1A) show that his conclusions are not really justified, except in the case of the Avena Coleoptile, for all other tissues

TABLE 1A

show active extrusion of both K and Na at all the external concentrations he used, though the "active transport potentials", $(E_m - E_k)$ and $(E_m - E_{na})$ do seem to increase. It seems doubtful whether all his tissues were in flux equilibrium, and thus the natural "homeostatic adjustments" of the cation pumps which he suggests require more adequate demonstration.

AVENA:-

	COLEOPTILES			ROOTS		
Soln.	E_k	E_{na}	$E_m(x)$	E_k	E_{na}	$E_m(x)$
0.1x	-145	-105	-106	-	-	-
1x	-109	- 59		-92	-63	
3x	- 79	- 28		-78	-47	
10x	-91	- 35		-87	-53	-62

(Calculated using the Nernst equation from Etherton,[50] who quotes intracellular concentrations in various bathing solutions, x, 3x and 10x. x is a standard solution 1.0mM for sodium and potassium).

TABLE 1A

ION TRANSPORT IN PISUM AND AVENA

PISUM:-

	STEM			ROOT		
Soln.	E_k	E_{na}	$E_m(x)$	E_k	E_{na}	$E_m(x)$
1x	-96	-35	-120	-109	-44	-112
3x	-72	-10		-80	-17	
10x	-45	+13		-49	+15	

AVENA:-

	COLEOPTILES			ROOTS		
Soln.	E_k	E_{na}	$E_m(x)$	E_k	E_{na}	$E_m(x)$
0.1x	-145	-105	-106	-	-	-
1x	-109	- 59		-92	-63	
3x	- 79	- 28		-78	-47	
10x	-51	- 35		-57	-53	-82

(Calculated using the Nernst equation from Etherton[50], who quotes intracellular concentrations in various bathing solutions, X, 3x and 10x. x is a standard solution 1.0mN for sodium and potassium).

It is tempting to assume that a sodium - potassium coupled exchange pump of the type found in many animal cells is operating in plant cells, but that it can lose the ability to transport either sodium or potassium, so becoming an electrogenic pump.[64] Dainty has suggested that a sodium extrusion pump could lose its specificity and become a potassium extrusion pump; the reverse is certainly known, in nerve[65] and possibly in yeast.[66] This would certainly explain the potassium extrusion by Halicystis osterhoutii. Dainty has also suggested that a pump can be rendered ineffectual by a large membrane permeability to the ion in question, as may well have happened in the case of Valonia macrophysa. If this is so the tonoplast should behave as a chloride electrode when the vacuole is perfused, which it apparently does not,[29] but the general principle is still valid. Such pumps would however be detected by a short-circuit technique as used by Blount and Levedahl,[43] for the ion transport current is quite independent of membrane resistance.

TABLE II

Classification of ion pumps in plant cells.

Note: the ions are given a subscript oi or io to designate the direction of transport i.e. oi = outer solution to inside of cell. Where the ion pump has been localised reasonably well, the site (tonoplast or plasmalemma) is mentioned by insertion of (T) or (P), respectively. Demonstrations of passive transport are included, and question marks refer to findings which are debateable.

Chara australis [33]	Na_{io}, (P). Ca_{io}. (P)
	K passive. Cl_{oi}.
Chara globularis [45]	Na_{io} K passive. Cl_{oi}.
Halicytis osterhoutii [46,29]	Na_{io}. K_{io}. Ca_{io}.
	Mg passive. Cl_{oi}.
Halicytis ovalis [43]	Na_{io}. K passive. A_{oi}.
Nitella translucens [35,39]	Na_{io}. (P)
	K_{oi}. (P)
	cl_{oi}. (P)
	cl_{oi}. (T) ?
Nitella translucens [40]	Na_{oi}. (T) K passive (T)
	cl passive (T) ?
Nitellopsis obtusa [27]	Na_{io} (P). K_{oi} (P)
	Cl_{oi}. (T)
Rhodymenia palmata [42]	Na_{io}. K_{oi}. Cl_{io}.
Valonia macrophysa [47,48]	Na_{io}. K_{oi}. Ca_{oi}. Mg_{io}.
	Cl passive.
Avena, Pisum. [50]	(Na passive; Na_{io}) (K_{oi}, K passive, K_{io}) ?

SHORT CIRCUIT STUDIES.

(iii) The short circuit technique was devised for studying the transport of ions across epithelial tissues,[51] and bears a relationship to the voltage clamp technique used by nerve phsyiologists.[52] It is in fact a very low frequency voltage clamp. The principle is to hold the membrane potential at zero by means of an external short-circuit, during which operation the current flowing is measured. The fluxes of certain ions through the preparation are then measured (usually by isotopic tracers when available),[53] and their contribution to the total current is assessed. Thus it is possible to find out if the movement of all ions have been detected, for unless all the ion transports are measured, the total coulombic flux cannot be accounted for. In the application of voltage clamping to certain algal cells which show electrical excitability,[23,45] the aim has been to measure the transitory diffusion currents set up when the membrane undergoes permeability changes towards certain ions and thus to describe the time course of the specific ion conductance changes. These short-circuit currents are therefore passive ion currents of a temporary nature, and these studies do not give direct information about metabolic ion transport. Action currents can often be in the range of 1 - 100 m Amps./cm^{-2}, but the current

created by ion pumps is of the order of 1 - 50 $\mu A/cm.^{-2}$ [51].

The study of ion transport in Halicystis ovalis by Blount and Levedahl [43] is an example of a short-circuit experiment during which the cell interior is perfused with artifical sea-water. This system is therefore similar to that set up during studies on epithelia [51] and on perfused axons [54], but differs in that while the former represents transport across whole cells and the latter transport across a single membrane, here transport is across a double membrane system, the cytoplasm. Under these conditions there is no gradient of temperature or osmotic pressure across the membrane; the use of identical solutions inside and outside, together with the abolition of any gradient of electrical potential by the external circuit, ensures that there is no gradient of electrochemical potential, $\bar{\mu}$, for any ion in the system. Thus any net movement of ions which takes place under these conditions must be due to an ion pump.

Blount and Levedahl perfused the vacuole of Halicystis with sea water containing radio-sodium (Na^{22}, Na^{24}) and measured the rate of its appearance in the external bathing solution. This gave a measure of the sodium efflux, ϕ_{io}; similar experiments with radiosodium in the bathing fluid gave values of the sodium influx, ϕ_{oi}.

The net flux $\phi = \phi_{io} - \phi_{oi}$, and as this was appreciable, metabolic transport of sodium was unambiguously demonstrated, with one proviso however; that the flux of water across the membrane was zero. With the bathing solution isotonic to the perfusion solution, and the vacuole opened by tubes, no gradient of osmotic or hydrostatic pressure could have existed, and the absence of a potential difference ruled out any electro-osmotic contribution.

Outward sodium transport and inward transport of chloride ions was demonstrated, and Blount and Levedahl expressed the magnitude of the pumps by calculating their percentages of the total short-circuit current. The measured currents are quite variable, but the percentage values show good correalation, amounting to 97% of the total current. Table III.

TABLE III.

DETAILS OF SHORT CIRCUIT MEASUREMENTS

ON HALICYSTIS OVALIS BY[43]

BLOUNT AND LEVEDAHL.

TABLE 3.

Short-circuit studies on Halicystis ovalis,[43] taken from Blount and Levedahl.

Ion	Efflux*	Influx*	Short-circuit current (micro amps)	Net ion current x 100 / shorting current.
Na^+	74	10	11.7	39.2%
Cl^-	102	426	44.5	57.6%

* p. mol. per cm^2. second.

(iv)

Many workers have measured the permeabilities of cell membranes, by various methods. These fall under three distinct heads

(i) Flux studies. These are based on the fundamental relationship

$$F = P\ (A_o - A_i\, e^{\frac{-zFE}{RT}})$$

where F = the net flux, P = a permeability coefficient, and $A_{o,i}$ are the activities in the two phases o, i, differing in potential by E. This equation is of general validity and makes no assumption about the condition of the membrane. The permeability coefficient P, defined in this way is not a priori assumed to be constant. An interesting method for determining permeabilities has been employed by Hope and Walker.[33] They passed currents through the tonoplast and measured the sodium flux, which did not alter to any extent. Thus, they concluded that the tonoplast is virtually impermeable to sodium ions. This method has not been developed in a quantitive way.

(ii) Potential-change studies:

These are based on the application of either the Planck or the Goldman equation to the passive ion distribution:[55,56]

$$E = \frac{RT}{zF} \log_e \frac{P_K.K_o + P_{Na}.Na_o + P_{Cl}.Cl_i}{P_K.K_i + P_{Na}.Na_i + P_{Cl}.Cl_o}$$

where P_{Na}, P_k and P_{Cl} are the permeability coefficients for the three ions Na, K, and Cl. Non-passive ion transport is usually present as well and this, when electrogenic, gives rise to an additional potential. For this reason changes in membrane potential are usually studied: the external solution is changed abruptly, and a new membrane potential is set up. The non-passive ion transport is assumed to be unaffected. Any Donnan potentials due to the contiguity of the wall, a fixed-anion phase, and the plasmalemma, are kept constant by maintaining a constant salt concentration, varying only the cation proportions. This approach has been well investigated for Chara australis by Hope and Walker[57], and they concluded that the results of such changes are adequately explained by the Goldman theory, which leads to reasonably accurate quantitative predictions over a considerable range of external concentrations of Na and K. These methods have been used where P_{Cl} is negligible. The Goldman equation then gives:

$$E = \frac{RT}{F} \log_e \frac{K_o + \alpha Na_o}{K_i + \alpha Na_i}$$

where $\alpha = P_{Na} / P_K$. This may well be a membrane property which is independent of the absolute values of P_{Na} and P_k, and Hope and Walker derived a value of α and $(K_i + \alpha Na_i)$, an "internal concentration factor", which were assumed constant during the experiments.

can of course be derived from individual flux measurements which give P_{Na} and P_k separately as in (i).

(iii) Resistance Measurements.

The Goldman theory predicts that when ionic solutions of similar composition but different strength are separated by a membrane, resistance measurements will show rectification of the system. The extent of the rectification caused by an ion is a function of the permeability of the membrane to that ion, and thus permeability coefficients can be calculated by a study of the rectification of a membrane in different ionic solutions. This has been done in a quantitative manner.[57]

Differentiation of the Goldman equation for ion flux to obtain $\left(\frac{\partial E}{\partial I}\right)_{I \to o}$ gives a relationship linking membrane resistance, $\frac{dE}{dI}$ ($= R_m$) and ion flux. This takes several forms, from which we may note

$$1/Rm = \frac{F^2}{RT} \cdot \left(\phi^- + \phi^+\right) \ldots\ldots\ldots (a)$$ [101]

and $$Rm = \frac{RT}{F^2} (1/C_o - 1/C_i)/\log_e (C_i/C_o) \ldots (b)$$ [57]

where $C_o = \sum_o \sum_j P^j A^j{}_o$ and $C_i = \sum_o \sum_j P^j A^j{}_i$

Equation (a) has been used by several workers to predict membrane resistances,[27,35,58] whilst equation (b) has been used to calculate P_k and P_{Na} from resistance measurements,[57] using values of α and $(P_k.K_i + P_{Na}.Na_i)$ obtained from potential - change measurements.

The problem of ensuring that the applied current density over a cell surface is fairly uniform has been dealt with at length by Walker,[59] Dainty et.al.,[58] and Hope and Walker.[57] If this condition is not met, quite erroneous values of membrane resistance and capacitance can be obtained.[60] This problem is also very important in short-circuit techniques, and will be discussed below.

The most interesting fact to emerge from resistance studies is that the values of membrane resistance given by direct electrical measurement do not agree with those calculated from flux data, the latter often being larger by a factor of ten or more. This has been observed in many biological tissues and has been attributed by Hodgkin and Keynes[61] to the filing of ions through membrane pores; this will modify the ion conductance equation (a) by a factor n,

$$G_m = n. \frac{F^2}{RT}. (\phi^- + \phi^+).$$

where n is the number of ions in the pore. Thus n appears to be about 10. Discrepancies between calculated and observed resistance of the order of 20 have been found in algal cells, and pores of this size seem doubtful;[35] the fundamental assumption of independent ion movement probably needs to be questioned, as has been done by Sjodin.[63]

The values of tonoplast resistance calculated from flux data are often much lower than those calculated for the plasmalemma[35]., although the tonoplast has low selectivity towards ions in general.[29] All direct resistance measurements have so far shown that the tonoplast resistance is very much lower than that of the plasmalemma.[57,59].

The discrepancy between flux -measured resistance and potential-measured resistance, when finally resolved, will no doubt shed new light on the problem of membrane structure. To this end, it is worthy of considerable study.

Values of P_{Na}, P_k, P_{Cl}, α, and R_m quoted by various authors are collected in Table IV. It can be seen that the resistance anomaly exists for the tonoplast and the plasmalemma. Measurements were all made with inserted microelectrodes, The resistances of internodal cells of the Characeae has been reviewed by Dainty et.al,[58] who give the ionic composition of the bathing medium in which the measurements were made, a most important point.

TABLE IV.

Permeabilities and Resistances of giant algal cells

Note:- the values of approximately 100 KΩ/cm.^{2} for the resistance of the cytoplasm in Nitella flexilis[88], Chara coronata[88], Nitella micronata[89] and a Nitella spp.[60] are not included. These results, together with those of Weidmann[90] are not corroborated in recent studies, and are regarded, for a variety of reasons, as being anomalous.

The values quoted are marked,

(A) - derived from flux data

or (B) - derived from electrical measurements.

The only cell on which both types of measurement have been made to date is Nitella translucens.

Permeabilities are given as cms. sec.$^{-1}$ and resistances as kilohms. cm.$^{-2}$

TABLE IV.

Electrical Resistances and Ion Permeabilities in Plant Cells.

Plant	Measurement	Author
Nitella spp.	R = 27 K depolarising 46 K hyperpolarising - plasmalemma (B)	Findlay [91]
"	R = 54 K (B)	Findlay [94]
Nitella spp.	R = 5 - 50 K plasmalemma (B) R = 1 K - tonoplast (B)	[59]
Chara australis	R = 15 K plasmalemma (B)	Walker
Chara australis	$P_K = 10^{-5}$ $P_{Na} = 10^{-6}$ α = 0.06 (B)	Hope & Walker [57]
*Chara globularis	$P_K = 3 \times 10^{-7}$ $P_{Na} = 10^{-8}$ - plasmalemma (A)	Gaffey & Mullins [45]
Chara braunii	R = 30 K (B)	Oda [93]
Nitellopsis obtusa	R = 250 K - tonoplast (A) α = 0.05	MacRobbie & Dainty [27]

* Calculated from flux data by Hope and Walker [57]

TABLE IV (Cont'd)

Plant	Measurement	Author
Nitella axillaris	$P_K = 1.3 \times 10^{-6}$ - plasmalemma (A)	Diamond & Solomon[28]
Nitella translucens	R = 360 K - plasmalemma (A)	MacRobbie[35]
	α = 0.18	
Nitella translucens	R = 19 K (B) - plasmalemma	Williams, Johnston & Dainty[58]
Nitella translucens	α = 0.2 (B)	Spanswick & Williams[92]

(V) ION TRANSPORT STUDIES ON LIMONIUM

The study of ion transport in the cells of higher plants is greatly complicated by the small size of the cells. In the studies on Pisum, Zea, and Avena by Etherton,[49,50] it is quite impossible, as far as modern techniques permit, to obtain samples of vacuolar sap or pure cytoplasm. In any proceedure for extracting cellular fractions from whole tissues the rupture of cell membranes leads inevitably to the contamination of material from one cell compartment by that from another.

Limonium was chosen as a very suitable material for the study of ion transport in higher plants for two reasons:

(i) it is possible to apply short-circuit studies to the leaf.

(ii) the leaf will transport ions from one solution to another, and the composition of both these solutions can be varied at will.

The upper surface of the Limonium leaf consists of numerous multicellular glands embedded in a highly impermeable cuticle; there are about four to five times the number of glands on the upper surface as on the lower, in Limonium vulgare. The midrib also becomes

inconspicuous towards the tip of the leaf and this enables one to remove a flat disc of tissue with a punch; after suitable pretreatment (Materials and Methods) the disc can be used to separate two chambers containing salt solutions for a short circuit experiment, in a similar manner to many animal epithelia.[51]

The functioning of the glands has been studied by Arisz et.al.[87] in the closely related species Limonium latifolium, with respect to their ability to transport chloride.[96] Ruhland had previously described the glands of Limonium gmelinii in detail, but came to the conclusion that the glandular exudate was isotonic with the cell sap, and that as a consequence the glands do not perform osmotic work. Arisz has clearly demonstrated that the osmotic pressure of the exudate is higher than that of the leaf sap, and that the secretion of chloride ions is closely linked to metabolism. He has also shown that the rate of salt exudation is unaffected by increasing the osmotic pressure of the medium on which the leaf discs are placed. The earlier work has been succinctly reviewed by Helder.[102]

Short-circuit studies on the Limonium leaf can therefore be used to obtain information about chloride

transport through the gland cells, and the study can be extended to many other ions. This present work is concerned with the transport of several alkali metal ions and the halide ions, with respect to the direction and magnitude of the transport processes. The information obtained from such experiments can then be used to shed light on ion transport phenomena in other higher plant tissues. The electrical impedance of the Limonium preparation can easily be measured in various salt solutions, and it has in fact proved possible to construct an analogue of the leaf, using resistors and capacitors. The ease of working with Limonium leaf discs is only counterbalanced by the great variability of the secretory process. In all experiments the leaf discs used were taken from the same plant, where this was possible. It is evident that the only comparison which cannot be made between the results of experiments with this plant and algal cells is that of membrane properties per unit area of surface. Whilst it is possible to derive values for resistance, capacitance and permeability for unit areas of leaf surface, these cannot be corrected to absolute membrane coefficients, for the membrane surface of the

glandular cytoplasm is quite unknown; it may be enormous in spite of the small size of the cells. Determinations of α and comparisons of electrical resistances with ion conductances are still perfectly valid, however.

MATERIALS AND METHODS:-

Limonium vulgare, Miller., (syn. Statice limonium L., Sea Lavender) was collected in October from a Kent saltmarsh, and grown for three years in the greenhouse; plants grew well only when cultured in a polythene moisture tent, in pots. Lighting was provided by mercury vapour lamps above the tent, and the plants were watered with tapwater two or three times a week. Leaves were selected for experiments preferably all from a single plant to ensure uniformity, each leaf yielding one leaf disc. In a few experiments, very big leaves yielded two or even three discs from either side of the midrib. The small size of the midrib at the top of the leaf enabled a flat lamina of 1.8 cm. diameter to be punched out. The old leaves (dark green) and very young leaves (bright green) were not used.

The pretreatment of leaf discs consisted of two stages; to begin with the disc was laid face downwards onto a wet rubber bung, and the lower cuticle gently scraped all over with a very sharp scalpel. This destroys the high impermeability of the lower cuticle, and also removes the few glands on the lower surface (1/5th in number of those on the upper surface). This treatment can be shown to be sound by measurement of the

impedance of the leaf disc (discussed in Part IV); the leaf now behaves as a "transport surface" comprising the upper cuticle with its glands, attached to a fairly homogeneous diffusion layer consisting of leaf parenchyma, which has no polar transport properties. The resistance of the parenchyma layer is about 1 KΩ, whilst that of the upper surface roughly 10 KΩ. The discs were transferred to small plastic vials containing the salt solutions, which were aerated by fine polythene air-tubes; they were left in these solutions for about 15 - 20 hours under bench illumination (60 watt. tungsten bulb at 6 inches through plate glass), after which time it was assumed that flux equilibrium had been attained. The transport of any particular ion across the glands in either direction was then determined as follows. In the efflux experiments, (i.e. transport of an ion from the parenchymatic surface to the outside) the discs were pretreated in a salt solution labelled with the radioisotope of the ion, and mounted in the short circuit chamber with labelled solution on the parenchymatic side and unlabelled solution on the other. The unlabelled solution was then sampled for the appearance of radioactivity. In the influx experiments, (i.e. transport of an ion from the outside through to the

parenchyma) the discs were pretreated in an unlabelled salt solution and mounted in the chamber with unlabelled solution on the parenchymatic side and labelled solution on the outside. After the experiment the discs were removed and rinsed, blotted dry and stuck lightly to a planchette with vaseline, for direct radio-assay. All the radioactivity crossing the glandular surface is trapped in the parenchymatic layer, through which diffusion is slow.

The chambers are illustrated in Fig. 1. Each chamber had a volume of about 2 ml., and the assembly, which was made from perspex, was clamped onto a perspex table holding the electrodes. Each chamber was radially symmetrical, and the leaf disc (L) was held by a pair of synthetic rubber O-rings, let into the face of each chamber, (D). The electrodes for impedance studies were flat spirals of anodised platinum (C,C) at the ends of each chamber and parallel to the leaf disc, thus ensuring even current density through the tissue. Salt bridges (A,A) consisted of polythene tubes filled with 3M KCl agar-gel, let into each chamber through closely fitting holes in the top and connected directly to calomel electrodes; these

were used to record the transglandular potential. A similar set (B,B) also connected to Calomel electrodes, was used to pass the short circuit current through the chamber. The area of leaf disc exposed to each chamber was 1.76 cm^2. Stirring was done by electrically driven shafts, (S,S). For efflux experiments the chamber facing the outside of the leaf disc was filled with a graduated pipette and the sampling at the end of the experimental time (0.5 ml.) was made with a graduated syringe. For all other work, simple teflon syringes were used to transfer solutions to and from the chambers.

All the radioisotopes were obtained from the Radiochemical Centre, Amersham, England. Sodium, potassium, caesium and rubidium were used in solution as the chlorides, $Na^{22}Cl$, $K^{42}Cl$, $Cs^{137}Cl$ and $Rb^{86}Cl$, whilst chloride bromide and iodide were used as sodium salts, $Na\ Cl^{36}$, $Na\ Br^{82}$ and $Na\ I^{131}$. In almost all the experiments the ionic concentrations were 100 mM. Specific activities were all adjusted to give a solution activity of 500 μC/litre approximately. No buffers were used.

Counting was done with a Panax scintillation counting unit (Scalar type D. 657., Timer type T.300); in one experiment where sodium (Na^{22}) and chloride (Cl^{36})

ions were assayed mixed, the chloride beta radiation was removed with a Panax aluminium filter; Cl^{36} has no gamma emission, and so the Na^{22} can be assayed by its residual gamma particles which pass the filter. The 0.5 ml. liquid samples were dried down on four planchettes and the total count obtained by addition; leaf discs were allowed to dry under ambient conditions, and assayed whole. The self-absorption of the thin dry lamina was negligible (less than 2%).

The electrical circuit is shown in Fig. 2. The transport chambers (TC) were connected by their electrode systems to:

(a) The impedance measuring circuit. This comprised the A.C. bridge (AC), which was powered by a simple oscillator (OSC) of frequency range 4,000 - 20 c.p.s. (sine). The output from the bridge was fed to the y-plates of a cathode ray oscilloscope (CRO) with its time-base disconnected, to generate a Lissajou figure; the X-plates were connected to the oscillator via a step down transformer (10 : 1), and the output from the bridge was preamplified in the oscilloscope. At the balance point a straight line was obtained.[55] The platinum spirals at the ends of the chambers were occasionally replated in $PtCl_2$ solutions. The resistance of

the chambers without the leaf disc, when filled with 100 mM. sodium chloride solution, was 300 ohms.

(b) The potential measuring circuit. The calomel electrodes attached to the salt bridges AA (Fig. 1) were connected to a high impedance millivoltmeter, the output of which was fed to a moving spot galvanometer (GD), thus imparting considerable sensitivity (0.2 mv. per cm. spot deflection). At zero potential the millivoltmeter output was zero. The lead to the millivoltmeter input was double shielded, the inner shield going to the cathode-follower cathode; the outer shield was earthed, as was the other calomel electrode. Interference was reduced by siting the whole apparatus on an earthed sheet, surrounded by a wire cage.

(c) The shorting circuit. A potential divider (P) was used to pass current through the current electrodes B,B, (Fig. 1) in series with a calibrated moving spot microammeter (MA). Also included in the circuit was a standard 100 K ohm resistance (R), and the potential drop over this was amplified (A), and fed to a recorder (PW). The short circuit current was adjusted every five minutes, the zero potential being observed on the millivoltmeter galvanometer display.

The recorder trace provided an accurate record of the time, most experiments lasting from half-an-hour to an hour.

Ion fluxes were calculated as ion currents (microamps.) from the formula:

$$\text{ion current (microamps.)} = \left(\frac{MC}{AS}\right). 96.5 \times 10^{6}$$

where

M = Moles/litre of ion in the bathing solution.

C = total count/unit time., of ion transported.

A = counts/ml./unit time., of the radio-bathing solution.

S = seconds (duration of experiment)

The average short circuit current was obtained by graphical integration of the recorder trace.

Fig. 1. Short-circuit chambers.

A.A. Agar-gel 3 M KCl bridges to calomel electrodes and millivoltmeter.

B.B. Agar-gel 3 M KCl bridges to calomel electrodes and shorting circuit.

C.C. Platinum black spiral electrodes via external leads to A.C. bridge.

S.S. Electrically driven stirrers.

LL. Leaf disc.

D. O-rings holding leaf disc.

Fig. 2. MV. Marconi TF 1093 Millivoltmeter.

GD. Galvanometer display - Tinsley S.R. 4/45 moving spot.

OSC. Advance H - 1 oscillator (20 - 4,000 c.p.s.).

AC. Muirhead Universal Bridge, A-154-A.

CRO. Oscilloscope - Solatron C.D. 1014.2.

MA. Microammeter - Pye "Scalamp" 7906/5.

R. 100 K ohm. resistor.

P. Variable source (potential dividers plus battery).

P.W. Penwriter - Evershed Mark 2. Minature recorder.

A. D.C. Amplifier, 1000: 1 gain.

T.C. Transport chambers.

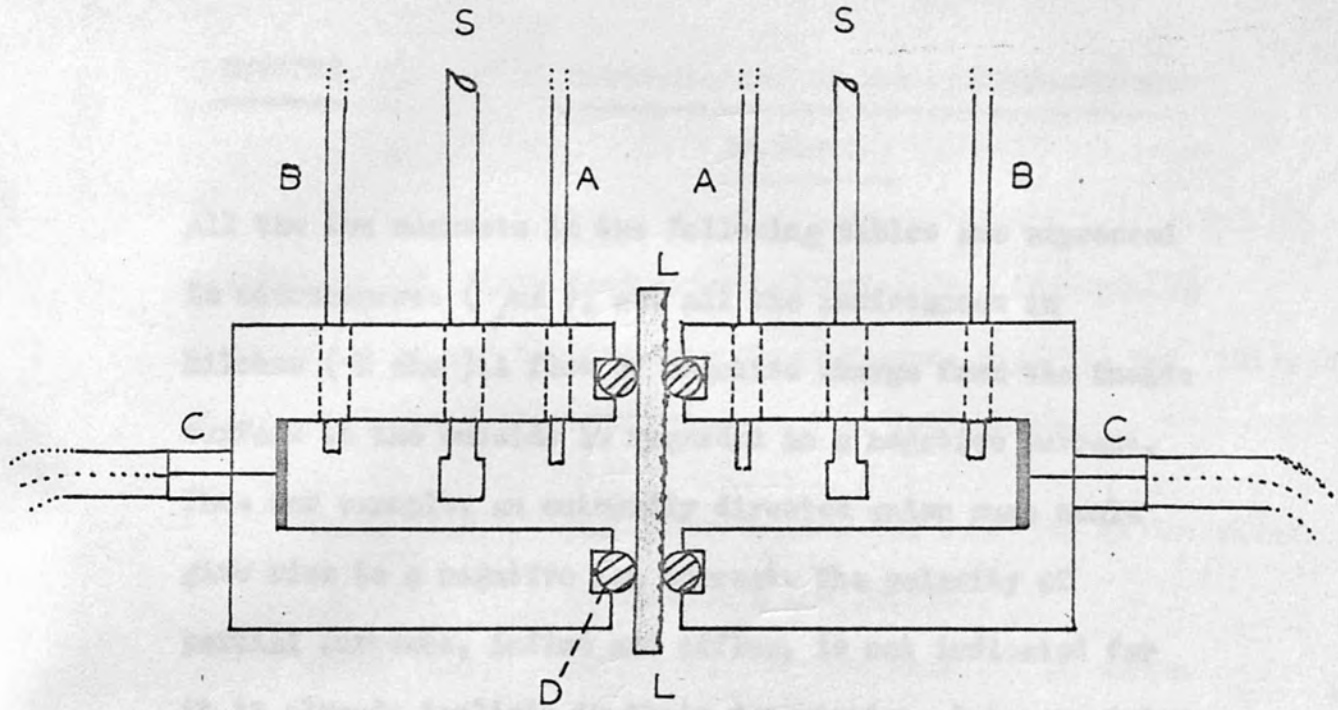

fig 1.

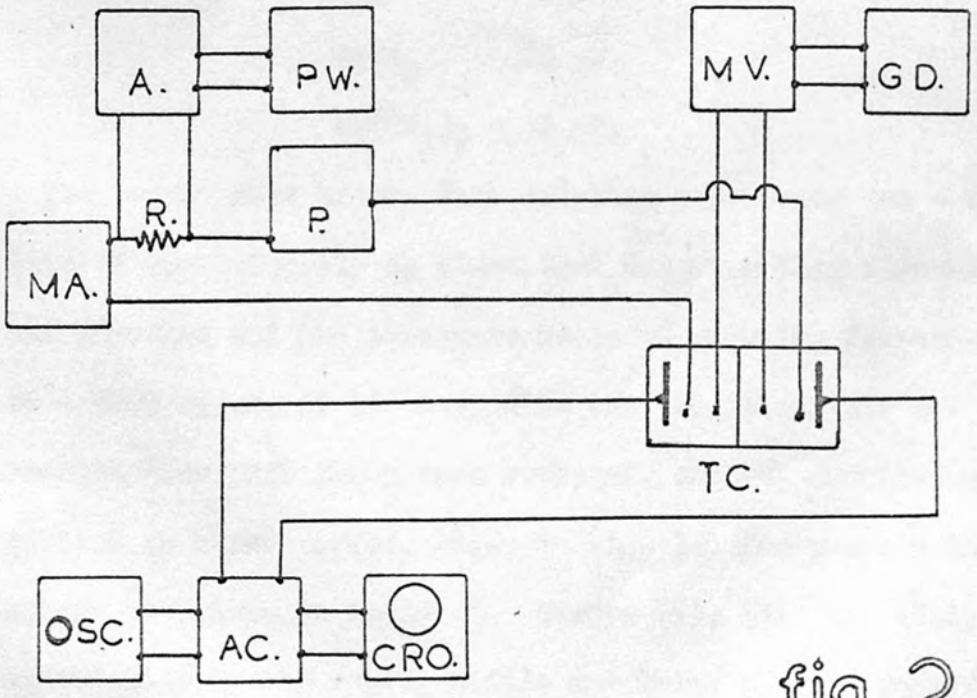

fig 2.

RESULTS

All the ion currents in the following tables are expressed in microamperes (μA), and all the resistances in kilohms (K ohm).A flow of negative charge from the inside surface to the outside is regarded as a negative current. Thus for example, an outwardly directed anion pump would give rise to a negative ion current. The polarity of partial currents, influx and efflux, is not indicated for it is already implicit in their description, but a reminder of the experimental arrangement is included before each table.

Experiment 1. Determination of the transglandular impedance.

A selection of thirty leaf discs collected from four plants was randomised and divided into three groups, (i),(ii) and (iii). Groups (ii) and (iii) were pretreated as follows :-

Group (ii) The lower cuticle was removed, as described in Materials and Methods.

Group (iii) Both cuticles were removed, as above.

All the groups were incubated with a solution of composition,

NaCl	100 mM.
KCl	2 mM.
$MgCl_2$	10 mM.
$Ca(NO_3)_2$	2 mM.

for twenty four hours. This solution represents sea - water diluted approximately 4½ times. Each disc was then clamped into the chambers and its impedance measured over the frequency range 20 - 2000 c.p.s. at 100 mv., with the bridge circuit and oscillator. Results from each group were averaged, and the results were plotted as three curves, shown in Fig. 3. The average impedance values are given in Table 5. Groups (i), (ii) and (iii) are represented by the upper, middle and lower curves, respectively,

(solid line). It can be seen that each cuticle with its associated glands behaves as a resistance which is effectively shunted at high frequencies by a capacitative element in parallel. The relatively frequency - independent impedance of the leaf parenchyma (group (iii), lower curve), and its low value indicate that it is, as would be expected, a slow diffusion zone presumably via the apoplasm.

TABLE V

Experiment 1. (Impedance of the Limonium leaf as a function of frequency.)

Group (i), K ohm.	Group (ii), K ohm.	Group (iii), K ohm.	f, c.p.s.
47.75	11.26	1.90	20
41.00	10.94		50
		1.72	100
21.70	8.34		200
10.20	4.46	1.56	500
4.67	2.60		1000
2.30	1.55	1.28	2000

Area of leaf disc = 1.76 cm.^{2}

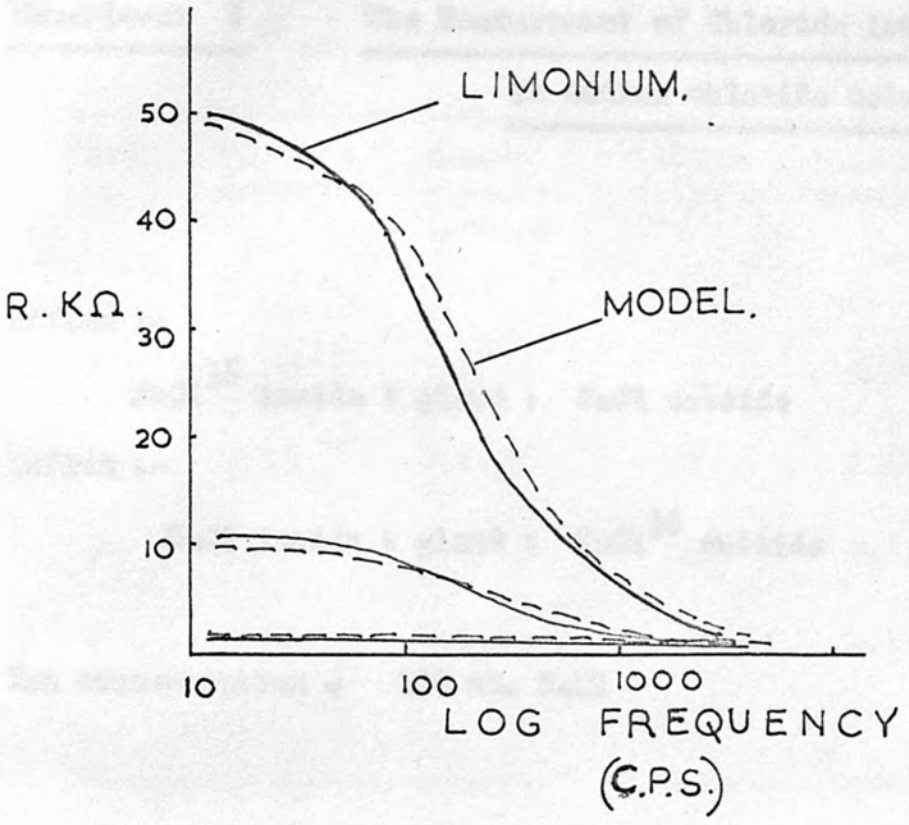
LIMONIUM.
MODEL.
R. KΩ.
50
40
30
20
10
10
100
1000
LOG FREQUENCY
(C.P.S.)

fig 3.

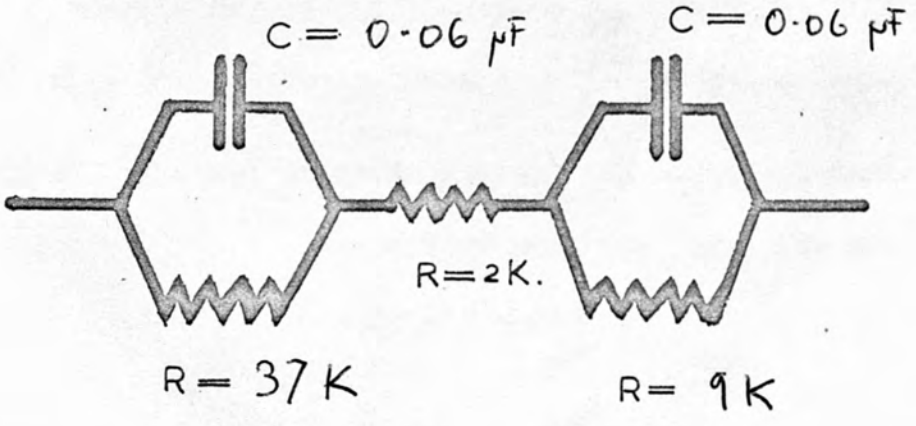
C = 0·06 μF
C = 0·06 μF
R = 2K.
R = 37K
R = 9K

fig 4.

Experiment 2 The Measurement of Chloride ion transport in sodium chloride solution

Efflux :-

$NaCl^{36}$ inside : gland : NaCl outside

Influx :-

NaCl inside : gland : $NaCl^{36}$ outside

Ion concentration = 100 mM. NaCl

Chlorine^{36} has a half-life of 3.03×10^{5} years, and emits no gamma radiation, only beta.

Cl^{36} efflux			Cl^{36} influx		
Disc No.	chloride current μA	short-circuit current μA	Disc No.	chloride current μA	short-circuit current μA
1	6.28	-2.50	5	0.46	-1.53
2	7.11	-2.87	6	2.29	-2.20
3	5.25	-1.86	7	0.43	-1.50
4	4.22	-1.43	8	0.10	-1.55
Average chloride current =	5.72		Average chloride current =	0.82	

Net average chloride ion current = -4.90 μA

Average short-circuit current = -1.93 μA

Note: The net chloride current was negative, indicating the outward transport of chloride ions; the short-circuit current is negative also.

Experiment 3 The measurement of sodium ion transport in sodium chloride solution

Efflux :-

$Na^{22}Cl$ inside : gland : NaCl outside

Influx :-

NaCl inside : gland : $Na^{22}Cl$ outside

Ion concentration = 100 mM. NaCl

Sodium^{22} has a half-life of 2.6 years, emitting gamma and + beta (positrons).

Na^{22} efflux			Na^{22} influx		
Disc No.	sodium current	short-circuit current	Disc No.	sodium current	short-circuit current
	/uA	/uA		/uA	/uA
1	3.37	-1.42	5	0.25	-3.4
2	2.9	-3.5	6	0.34	-1.4
3	1.1	-0.7	7	0.38	-0.6
4	4.65	-1.65	8	0.33	-2.2

Average sodium current = 3.03

Average sodium current = 0.33

Net average sodium current = 2.70 /uA

Average short-circuit current = -1.86 /uA

Note: The net sodium current was positive, indicating an outward transport of sodium ions.

Experiment 4 — **The measurement of potassium ion transport in potassium chloride solution**

Efflux :-

$K^{42}Cl$ inside : gland : KCl outside

Influx :-

KCl inside : gland : $K^{42}Cl$ outside

Ion concentration = 100 mM. KCl

Potassium^{42} has a half-life of 12.45 hours, and emits gamma and beta.

K^{42} efflux			K^{42} influx		
Disc No.	potassium current	short-circuit current	Disc No.	potassium current	short-circuit current
	μA	μA		μA	μA
2	0.48	0.08	1	0.50	0.51
3	2.37	0.38	6	5.72	-0.22
4	1.18	0.41	7	10.84	-0.27
5	1.49	-0.12	8	2.09	-0.29
Average potassium current =	1.38		Average potassium current =	4.79	

Net average potassium current = 3.41 μA

Average circuit current = 0.06 μA

Note: The net short circuit current is virtually zero, being sometimes positive and sometimes negative. The potassium ion transport, which is demonstrated, is <u>inwards</u>; this is in contrast to sodium, which shows an <u>outward</u> ion current of roughly similar magnitude.

Experiment 5 The measurement of rubidium ion transport in rubidium chloride solution

Efflux :-

$Rb^{86}Cl$ inside : gland : RbCl outside

Influx :-

RbCl inside : gland : $Rb^{86}Cl$ outside

Ion concentration = 100 mM. RbCl

Rubidium^{86} has a half-life of 18.7 days, emitting gamma and beta.

Rb^{86} efflux			Rb^{86} influx		
Disc No.	rubidium current	short-circuit current	Disc No.	rubidium current	short-circuit current
	μA	μA		μA	μA
1	3.09	2.30	5	0.46	0.16
2	10.35	0.40	6	0.81	0.37
3	2.59	0.05	7	1.71	0.12
4	42.0	0.30	8	5.84	0.17
Average rubidium current =	14.51		Average rubidium current =	2.21	

Net average rubidium ion current = 12.30 μA

Average short-circuit current = 0.48 μA

Note: The <u>outward</u> transport of rubidium ions is demonstrated. The ion current is quite large, some four times that of sodium or potassium. The short-circuit current is consistently positive.

Experiment 6 The measurement of caesium ion transport in caesium chloride solution

Efflux :-

$Cs^{137}Cl$ inside : gland : CsCl outside

Influx :-

CsCl inside : gland : $Cs^{137}Cl$ outside

Ion concentration = 100 mM. CsCl

Caesium^{137} has a half-life of 30.0 years, emitting gamma and beta.

Cs^{137} efflux			Cs^{137} influx		
Disc No.	caesium current	short-circuit current	Disc No.	caesium current	short-circuit current
	μA	μA		μA	μA
3	9.60	0.44	1	1.44	0.30
4	13.93	0.98	2	6.87	0.17
5	22.23	1.24	7	3.36	0.21
6	15.65	0.74	8	4.07	0.53
Average caesium current =	15.35		Average caesium current =	3.94	

Net average caesium ion current = 11.41 μA

Average short-circuit current = 0.58 μA

Note: Very similar to rubidium; a positive short-circuit current, and a large net caesium current, directed outwards.

Experiment 7 The measurement of bromide ion transport in sodium bromide solution

Efflux :-

$NaBr^{82}$ inside : gland : NaBr outside

Influx :-

NaBr inside : gland : $NaBr^{82}$ outside

Ion concentration = 100 mM. NaBr

Bromine^{82} has a half-life of 36 hours, emitting gamma and beta.

Br^{82} efflux			Br^{82} influx		
Disc No.	bromide current	short-circuit current	Disc No.	bromide current	short-circuit current
	μA	μA		μA	μA
5	3.50	0.04	1	4.73	-0.5
6	3.39	-0.20	2	0.10	-0.5
7	17.77	1.26	3	0.41	-0.93
8	3.63	-0.90	4	4.50	0.65
Average bromide current =	7.07		Average bromide current =	2.44	

Net average bromide ion current = -4.63 μA

Average short-circuit current = -0.14 μA

Note: The short-circuit current was again of variable polarity and the bromide currents show considerable variation; the net ion current is very similar to that in the chloride transport experiments.

Experiment 8 The measursement of iodide ion transport in sodium iodide solution

Efflux :-

NaI^{131} inside : gland : NaI outside

Influx :-

NaI inside : gland : NaI^{131} outside

Ion concentration 100 mM. NaI

$Iodine^{131}$ has a half-life of 8.04 days, emitting gamma and beta.

I^{131} efflux			I^{131} influx		
Disc No.	iodide current	short-circuit current	Disc No.	iodide current	short-circuit current
	μA	μA		μA	μA
5	5.12	0.04	1	0.51	-0.35
6	3.76	0.14	2	0.75	0.16
7	1.67	0.02	3	1.54	*0.021
8	8.69	0.29	4	0.57	-0.06
Average iodide current =		4.81	Average iodide current =		0.84

Net average iodide ion current = -3.97 μA

Average short-circuit current = 0.03 μA

Note: Similar to bromide; the short-circuit current is of variable polarity, with a net <u>outward</u> iodide transport similar to chloride and bromide.

* Circuit current actually reversed polarity during this experiment.

Experiment 9 The measurement of chloride ion transport in a sodium/potassium chloride solution

Efflux :-

$-Cl^{36}$ inside : gland : -Cl outside

Influx :-

-Cl inside : gland : $-Cl^{36}$ outside

Ion concentrations = Na^{+} 100 mM

K^{+} 2 mM

Mg^{2+} 10 mM

Ca^{2+} 2 mM

Cl^{-} 122 mM

This is the same solution as was used in Experiment 1., and represents sea-water diluted some 4 - 5 times.

Cl^{36} efflux			Cl^{36} influx		
Disc No.	chloride current	short-circuit current	Disc No.	chloride current	short-circuit current
	μA	μA		μA	μA
1	3.01	-1.28	1a	0.12	-1.20
2	3.13	-1.12	2a	2.83	-2.03
3	3.47	-2.58	3a	0.80	-3.16
4	3.34	-1.41	4a	0.00	-2.23
5	2.63	-0.39	8	0.04	
6	0.90	-0.31			
7	0.92	-0.17			
Average chloride current =		2.42	Average chloride current =		0.76

Net average chloride current = -1.73 μA

Average short-circuit current = -1.39 μA

Note: The net chloride ion current is reduced to about a third of its value in 100 mM NaCl solutions (Exp. 2). The short-circuit current is approximately normal, and always negative.

Experiment 10 — The measurement of short-circuit current in solutions of sodium and potassium borate.

The borate ion was chosen as being one which is not 'actively' transported by the gland, a reasonable assumption. It is not known to participate directly in respiratory metabolism, and is non - toxic.

Note : Far from there being measureable short - circuit currents and potentials due to sodium or potassium ion transport, the tissue seems to be electrically dead. The resistances, which were also measured, indicate that the glandular membranes are fairly permeable to borate ions for their value is not high.

Currents and resistances in borate solutions

Sodium borate. Na 100 mM.

R.
12 K p.d. 0
12 K microamps. 0
16 K
13 K

Potassium borate. K 100 mM.

R.
10 K p.d. 0
14 K microamps. 0

Sodium 50 mM. potassium 50 mM. borate

R.
18 K p.d. 0
11 K microamps. 0
14 K

Experiment 11 — **The simultaneous measurement of electrical resistances and ion conductances in sodium chloride solution**

Influx :-

NaCl inside : gland : $Na^{22}Cl^{36}$ outside

Ion concentration = 100 mM. NaCl.

Note : Assuming that the influx of sodium and ~~potassium~~ chloride ions is passive (Exps. 2 & 3), the measurement of these fluxes leads to a calculated value of the total ion conductance of the discs in sodium chloride solution (page 32). The D.C. resistance of the tissue should be equivalent to the total ion conductance.

Na^{22} and Cl^{36} influxes were measured in short-circuit experiments by combined assay ; after counting the mixed isotopes in a disc, the soft Cl^{36} betas were filtered out with an aluminium filter. The two isotopes could therefore be assayed separately. Impedance measurements were made on each disc at 20 c.p.s.

DISCUSSION

Disc no.	Resistance (K ohms)	Cl^{36} influx μA	Na^{22} influx μA
1	40	0.039	0.033
2	22	0.057	0
3	29	0.057	0.020
4	11	0.066	0.852

DISCUSSION

The complex impedance of the Limonium leaf is equivalent to a circuit shown in Fig. 4; having measured the low frequency impedance of the leaf disc with one or both cuticles removed, it is possible to calculate the magnitude of the capacitative element which shunts the resistances associated with the surfaces. An example will make this clear; the low frequency impedance of the disc with lower surface removed is (on average) 11 K ohm. Removing the remaining upper surface reduces the low frequency impedance to 2 K ohm, and this low impedance is relatively frequency-independent. The disc without lower surface is thus represented by the equivalent expression

$$Z = R_{(\text{parenchyma})} + \frac{R_{(\text{upper surface})}}{\sqrt{1 + w^2.C^2.R^2_{(\text{upper surface})}}}$$

where $w = 2\pi f$, f being the A.C. frequency

C = the capacitance.

Inserting the two known values, this reduces to

$$Z = 2 + \frac{10}{\sqrt{1 + 100.w^2.C^2}}$$

and as we have several values of Z at different frequencies (Table 5), an average value of C can be calculated. There are

certainly more accurate methods of obtaining C (e.g. graphical ones) but the capacitance does not really concern us in this study, and a value of C = 0.06 microfarads probably represents a good approximation.

An analogue of Fig. 4 was constructed from electrical resistors and capacitors, and its frequency characteristics were examined by using exactly the same apparatus as shown in the lower part of Fig. 2, with the analogue replacing the disc in the transport chamber. The dotted curves of Fig. 3 were obtained, showing that the fit is very good; the two RC elements of Fig. 4 represent the surfaces. A basic assumption in this frequency-analysis is that the low frequency impedance (20 c.p.s.) is equivalent to the D.C. resistance. In fact to test this, the D.C. resistance was measured over a range of 200 millivolts by passing a current through the leaf with the shorting circuit (Fig. 2), and the calculated resistance is here compared with the impedance of the leaf disc measured with A.C. at 20 c.p.s.

A.C. impedance	12.1 Kilohms
D.C. resistance	12.3 Kilohms

The A.C. impedance is 1.6% lower than the D.C. resistance, and this discrepancy is neglected in this study.

The impedance of the upper surface is thus 9/37 that of the lower surface, and expressing this as a conductance, we can say

that the upper surface has 4 times the conductance of the lower; it is now possible to predict that there should be some structural difference between these surfaces to account for these conductances, and it is indeed a fact that the upper surface has 3-5 times as many glands as the lower. Thus the value of 9 kilohms./cm.^{2} is identified with the transglandular resistance per cm.^{2} of upper surface, and the transcuticular conductance is neglected. The relationship of the electrically determined resistance to the ion conductance in the Limonium gland is investigated in Experiment 11.

It is essential to begin any interpretation of the transport experiments by accounting for the short-circuit current which flows during a typical experiment with a Limonium leaf disc. If this can be satisfactorily done, then all the ion transport processes contributing to the current will have been recognised, and it will also demonstrate that the experimental techniques are sound.

In Experiment 1, the net transport of chloride ions was determined in a sample of eight leaf discs, in pure sodium chloride solution. The average net ion current was 4.90 μA, and this represents an 'active' outward transport of the ion. The short-circuit current was 1.93 μA, and this was negative, and is thus equivalent to the outward movement of a negative ion,

or the inward movement of a positive one. We require, therefore, a transport of sodium ions in an outward direction, and of magnitude 4.90 - 1.93 = 2.97 μA, to account for the short-circuit current. The simultaneous transfer of both sodium ions and chloride ions in single leaf discs was not measured, and so it is only possible to compare the sodium currents in another sample of eight discs with the ion currents in Experiment 2.

Experiment 3 represents the measurement of the sodium currents in a sample of discs. The net average sodium current was 2.67 μA, and this represents an outward transport. In fact the average short-circuit current during this sodium transport experiment was -1.86 μA, and so the average short-circuit current for the two Experiments 2 and 3, is -1.89 μA, i.e. (1.93 + 1.86)/2. These results have been analysed statistically in the Appendix, where a sample calculation of a short-circuit experiment is also included. There is no significant difference between the average short-circuit current and the sum of the chloride and sodium net transfers, at $p = 0.05$; the summary of these results is set out below in Table 6.

It is possible to say, therefore, that the short-circuit current represents the difference between the net ion currents of sodium and chloride ions, and that the movement of no other ions is required to explain the observations. The 'active'

sodium efflux is about 80% that of the 'active' chloride efflux, in sodium chloride solution.

If we assume that the gland is a desalting organ, it seems surprising at first that there are two ion transport mechanisms operating; one would be quite sufficient for the purpose. An outwardly directed chloride pump would cause the passive efflux of cations, and the proportions of these in the exudate could be regulated by the selectivity of the membranes towards positive ions. The small size of the short-circuit current is due as we have seen, to a sodium and chloride pump acting in the same direction; where they opposed, as is the case in Halicystis ovalis[43] (Table 3), the current would have been about 8 microamps (2.68 μA for Na + 4.9 μA for K), i.e. 4.3 μA/cm.^{2} taking account of the disc area (1.76 cm.^{2}), and this is comparable with the smallest currents obtained from Halicystis.

As the other halide ions usually have similar physiological properties to chloride ions, their transport was studied, using sodium radiobromide and sodium radio-iodide, Experiments 7 and 8. It can be seen that both ions are <u>outwardly</u> transported in a similar manner to chloride, and to a similar extent: in fact, there is no reason to believe that they do not substitute for

chloride and that they are transported by the same pump. Of especial interest is the polarity of the circuit current which appears to vary from disc to disc, giving a very small average value.

The transport of potassium ions is different from the other alkali metal ions, Experiment 4. There is certainly a net ion current, indicating the operation of a potassium pump, but it is an inward transport, of apparently equal size to that of sodium. It is difficult at first to reconcile this inward potassium transport with any mechanism of desalination. The concentration of potassium ions in seawater is quite low (roughly 10 mM.) compared to 100 mM. KCl solution, whilst the sodium concentration is much higher than 100 mM. (400-500 mM.), and thus the most pressing requirement is to lower the sodium ion concentration in the tissue. In view of the fact that most cells excrete sodium, but often accumulate potassium to high levels, one might venture the suggestion that (here at any rate) desalination really implies a lowering of the intracellular sodium concentration, and that the potassium accumulation mechanism therefore still operates. In view of the fact that potassium accumulation is often 'linked' to sodium excretion[37,38], it is interesting to compare the relative magnitudes of the two

transports. MacRobbie[35] has shown in Nitella translucens that the ratio of active sodium efflux to active potassium influx lies in the range 1.1-1.4, and Spanswick and Williams[40] have shown that in this species the specific inhibitor of the coupled sodium/potassium pump, the cardiac glycoside ouabain, does not affect the membrane potential; thus 'neutral' pumps do seem to exist in plants. Although in Limonium the separate ion transport mechanisms can apparently operate in the absence of each other it is interesting to note that the ratio of net sodium current in 100 mM. NaCl to net potassium current in 100 mM. KCl is 1.1 in Limonium. Does this represent the separate electrogenic action of two pumps which are usually linked together as a 'neutral' exchange pump?

Experiments 5 and 6 indicate that rubidium and caesium ions are actively transported in an <u>outward</u> direction. The net average ion currents of these two alkali metal ions are some three times larger than the net sodium current in sodium chloride solution (Experiment 3), but their similarity indicates that they are most probably transported by the same mechanism; there is indeed no reason why the Limonium gland should not possess a general cation pump with differing affinities for the various alkali metal ions. Considering the fact that rubidium and caesium are present in seawater in low concentration (less than

10^{-3} x potassium concentration), it would seem mildly unusual for a separate mechanism to exist for their transport. The average short-circuit is small in Experiments 5 and 6, but the fact that it is positive shows that cation transport was always greater than the chloride transport.

The magnitude of the short-circuit current can provide information of the greatest interest once the assumption is made that it represents the disparity between the individual cation and anion transport; we have seen that this assumption is most probably correct by analysis of the transport in sodium chloride solutions (Experiments 2 and 3). Examination of the short-circuit current in potassium chloride solution (Experiment 4), now shows that its value is quite minute (+ 0.06 μA) and this must mean that the chloride pump in this experiment was compensating for the potassium ion current, and was in consequence directed inwards. The chloride pump seems to reverse itself during potassium ion transport in pure potassium chloride solution.

Reference to the short-circuit current in rubidium chloride (Experiment 5) and caesium chloride (Experiment 6) indicates another effect. Here the small value of the currents indicates that the chloride ion current must have been of similar

magnitude to that of rubidium and caesium, and directed outwards. The chloride ion transport seems therefore to have increased in magnitude by a factor of three or so, when compared with its value in sodium chloride solutions (Experiment 3). The cation transport thus seems to determine both the direction and the magnitude of the chloride transport, or assuming physiological equivalence of bromide and iodide ions, the halide transport process. Transport of anion and cation are linked together in such a way that they are fairly evenly matched, and consequently the measurable potentials and currents in Limonium are very small. The position is set out in Table 7. It is as well to consider what this implies from a general standpoint; in a situation where the anion is not 'actively' transported at all, the movement of anions is always in the same direction and of equal magnitude to the cation transport. This much is demanded by the principle of electroneutrality. The polarity of any potential or short-circuit current measured in such a situation is dictated by the cationic charge (+ve) and the magnitude of the cation transport process. We say that the anion passively 'accompanies' the 'active' transport of cations. The situation which seems to operate in Limonium, however, is one in which the 'active' transport of anions (chloride ions and probably halide ions in general) is geared to the transport of cations, both with respect to direction and magnitude.

The extent to which this matching process operates can be judged from two important observations:

(i) The circuit currents in Experiments 4, 7 and 8 are of variable polarity, although the net short-circuit currents are small (0·06, 0·48 and 0·58 respectively). It seems that when the matching is fairly good corresponding to a small short-circuit current, either of the transports, cation or anion, can exceed the other, leading to either positive or negative currents.

(ii) The circuit current drifts the whole time during an experiment, and where it is small, it can change polarity during an experiment; in fact this actually happened during Experiment 8 (iodide transport) in Disc no. 3, whose current slowly drifted over the zero-line, ending up negative.

The matching process therefore seems to be only approximate, and we can say that over short time intervals the extent of the matching appears to be a statistical process, subject to random fluctuation.

Experiments 9 and 10 must be considered before we can definitely assume that the ion transport mechanisms are not really independent, and in fact they strongly support this assumption. In Experiment 9 the transport of chloride ions

was studied with Cl^{36} in a medium representing diluted sea-water, as opposed to pure sodium chloride solution (Experiment 2). The net chloride ion current was considerably reduced in this medium, but it is interesting to note that the influx, which is presumably passive, was virtually unchanged (0.76 as compared with 0.82 μA in Experiment 2). The net chloride efflux has in fact been reduced to 35% of its value in sodium chloride solution. Bearing in mind that the chloride transport seems to reverse in pure potassium chloride media, it is a possibility that the presence of potassium is causing a reduction in the 'active' chloride efflux, and there could come a point where a high potassium/sodium concentration ratio would reduce the net chloride transport to zero. This is obviously a point to be settled by future experiments, but Experiment 9 nevertheless shows that the chloride transport is reduced by the presence of other cations in the medium.

Experiment 10 is a study of the short-circuit currents in solutions of sodium and potassium borate. Borate was chosen as an ion which is presumably not transported by a specific ion pump, and which is not toxic or actively metabolised. If there exists an independent sodium ion pump whose function is to excrete sodium ions from the Limonium gland, then the short-circuit current should increase to a positive value of about 4 μA, and the reverse should occur in potassium borate solutions. There was

in fact no electrical activity whatsoever; the probability that borate transport is taking place, and to an exactly similar extent to sodium ion transport in all discs studied, thus giving rise to no measurable short-circuit current, must surely be remote. The resistances are comparable to those obtained from discs in pure sodium chloride solution, indicating that the glandular membranes cannot really be any less permeable to borate ions than chloride ions.

If we abandon the picture of independent ion pumps in Limonium, what mechanism can possibly explain the linkage observed between cation and anion transport? If we regard a neutral transfer pump (i.e. a pump which would transfer a cation and an anion outwards with perfect 1 : 1 stoichiometry in a similar manner to the neutral potassium/sodium exchange pump) as being a molecular linkage, and reject this explanation on the grounds that it is far from stoichiometric, and certainly electrogenic, we are left with two strong possibilities: metabolic linkage, and pinocytosis[21].

Coupling of ion transports by metabolic linkage is illustrated by sodium and potassium transport in Ulva lactuca[71]. Here the two transports are of similar magnitude and opposite direction, and when the tissue is put into darkness, both pumps cease to function. Scott and Hayward have shown that the

sodium pump seems to be linked quite directly to light utilisation, probably via photophosphorylation, whilst the potassium pump is geared to glycolysis via the dark reactions of photosynthesis; during illumination these two ion pumps seem to function together and at equal rates.

This view of linkage, if applied to the present results, would require two separate chloride pumps to explain the reversal of chloride ion transport in pure potassium chloride solutions. This is not so unusual as it might seem; many workers have postulated the existence of separate chloride pumps to account for chloride transport over the plasmalemma and the tonoplast[25,24,98]. The two chloride pumps would then be linked to the transport of sodium ions and potassium ions in different directions. The nature of the metabolic linkage must on one hand be that of a 'tightly' coupled one, for cation transport does not take place in the absence of halide ions as we have seen, and yet on the other, the coupling must be 'loose' enough to allow for considerable differences in rates, which manifest themselves as the constant fluctuations in short-circuit current. Experiment 9 also shows that the presence of other cations can reduce the efflux of chloride ions, leaving the influx unchanged, and this is difficult to reconcile with two chloride pumps working in opposite directions.

(a) Pinocytosis would provide a simpler explanation of the apparent linkage of cation and anion transport, in that the vesicles would possess approximate electroneutrality, and thus they would transfer mainly salt, not separate ions. The pinocytotic mechanism envisaged is that of membrane bounded vesicles being formed from a pre-existing membrane, either the inner plasmalemma of a gland cell or one inside the cytoplasm, and these vesicles crossing the cytoplasm and fusing with the outer plasmalemma of the gland cell to liberate their contents through the gland pores, Plate 2. This outer plasmalemma has an area which has been anatomically restricted to that exposed through minute pores, and this fact suggests that the ion transport mechanism is associated with this outer membrane. If this were not so, the pore-restricted membrane would act as a serious diffusion barrier to ions leaving or approaching the transport sites, and it would seem to be a basic principle that the membrane at which an 'active' ion transport mechanism is operative should possess low conductance to the ion in question or else the high permeability will 'short-circuit' the transport process. An example of this principle operating in another tissue is that of the frog skin permeability[2,95].

The weak electrogenic nature of the vesicular transport could be explained by

(a) The operation of cation or anion pumps across the vesicle membrane, after their detachment from the mother membrane. Thus the vesicles could arrive at the opposite cell membrane with a cation or anion imbalance giving rise to ion transport with measurable circuit current,

or

(b) The existence of an electrical double layer at the mother membrane. If the dimensions of the vesicle were comparable to those of this minute layer the vesicles might contain net charge i.e. an excess of cation or anion due to ionic asymmetry in the layer. (The theory of the double layer is given by Briggs et al.[44])

Transport by membrane bounded vesicles implies contrary movement of vesicles containing sodium chloride to those containing potassium chloride, but this is quite feasible. It is well known that certain cations are required for the induction of pinocytotic drinking in Chaos chaos, the giant amoeba[73], and that a preliminary phase to drinking is ion binding on the cell surface. It is quite conceivable that the two plasmalemma surfaces (outer and inner) of the gland cells are differently activated by sodium and potassium; halide ions would obviously be required to complete the process,

whereas borate ions would be incapable of this. The random fluctuations of short-circuit current still have to be explained, and this may be due to a combination of (a) and (b).

It is impossible to decide between the two major possibilities, pinocytosis or metabolic linkage on the present evidence. A conclusive test of membrane vesiculation would be the measurement of the electrogenic drift during a transport experiment and the demonstration that it could be resolved into 'transport noise', which would be quantal in nature: this has been done with the transport of acetylcholine in synaptic vesicles by nerve physiologists[99]. It is also of interest that the unexplained linkage between plasmalemma fluxes of chloride ions and tonoplast fluxes of potassium and chloride ions, found by MacRobbie in Nitella translucens[39] can be interpreted as evidence for pinocytotic transfer into the vacuole. Sutcliffe[62] has also claimed that electron micrographs of Limonium glandular tissue reveal many minute vesicles scattered throughout the cytoplasm; thus pinocytosis seems a strong candidate.

Experiment 11 is a study of the ion conductances in Limonium. As described in Part 4 of the Introduction, there exists a serious discrepancy between ion conductances and

electrical resistances in many algal cells, as well as animal ones. From the expression for ion conductance

$$1/R_M = \frac{F^2}{RT}(\Sigma\phi^- + \Sigma\phi^+)^{101}$$

we can derive the equivalent expression

$$1/R_M = \frac{i_{(total)}}{25.3}$$

where R_M = membrane resistance in kilohms

$i_{(total)}$ = sum of the effluxes (or influxes) expressed in microamperes.

It may be argued that the above expressions are derived under the conditions of 'zero current flow' and are therefore inapplicable to any of the data presented above. This is not so, however, for the conditions of 'zero current flow' really imply the absence of passive ion current, whilst the short-circuit current represents a non-passive ion current due to transport mechanisms. In the special case of a tissue which has been voltage-clamped at zero with no activity gradients operating, the above expression for membrane resistance is perfectly valid. Direct calculation of the total inward currents and thus ion conductances for the four discs indicate that the discrepancies noted in other systems also exist here, Table 8.

Dainty, Croghan and Fensom[100] have proposed that an alternative explanation to ion filing is that electro-osmosis is taking place, and that the flow of solvent through the pores can alter the effective ionic mobilities in the membrane.

It seems from the results obtained here that neither explanation is really applicable. The size of the discrepancy varies from about two to above twenty, and this variability makes the ion-filing hypothesis rather unlikely, although it does not rule it out completely. Any electro-osmotic effect is however extremely unlikely, for under a zero voltage-clamp the occurrence of electro-osmosis is rather improbable. Perhaps another explanation for the discrepancy is that the membrane possesses channels through which charge can flow, but not ions; it is interesting to note in support of this that frog skin appears to have an impedance which is increased by inhibition of the sodium pump[103]; the skin is quite impermeable to sodium under normal conditions.

TABLE VI

Average net chloride ion current (Experiment 2) (ion flux outwards)	=	-4.90 μA
Average net sodium ion current (Experiment 3) (ion flux outwards)	=	+2.67 μA
Average short-circuit current for the two experiments	=	-1.89 μA
Net ion current outwards (chloride current + sodium current)	=	-2.23 μA

Table VII

Cl^- transport in:	Magnitude and Direction μA	Comment
NaCl	4.90 outward	Measured with Cl^{36}
KCl	3.47 inward	Difference between K^{42} current and short-circuit current
RbCl	11.82 outward	Difference between Rb^{86} current and short-circuit current
CsCl	10.83 outward	Difference between Cs^{137} current and short-circuit current

TABLE VIII

Electrical resistance and ion conductance in Limonium

Disc No.	R_M Kilohms	Impedance at 20 c.p.s.
1	351	40
2	444	22
3	328	29
4	28	11

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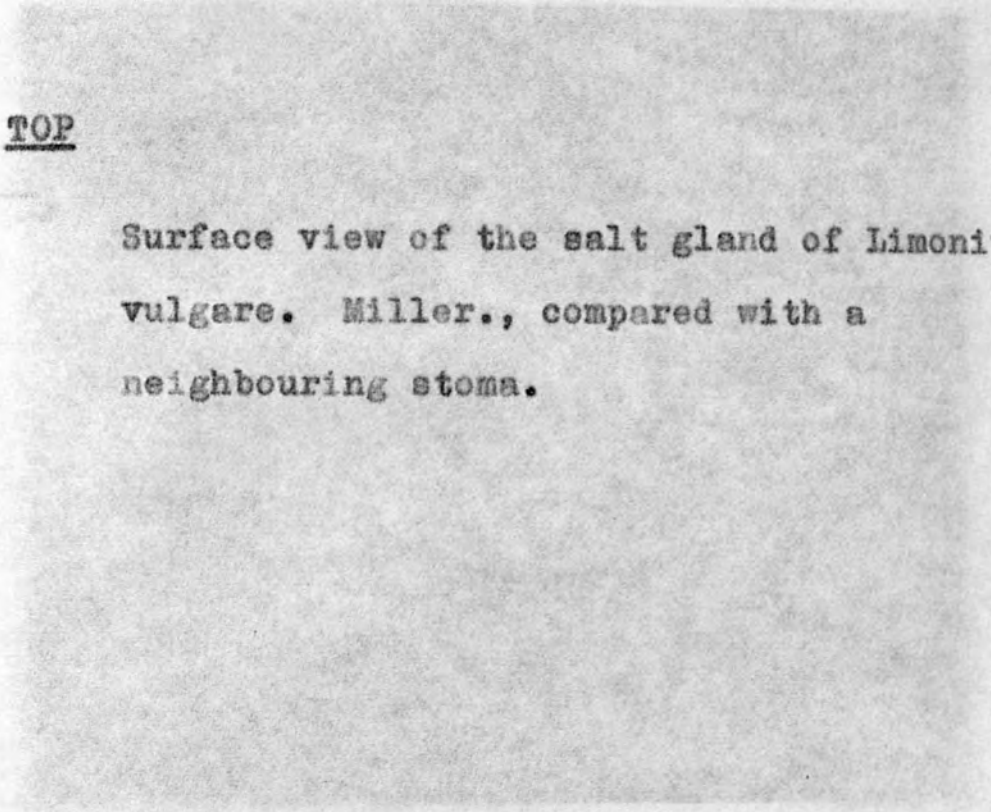
APPENDIX

Plate I

Plate II

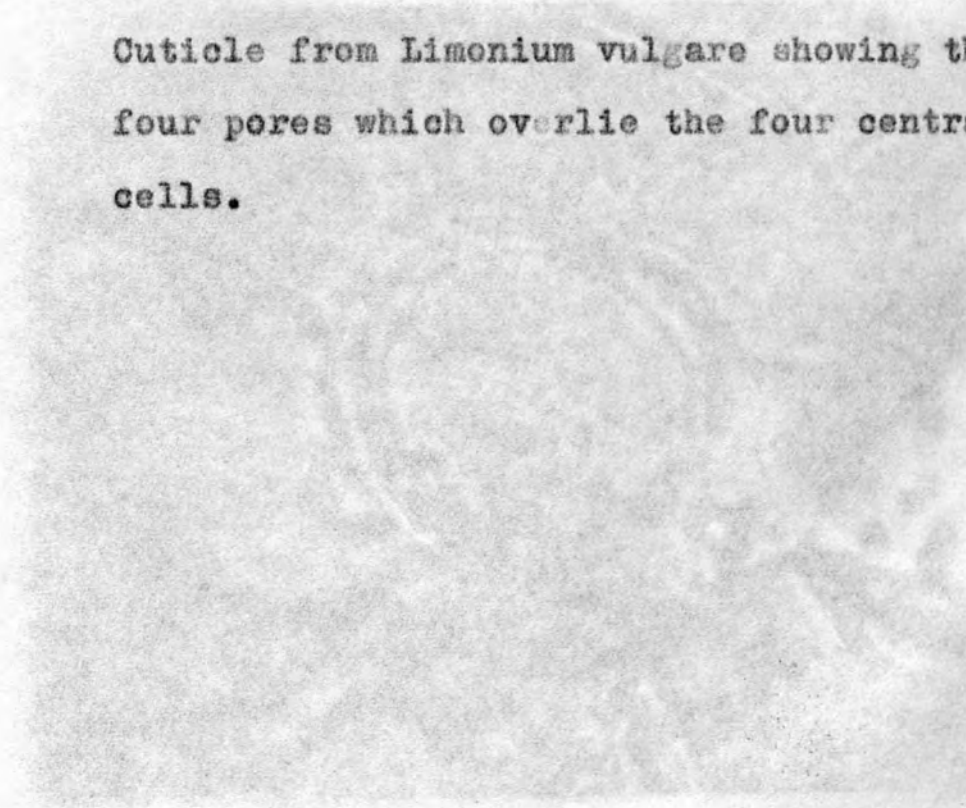
(i) Example of result sheet and calculation of short-circuit current.

(ii) Analysis of variance in Experiments 2 and 3.



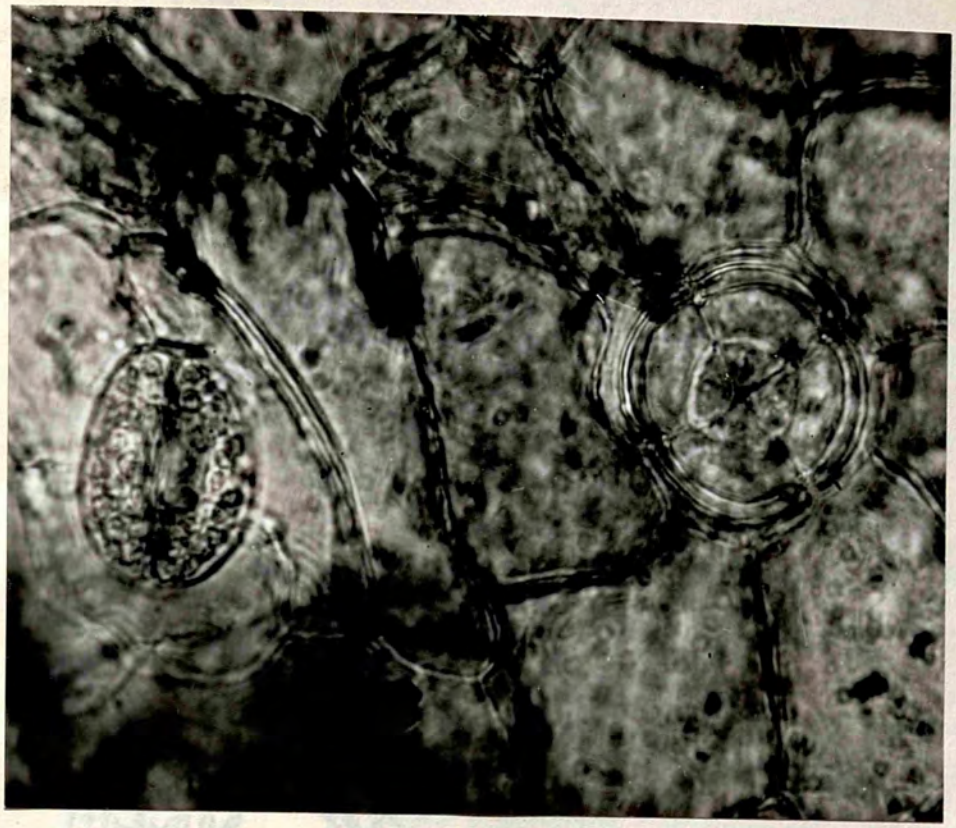
TOP

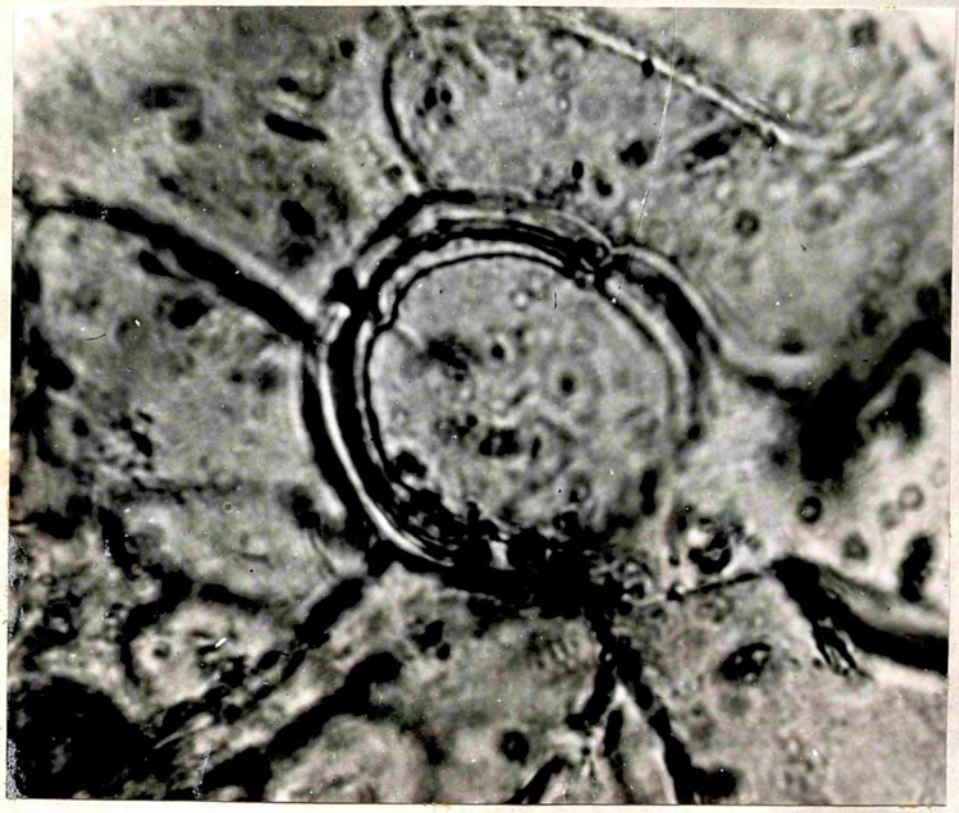
Surface view of the salt gland of Limonium vulgare. Miller., compared with a neighbouring stoma.

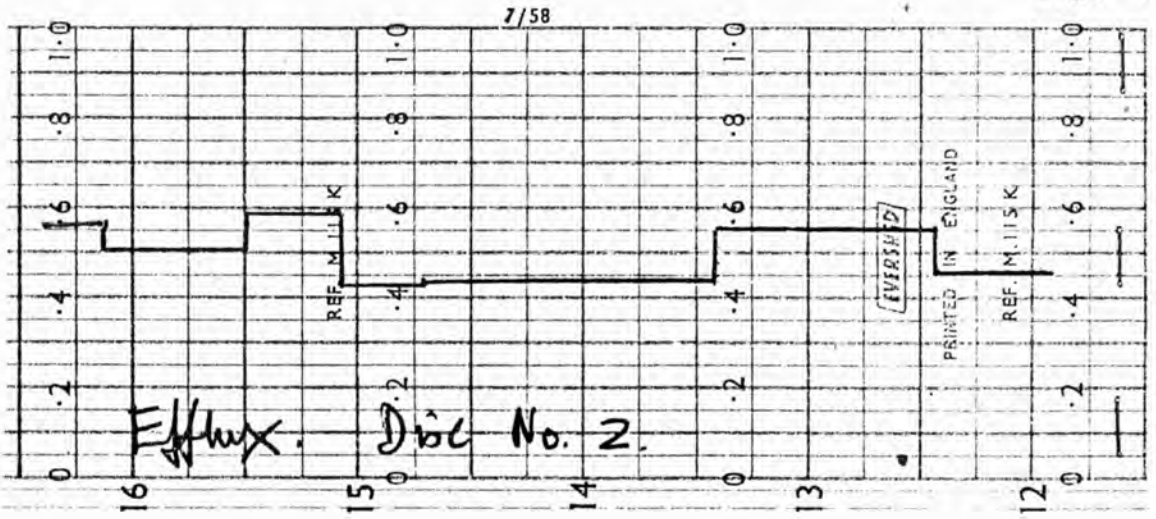


BOTTOM

Cuticle from Limonium vulgare showing the four pores which overlie the four central cells.







Cl^{36} transport 29/8/63.

100 mM NaCl solution.

Average short-circuit current = 0.5 μA

Time of experiment = 2700 secs.

Count 640 / 10^2 sec.

Background 26 / 10^2 secs.

Net count :— 614 / 10^2 secs.

Activity of sol^{n} — 845500 c.p. / 10^2 secs. per. cc.

mV = 9.5.

Cl^{36} experiment :— (100 mM $NaCl^{36}$)

24/8/63.

Efflux currents.

Disc. No. 2.

$$Cl(\mu A) = \frac{MC}{AS} \cdot 96.5 \times 10^6$$

$$= \frac{0.1 \times 614 \times 96.5 \times 10^6}{845500 \times 2700}$$

$$= 2.595$$

$$= 2.60 \ \mu A.$$

APPENDIX

(ii) Analysis of variance ; the results of experiments 2 and 3.

Four values of the average net ion current, i.e.

(chloride efflux - chloride influx) minus

(sodium efflux - sodium influx) ,

were obtained by random selection, and these were paired with their corresponding average short-circuit currents.

Cl - Na	/μ A
- 2.70	- 2.21
- 2.26	- 2.49
- 0.50	- 1.17
- 3.40	- 1.17
- 8.86	- 7.58

Analysis of Variance, cont.

	D.F.	S.S.	M.S.	V.R.
S.S. between samples	1	0.22	0.22	0.23
S.S. within samples	6	5.59	0.93	
S.S. total	7	5.81		

For 6 degrees of freedom, $P = 0.05$, $t = 2.447$.
Since the calculated value of t is 0.23, the difference between the average net ion current and the short-circuit current is not significant. The short-circuit current can therefore be said to represent the difference between the net transport of chloride and sodium ions, in pure sodium chloride solution.