THE ELECTRIC DOUBLE LAYER AND THE DONNAN EQUILIBRIUM IN RELATION TO PLANT CELL WALLS

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Summary

The division of free space in plant tissues into "water free space" (W.F.S.) and "Donnan free space" (D.F.S.) is examined in systems which contain electrically charged surfaces separated by various distances. It is suggested that plant cell walls should be described in terms of a system of electric double layers and not by classical Donnan equations. An approximate theory is presented which results in an expression for the equivalent width of D.F.S. in terms of the external concentration but which is independent of the surface charge density.

The theory is applied to experiments on the replacement of calcium by sodium in cell-wall segments of *Chara australis* and to data in the literature on solutions of gum arabic and on jute fibres. The variation in apparent concentration of indiffusible ion in these systems with external concentration is consistent with the double-layer theory and reaffirms that use of classical Donnan theory, which assumes a *homogeneous* charged system, is only permissible in limiting cases.

The double-layer theory leads to a value of 4×10^{-5} C . cm^{-2} for the average charge density in cell walls from *C. australis*.

I. Introduction

Cation exchange between plant cells and an external medium has been observed for many years, but only recently have attempts been made both to put it on a quantitative basis and to establish the site of the initial cation exchange in plant cells. This site has been assumed by some to be the cytoplasm of the cell (containing proteins, lipids, phosphate compounds, etc., with a predominantly negative net charge) and by others to be the cell wall, which contains cellulose organized in microfibrils of various orientations, together with appreciable amounts of "pectins" and "hemicelluloses". The unmethylated form of pectin is polygalacturonic acid, and the hemicelluloses also contain many uronic acid residues, probably mainly glucuronic acid.

The use of preparations of cell walls isolated from large algal cells (*Chara*: Gaffey and Mullins 1958; Dainty and Hope 1959; Dainty, Hope, and Denby 1960; *Nitella*: Diamond and Solomon 1959), and from coleoptiles (Jansen *et al.* 1960), has shown conclusively that the site of the initial cation exchange is the cell wall and, moreover, that the "exchange capacity" can be accounted for by uronic acid, the presence of which has been established by chemical analysis.

In plant cells and tissues a division can be made of the space freely accessible by diffusion from the external medium into "water free space" and "Donnan free

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space" (Briggs and Robertson 1957; Briggs, Hope, and Pitman 1958). "Water free space" (W.F.S.) is that part of the space in which mobile cations and anions are in equal (equivalent) concentration, and corresponds to the extracellular space referred to by workers on animal cells or tissues. "Donnan free space" (D.F.S.) has in the past been assumed, for the purposes of analysis, to be a homogeneous space containing indiffusible or restrained ions and to which the classical Donnan equations relating internal and external ionic activities can be applied.

Considerable error may be involved in the assumption that any fixed part of plant cell walls corresponds to either W.F.S. or D.F.S., as defined. The polysaccharide chains, some of which contain uronic acid residues, are probably separated—to judge by electron-micrographs and by cell-wall permeability—by distances such that the classical Donnan equations would not be applicable, for which reasons have been stated by Overbeek (1956).
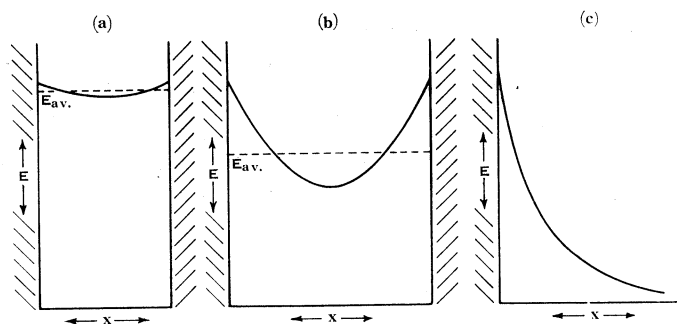

Fig. 1.—Variation in electric potential (E) with distance (x) between charged surfaces: (a) when the surfaces are relatively close together; (b) when the surfaces are further apart; (c) when the surfaces are infinitely separated. $E_{av.}$ is the average electric potential.

The argument can be illustrated by Figure 1. This shows the variations in electric potential E with distance (x) between charged surfaces, (a) in what would be an approximately homogeneous Donnan phase; (b) in a more "dilute" system where the potential approaches zero at the points of symmetry; and (c) for a single charged surface.

If the variation in potential between the surfaces is small, as in Figure 1(a), it is reasonable to suppose that the average potential will be related through a Donnan equation to the average concentrations of the cations ($\bar{C}_+$) and anions ($\bar{C}_-$); i.e. for this situation:

$$\bar{C}_{\pm} = C_o\{\overline{\exp(\mp FE/RT)}\} = C_o\{\exp(\mp FE_{av.}/RT)\}, \tag{1}$$

where C_o is the concentration of electrolyte, assumed uni-univalent and at some distance from the charged system, and $E_{av.}$ is the average potential (e.g. measured by a probe electrode) in the liquid phase of the charged system. F, R, and T have their usual significance. Activities are assumed equal to concentrations throughout.

If the variation in potential is large, as in Figures 1(b) and 1(c), it is not permissible to say that the average of $\exp(\mp EF/RT)$ is equal to $\exp(\mp E_{av.}F/RT)$. Since the physical situation in the cell wall is more likely to correspond to Figure 1(b) or 1(c) the question arises: where does W.F.S. end and D.F.S. begin? A theoretical treatment, based on Overbeek's remarks, has been developed to deal approximately with this situation and is described below.

II. THEORY

By use of Poisson's equation relating local electric potential to local volume charge density and Boltzman's statistical law (see equation (1) above), the Gouy–Chapman theory of the diffuse double layer near a uniformly charged plane surface leads to the following equations for the charge density on a surface and for the potential near a charged surface in the presence of an aqueous solution of a uni-univalent electrolyte:

$$\sigma = \sqrt{(\epsilon RT\, C_0/2\pi)}[\exp(E_oF/2RT) - \exp(-E_oF/2RT)], \qquad (2)$$

where σ = surface charge density in coulomb . cm^{-2},

ϵ = the permittivity of the medium [$80/(9 \times 10^{11})$ C.V^{-1} . cm^{-1} for water],

R = the gas constant ($8 \cdot 31$ J . mole^{-1}. deg^{-1}),

T = temperature in degrees Kelvin,

E_o = the electric potential of the charged surface, relative to the bulk phase, in volts,

C_o = the concentration in the bulk phase in equiv . cm^{-3},

F = 96,500 C . equiv.$^{-1}$,

and x, the separation distance in centimetres, is related to E, the potential at that distance by:

$$x = -\sqrt{\{\epsilon RT/8\pi F^2 C_o\}}\, \ln[\{\tanh(EF/4RT)\}/\{\tanh(E_oF/4RT)\}]. \qquad (3)$$

From these equations local concentrations can be calculated as functions of x. Analogous expressions can be derived for calcium sulphate or similar electrolytes in the bulk phase.

In a negatively charged double-layer system there is no sharp break into a D.F.S. and a W.F.S. — C_+ is greater than C_- from $x = 0$ to $x = \infty$. However, it is possible to calculate the formally equivalent D.F.S., for the system is equivalent to a D.F.S. extending from $x = 0$ to $x = x_D$ (to be determined) and a W.F.S. extending from $x = x_D$ to $x = \infty$. This is illustrated in Figure 2.

In the equivalent D.F.S. the concentration of indiffusible anions will be $\sigma/(F . x_D)$ equiv . cm^{-3} and the concentrations of the diffusible cations and anions C'_+ and C'_- will, by definition, obey the Donnan equations:

$$C'_+ . C'_- = C_o^2, \qquad (4)$$

and

$$C'_+ = C'_- - \sigma/(F . x_D). \qquad (5)$$

Outside the D.F.S. is W.F.S. where the concentration of both ions is C_o. Thus the amount of diffusible anion excluded per cm^2 according to this picture is $(C_o - C'_-)x_D$.

If this is to be equivalent to the effect produced in the real double layer, then:

$$(C_o - C'_-)x_D = \int_{x=0}^{\infty}(C_o - C_-)dx, \tag{6}$$

where C'_- is given by equations (4) and (5) and C_- by Boltzman's equation (1).
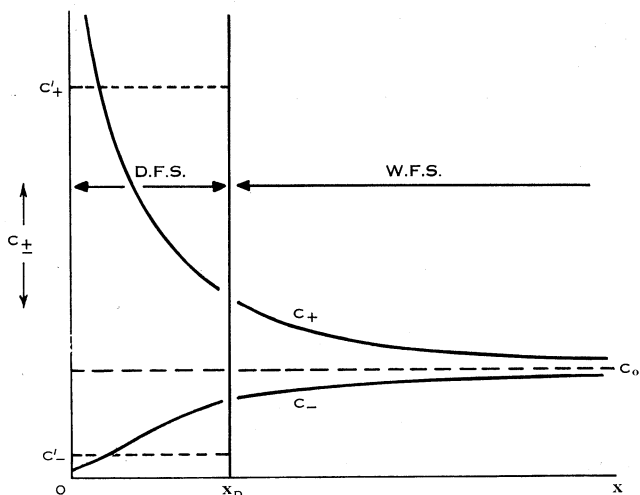

Fig. 2.—Diagrammatic representation of the extent of the Donnan free space (D.F.S.) and water free space (W.F.S.) near a negatively charged surface. $C_{\pm}$ are the local concentrations of cation and anion at distances x from the surface. C_o is the concentration very far from the surface. $C'_{\pm}$ are the equivalent concentrations in the D.F.S. which extends to x_D.

With the help of equations (1), (2), (4), and (5), equation (6) can be solved exactly for x_D, leading to the simple result:

$$x_D = \sqrt{(\epsilon RT / 2\pi F^2 C_o)}. \tag{7}$$

This is exactly twice the classical "width" of the electric double layer. Some values of x_D, which depends only on C_o and not on σ, are given in Table 1. For a bi-bivalent electrolyte the electric double layer is relatively compressed, the value of x_D being smaller by a factor of $\sqrt{2}$ (Table 1). The average concentration of indiffusible anions in the double-layer system is given in line 4 of the table, calculated as $\sigma/(F \cdot x_D)$. This formula and theory apply only to a single, charged, plane surface in an infinite volume of solution. The theory should apply to a cylindrical surface provided the radius of the cylinder is more than twice or thrice x_D, but the problem becomes mathematically difficult in the presence of other charged surfaces, as this causes mutual disturbance of the two double layers. However, the theory is applicable if the surfaces are separated by at least $2x_D$.

In order to investigate the validity of the above approach it is necessary to have data on the apparent volume (V_D) of the D.F.S. and on the apparent concentration of indiffusible charge (A) in the D.F.S., as functions of the external concentration. The small amount of material available in the present experiment (single cell walls of *Chara australis*) precluded the separate and accurate determination of diffusible cation and anion in the material (see Briggs 1957), and an alternative attack was necessary. Fortunately, for our purpose it is only necessary to determine the ratio of divalent to monovalent cations in the material when it is equilibrated with definite external concentrations of a mixture of salts of specified divalent and monovalent ions.

TABLE 1

EXTENT (x_D) OF D.F.S. AND AVERAGE CONCENTRATIONS OF FIXED ANIONS AS FUNCTIONS OF EXTERNAL ION CONCENTRATION

External ion concn. C_o (m-equiv/l)	0·1	1	10	100
x_D (uni-univalent) (Å)	608	192	60·8	19·2
x_D (bi-bivalent) (Å)	428	136	42·8	13·6
Av. concn. of fixed anions (m-equiv/l)*	17	54	170	540

* Calculated as $\sigma/(F \cdot x_D)$ for $\sigma = 10^{-5}$ C. cm^{-2}.

Although this approach requires extension of the double-layer theory to mixtures of ions of different valencies and there is then no simple expression for x_D, the value of x_D will lie between those values given in lines 2 and 3 of Table 1. The general argument, that because of the contraction of the double layer with increasing external salt concentration the apparent volume of the D.F.S. will decrease and the apparent A increase, is still correct.

Eriksson (1952) has shown that the ratio of bivalent ions to total counterions in a double-layer system containing sodium chloride and calcium sulphate (concentrations C_1 and C_2 equiv. cm^{-3}, respectively, in the bulk phase) is:

$$\mathrm{Ca}_i/(\mathrm{Ca}_i + \mathrm{Na}_i) = 1 - \{C_1/2\sqrt{(z \cdot C_2)}\} \cdot \arg\sinh\{2\sqrt{(Z \cdot C_2)}/(C_1 + u_d \cdot C_2)\}, \quad (8)$$

where

$$Z = \pi\sigma^2/\epsilon RT,$$

and

$$u_d = 2\cosh(-EF/RT),$$

($= 2$ when the charged surfaces are infinitely separated).

If the cell wall phase of plant cells could be regarded as a homogeneous Donnan phase then, in terms of the mean concentration of indiffusible anions (A), the ratio

of calcium ions to total counterions is:

$$Ca_i/(Ca_i+Na_i) = 1+C_1^2/C_2A-\sqrt{(C_1^4/4C_2^2A^2+C_1^2/C_2A)} \qquad (9)$$

(neglecting diffusible anions in the D.F.S.).

Comparison of observed successive replacements of calcium by sodium (Dainty, Hope, and Denby 1960) with the predictions of equations (8) and (9) may enable us to decide which system described best the cell wall phase.

III. EXPERIMENTAL AND RESULTS

Segments about 1 cm long of cell walls isolated from long internodal cells of $C.$ *australis* were placed in a solution of $CaCl_2$ (1 m-equiv/l) for about 24 hr, and then treated for 2–3 days in the same solution with tracer amounts of ^{45}Ca, sufficient to give a specific activity of about 0.1 $\mu c/\mu$-equiv. The segments were blotted and
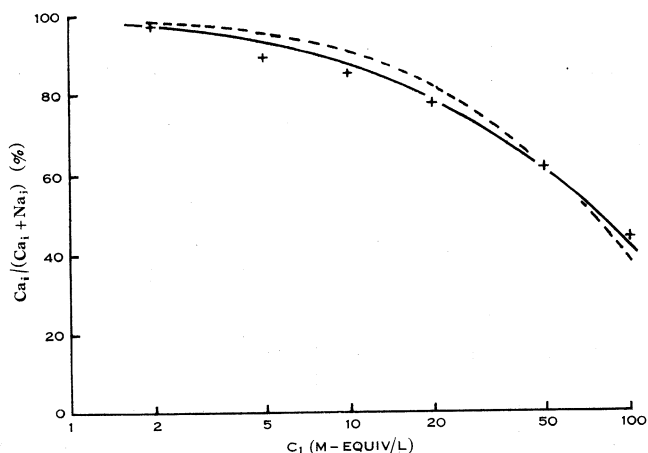

Fig. 3.—Variation in the exchangeable calcium (Ca_i) with external concentration of sodium or potassium (C_1) in segments of isolated cell walls from *Chara australis*, plotted as a fraction of the exchangeable calcium when sodium or potassium is absent (Ca_i+Na_i). Crosses are means of six determinations, half the height of the symbol being the standard error of the mean. The full and dotted lines are theoretical relations explained in the text.

counted under a thin end-window G.M. tube. Individual segments were then returned to solutions all containing $CaCl_2$ at a concentration of 1 m-equiv/l and with NaCl or KCl added to give concentrations ranging from 2 to 100 m-equiv/l. After 7 days to reach equilibrium (see Dainty and Hope 1959) the segments were again blotted and counted. These determinations, together with measurements of specific activity of the solutions after the segments had reached equilibrium, enabled the ratio $Ca_i/(Ca_i+Na_i)$ to be calculated as a function of $[Na]_o$ or $[K]_o$, the denominator being taken as the amount of exchangeable calcium in the first treatment, and the numerator being taken as that in the second.

Little difference was found in the replacing power of sodium and potassium except possibly when their concentration reached 100 m-equiv/l. Figure 3 shows the results of such an experiment, six values for each concentration of sodium or potassium being combined for each point. The full line is the line of best fit using equation (8) and putting $\sigma = 4\cdot2\times10^{-5}\ C.\ cm^{-2}$. Nearly all the points of such experiments were bounded by curves for $\sigma = 3\cdot3$ and 5×10^{-5}. The dotted line is the expected relation according to equation (9), with A put equal to 10 equiv/l.

The experimental results tend to be a better fit to the double-layer theory, all points except one being less than twice the standard error of the mean from the solid line. Other assumed values for A displace the dotted curve to left or right and do not give a better fit.

IV. DISCUSSION

The experimental results fit the expectations of the double-layer theory within reasonable limits. However, there are several assumptions involved:

(1) Equation (7) is intended for a mixture of uni-univalent and bi-bivalent electrolytes, whereas the data were obtained using chlorides. This should make little difference, since only minute amounts of mobile anion are to be found in the D.F.S. in the wall at the concentrations used.

(2) Ideal behaviour of ions is assumed throughout. It is well-known that the Gouy theory should be modified to account for ionic interaction, polarization of the ions, dielectric saturation, and finite ion size, which leads to a distance of closest approach between ions and the charged surface. Bolt (1955) points out that the differences between the corrected and simple theory largely cancel and the latter gives good agreement if the charge density does not exceed about $3\times10^{-5}\ C.\ cm^{-2}$.

(3) The curve in Figure 3 was calculated from equation (8) putting $u_d = 2$, i.e. assuming the charged surfaces are separated by distances such that the electric potential approaches zero at the point of symmetry. This is likely to be so more especially in higher concentrations of sodium chloride. At lower concentrations the effect of overlapping of the double layers is to raise the curve. For example, if $E = -100\ mV$ at the point of symmetry, then $u_d \simeq 54$ and the value at $[Na]_o = 2$ m-equiv/l is raised from $96\cdot8$ to $98\cdot4\%$.

When the cell wall D.F.S. is described in terms of an assembly of Gouy double layers, the parameter "concentration of indiffusible anions" (A) is no longer appropriate. The apparent $A\ \{= \sigma/(F\ .\ x_D)\}$ in the present experiments varies between about $0\cdot5$ equiv/l ($[Na]_o = 2$ m-equiv/l) and $2\cdot3$ ($[Na]_o = 100$), as x_D varies between 80 and 10Å (cf. Dainty, Hope, and Denby 1960). The charge density is apparently constant at $4\times10^{-5}C.\ cm^{-2}$.

When the double layers overlap enough, the A calculated from normal Donnan equations might not vary with external concentration. This may be the explanation of the results of Briggs, Hope, and Pitman (1958) who found the D.F.S. of disks

of red beetroot to have a constant volume of about 22 ml/kg fresh weight in concentrations of RbCl between 1 and 20 m-equiv/l. While this D.F.S. was ascribed to the cell cytoplasm, it has since (Pitman, personal communication) been located in the cell walls.

There are two sets of experimental data in the literature which illustrate the point we have tried to make in this paper that simple application of the Donnan equations to a charged colloidal system does not lead to values of D.F.S. and indiffusible charge concentration which are independent of external electrolyte concentration.

TABLE 2

VOLUME (V_D) AND EXTENT (x_D) OF D.F.S. IN GUM ARABIC SOLUTIONS AS FUNCTIONS OF GUM ARABIC CONCENTRATION

Total volume of solution = 1000 cm³

Potassium Bromide Concn. (m-equiv/l)	Gum Arabic Concn. (m-equiv/l)	V_D (cm³)	x_D (Å)	Av. Separation of Gum Arabic Particles (Å)
1·08	1	140	187	677
1·08	2	230	187	537
1·08	4	440	187	426
1·08	6	590	187	373
1·08	8	700	187	338
1·08	14	1000	187	281

Overbeek (1956) quotes some experimental results obtained by Klaarenbeck on the behaviour, as a Donnan system, of a colloidal solution of gum arabic, separated by a coarse membrane from a solution of potassium bromide. The concentration of bromide in the gum arabic solution was higher than expected from the assumption of a uniform Donnan system; in the phraseology of plant physiology the solution comprised a D.F.S. and a W.F.S. From Klaarenbeck's results we have calculated the volume of the D.F.S. (a), in Table 2, as a function of concentration of gum arabic solution at a constant potassium bromide concentration of 1·08 m-equiv/l; and (b), in Table 3, as a function of potassium bromide concentration at a constant gum arabic concentration of 1 m-equiv/l.

In the tables we also give the values of x_D (equation (7)), and the average separation of the gum arabic particles. The volume of the gum arabic solution is 1000 cm³.

It is clear from Table 2 that there is a rough proportionality between the volume of the D.F.S. and gum arabic concentration up to about 6 m-equiv/l when the average distance apart of the molecules is about $2x_D$. Thereafter the overlap of the double layers causes a marked departure from proportionality and when the gum arabic concentration is 14 m-equiv/l, the D.F.S. occupies the whole solution,

i.e. the solution is a uniform Donnan system. Note that this occurs when the average separation of gum arabic molecules is about $1 \cdot 5x_D$, i.e. three times the double-layer thickness. This simple approach is vitiated to some extent by the Brownian motion of the molecules, but the mutual repulsion of the negatively charged particles tend to counteract this thermal motion and gives a lattice-like structure to the solution.

Table 3 shows how the volume of the D.F.S. markedly decreases as the potassium bromide concentration is increased from $1 \cdot 08$ to 100 m-equiv/l. The D.F.S. has in fact decreased more than it should according to the simple Gouy–Chapman theory (equation (7)); this may be due to experimental error which cannot be judged from Klaarenbeck's data, but it may be due to theoretical errors arising as a consequence of treating a sphere of radius 100 Å as a plane surface when the double layer has a thickness of up to 100 Å.

TABLE 3

VOLUME (V_D) AND EXTENT (x_D) OF D.F.S. IN GUM ARABIC SOLUTIONS AS FUNCTIONS OF EXTERNAL POTASSIUM BROMIDE CONCENTRATION

Potassium Bromide Concn. (m-equiv/l)	Gum Arabic (Concn. (m-equiv/l)	V_D (cm³)	x_D (Å)	Av. Separation of Gum Arabic Particles (Å)
$1 \cdot 08$	1	115	187	677
10	1	25	61	677
100	1	6	$19 \cdot 4$	677

This analysis of Klaarenbeck's data illustrates our point that the calculated D.F.S. does vary in the expected way with external concentration. It also shows that the simple theory is adequate provided that the neighbouring surfaces are more than $2x_D$ apart and that when the surfaces are closer together than $1 \cdot 5x_D$, the whole system can be treated as if it were a uniform Donnan system.

The other set of results, to which reference has been made, is of more direct botanical interest. In a paper on "negative adsorption" of chloride ions, Schofield and Talibuddin (1948) express their results as the amount of water from which chloride is *absolutely* excluded per 100 g of dry jute fibre when equilibrated with various concentrations of potassium chloride. Here again, chloride is not excluded as much as it would be if the jute were a uniform Donnan system. From their results we calculate the data of Table 4. The total water volume is 75 cm³/100 g dry fibre and the total indiffusible charge is $22 \cdot 0$ m-equiv/100 g dry fibre.

From Table 4 it can again be seen that the volume of the D.F.S. in this plant material varies markedly with external solute concentration. Between $C_o = 1$ and $0 \cdot 05$ equiv/l the volume of the D.F.S. is inversely proportional to C_o but starts

to deviate from this at lower solute concentrations; since the extent of the D.F.S. is about 27 Å at $C_o = 0 \cdot 05$, the onset of deviation at this point implies that the charged surfaces must be 50–60 Å apart. If the material has pores, they must have diameters of the same order of magnitude. The last column of the same table gives the estimated fixed anion concentration in the D.F.S. and this of course also varies as the volume of the D.F.S. varies.

TABLE 4

VOLUME (V_D) AND EXTENT (x_D) OF D.F.S. IN JUTE FIBRES AS FUNCTIONS OF EXTERNAL POTASSIUM CHLORIDE CONCENTRATION

Potassium Chloride Concn. (C_o) (m-equiv. cm^{-3})	(V_D) (cm^3)	$V_D\sqrt{C_o}$	x_D (Å)	Apparent Indiffusible Ion Concn. (A) (equiv/l)
1·0	9·15	9·15	6·1	2·40
0·5	14·1	10·0	8·6	1·48
0·2	21·7	9·72	13·7	1·01
0·1	31·0	9·80	19·3	0·71
0·05	42·7	9·55	27·3	0·51
0·025	53·4	8·45	38·6	0·41
0·0125	61·5	6·88	54·6	0·36
0·00625	68·4	5·41	77·2	0·32
(0)	(75)			(0·29)

Since at low concentrations the volume of the D.F.S. is approaching the total water volume, it would seem that in jute fibre all the water is within about 100 Å of the fixed negative charges. Thus all the "pores" in jute fibres seem to be charged and have diameters in the range 50–200 Å. The situation seems to be quite different in the cell wall of *C. australis* (Dainty and Hope 1959), but it should be noted that "wet" jute fibre contains only 75 c.c./100 g dry weight whereas "wet" *Chara* cell wall contains 300 c.c./100 g dry weight.

V. ACKNOWLEDGMENTS

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