Permeation of Single Large Cells of *Nitella flexilis* by Amino Acids and Sugars

by

D. G. WALLEN

A thesis submitted to the Department of Biological Sciences
in conformity with the requirements for the degree
of Master of Science

Simon Fraser University
Burnaby, British Columbia

October, 1966

ABSTRACT

Single large internode cells of Nitella flexilis were put in water containing one of the following ^{14}C-labelled compounds at concentrations from 1 x 10^{-7}M to 1 x 10^{-6}M: glycine, alanine, valine, arginine, aspartic acid, glucose, and sucrose. "Cytoplasmic" and "vacuolar" fractions of the cell were analyzed separately for their ^{14}C content.

All compounds permeated into the cell. Uptake increased with time up to six hours, without an initial stimulation or lag in uptake. Rates of permeation of arginine and aspartic acid were 10 times that of the neutral amino acids. The rate of permeation of the neutral amino acids decreased in the order glycine>alanine>valine.

Kinetic analysis was applied to the uptake of arginine, aspartic acid, glucose and sucrose. This analysis indicated that a carrier system was operating for aspartic acid and glucose in the concentration range 1 x 10^{-7}M to 1 x 10^{-5}M. At higher concentrations, 1 x 10^{-5}M to 1 x 10^{-1}M, the carrier mechanism did not operate for glucose. The carrier mechanism did not operate for arginine at low concentrations.

Chromatographic analysis of cells allowed to take up either sucrose or glucose indicated that sucrose was probably converted to its constituent hexose sugars before permeation. Also, this analysis showed that the "vacuolar" fraction of the cell was probably contaminated with part of the cytoplasm.

ACKNOWLEDGEMENT

I am indebted to Dr. C. D. Nelson for permitting me the opportunity of pursuing graduate studies. For his encouragement, and direction of my thesis problem and graduate programme, I wish to express my thanks and appreciation.

Dr. Towers and Dr. Tregunna at the University of British Columbia will be remembered with appreciation. From them and Dr. Nelson I have received generously their principles and philosophy of research in Plant Physiology.

To Mr. R. Schmidt who was of great assistance in this investigation and who approached each problem with enthusiasm I wish to express my thanks. To my fellow students and professors at Simon Fraser University who discussed, criticized and encouraged my work, to Mr. P. Foreman who prepared the diagrams and graphs, and to Miss Jenny Turner, Miss Barbara Pizzuto and Miss Diane Whiffin who typed this thesis, I am indeed grateful.

Last, but certainly not least, I thank my wife, Donna Jean, for placing aside the comforts and security of home in order to accompany her husband on a most uncertain course.

Table of Contents

List of Tables Page

List of Figures

List of Figures Page

Glossary

1. Bioelectric potential difference: an electrical potential difference normally maintained in living matter.
2. Electro-osmosis: the movement of a fluid under the influence of a potential difference through a channel having charged walls.
3. Partition coefficient: the ratio of concentrations found in an organic solvent and water when a particular compound is shaken up in equal volumes of both, and the liquids are allowed to separate after equilibrium has been achieved.
4. Permeability: is a property of a membrane which controls penetration of substances.
5. Permeation: the process whereby a substance penetrates a membrane.
6. Permeation power: the ability of a substance to penetrate a membrane.
7. Pore: a channel existing in a membrane of a cell.

Introduction

The biological membrane is the fundamental unit of transport and governs all permeability processes, whether they are simple diffusion (25), exchange diffusion (2), ion exchange (2), or active transport (7) against a prevailing electrochemical gradient.

Pfeffer (3) advanced the hypothesis that the resistance to permeation is principally due to two extremely thin membranes, the one covering the outer surface of the protoplast, the other separating the cell sap or vacuole from the protoplasm. The outer membrane is now generally called the plasmalemma, while the inner one is called the tonoplast.

Overton (3) was the first to suggest that the cell surface is a lipid layer. The work of Gorter and Grendel in 1925 (2), showed that enough lipid was present in erythrocyte membranes to make a membrane about two molecules thick. They suggested that the membrane consisted of a bimolecular layer of lipid molecules, the molecules being oriented perpendicular to the membrane-water interfaces, and with their polar groups in the interfaces. For such a cell, the tension at the surface would be of the order of 10 to 20 dynes $cm.^{-1}$ Harvey and Cole, in 1932 (2), measured this tension and found it to be of the order 0.1 dynes $cm.^{-1}$ Danelli and harvey, 1934, therefore, suggested that the cell surface consisted of a bimolecular lipid

layer, with an adsorbed protein monolayer on each side. This model was compatible with all other observations available at that time. Later studies on the surface tension of cells and of protein adsorption indicated that in addition to the monolayer of denatured protein there was a secondary layer of globular native protein, which was reversibly adsorbed (2). These protein chains endow the membrane with a certain degree of elasticity, and mechanical resistance, as well as low surface tension.

Because the length of the lipid molecules is about 30 angstrom units and the thickness of a monomolecular protein layer is some 10 angstroms, Danielli fixed the total thickness of the membrane at about 80 angstroms. This model of the membrane has been supported by observations made with the electron microscope (22).

This model has received wide acceptance and has been used as the basis for discussion of the possible permeability mechanisms of cell membranes.

Frey-Wyssling (11) recently proposed a membrane model which he terms a block structure. He envisages this structure consisting of a bimolecular layer of globular lipoprotein macromolecules 40 - 50 Å in diameter. The lipid molecules are anchored in the loosely-constructed protein macromolecules with their helically wound polypeptide chains, and in the interstices between the globular macromolecules. The necessary polarity of the membrane is given by the intramolecular construction of the globular macromolecules.

Permeability mechanisms fall into five main groups or categories as follows:

1. Simple Diffusion: Two models have been proposed for the simple diffusion of permeants across a cellular membrane.

   a) The lipid-solubility principle. Overton (3) found that permeability in both plant and animal cells was a function of the oil/water partition coefficient of the penetrating molecules. Molecules with a high oil/water coefficient are able to permeate more rapidly than molecules with a small partition coefficient. From this, he reached the conclusion that compounds which are extremely lipophilic, such as hydrocarbons, have great permeation power and permeate into the cell by dissolving in the lipid component of the plasmalemma and diffusing across into the cytoplasm.

   Amino groups, hydroxyl groups, carboxyl groups, and keto groups decrease the lipid solubility and hence, the permeation power of a molecule. An increase in the length of the nonpolar portion of these molecules as well as an increase in the degree of methylation results in an increase in lipid solubility and hence, permeation power.

   b) The molecular-sieve principle. The extensive studies of the relationship between molecular size and permeability led to a picture of the cell surface as a membrane

containing different sized pores of diameters commensurate with the size of the penetrating molecules. Dainty (8) as a result of investigations of electro-osmosis in Nitella, has stated his hypothesis that the existence of electro-osmosis across cell membranes implies the existence of water-filled charged pores through the membrane. Solomon (23) regards pores in membranes as dynamic openings within a complex, hydrated lipoprotein framework. These are not necessarily fixed canals; as the membrane responds to changing conditions inside or outside the cell, some pores may open and others may seal up. Nevertheless, he conceives the membrane as containing on the average a uniform number of pores. In this regard Kavenau's new theory of membrane structure (16) has many attractive aspects. According to this theory the regularly arranged constituents of living membranes can change their structure reversibly from an open-type configuration with membrane pores to a closed-type configuration in which the pores are obliterated. In the open state the lipids form pillars within the envelope of membrane proteins and these pillars flatten out into discs in the closed state.

2. Active transport: When ions or molecules are moved across a membrane by a mechanism which is directly dependent on metabolic energy, they are said to be transported "actively."

There are certain important features of active transport. The rate of transport is not a linear function of the difference of concentrations on the two sides of the cell membrane. There is competition among substances having similar configurations. There is inhibition by enzyme inhibitors. The rate of transport may be different even for optical isomers. The temperature coefficients are often of the same order of magnitude as those of enzyme reactions.

Most of the hypotheses advanced to explain active transport involve reversible binding of ions or molecules to a constituent of the membrane which acts as a "carrier." According to the "carrier" concept (fig. 1), an ion or molecule reacts with its carrier (X) at, or near, the outer surface (M_1) of the membrane. This reaction might involve adsorption, exchange adsorption, or some kind of chemical combination. Neither the carrier nor the ion-carrier complex (IX) can move into the medium, but IX is mobile in the membrane, and moves to the other side (M_2). Here it breaks down, releasing the ion into the internal solution and forming a carrier precursor (X^1) which is incapable of leaving the membrane, or of reaccepting an ion. X^1 is transported back across the membrane and reconverted to X, where at M_1 it can combine with another ion.

One suggestion is that the carrier and ion or

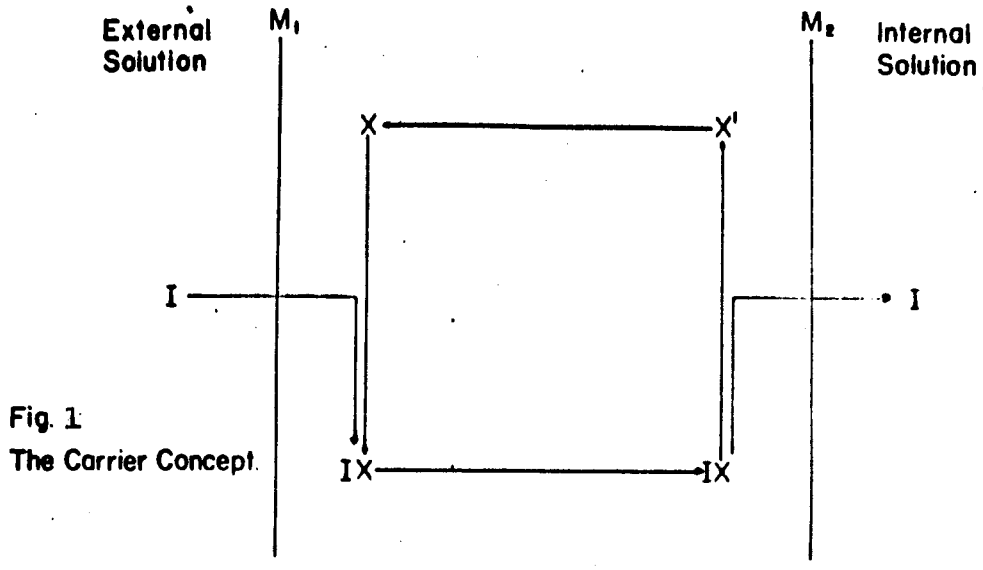
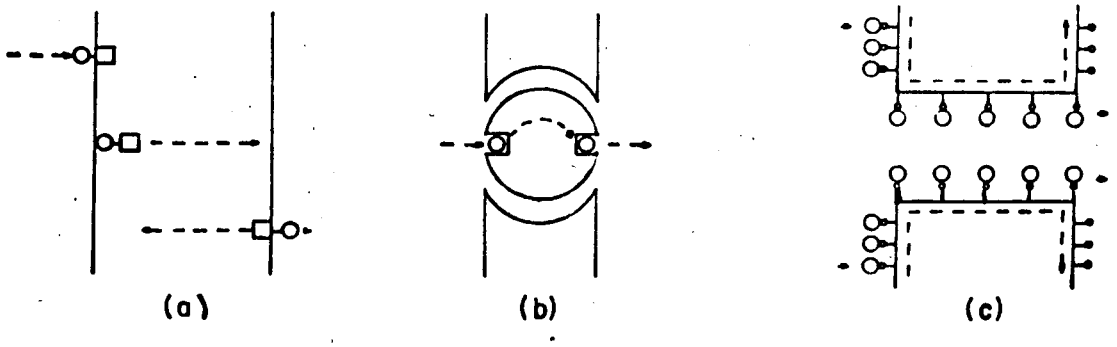


Fig. 1
The Carrier Concept.



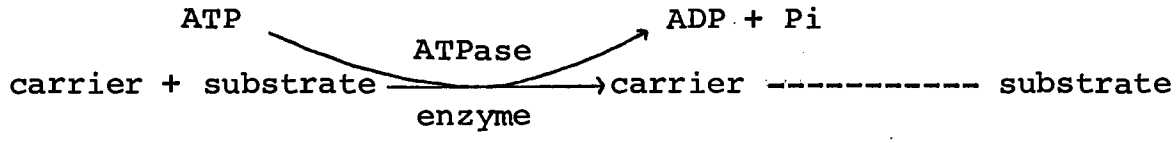
Diffusing carriers Rotating carriers Sliding carriers

Fig. 2 Hypothetical mechanisms of transport across membranes.

molecule may form a lipid-soluble complex which can diffuse across a lipoprotein membrane along a concentration gradient (Fig. 2). Another possibility is that the carrier or part of it is capable of rotating in some way in the membrane and thus of transfering ions or molecules bound at one surface across to the other. Some mechanism would be necessary to ensure that the binding site returns to the outer surface unloaded to receive another ion or molecule. Alternatively, the carrier might be a strongly surface-active substance which slides along the water-filled pores in the membrane with the polar head in the water phase, and the lipophilic tail associated with lipid materials in the membrane (13). Phospholipids appear to be well suited for such a purpose, and they are known to be constituents of biological membranes. Danielli (7) and Mitchell (19) have suggested the existence of spatially organized enzyme and catalytic carrier systems as components of the membrane complex. Such enzymes, have in common with other enzymes, the property of binding their substrates (the permeating molecules) by non-covalent bonds to form the classical Michaelis-Menten complex, the energy required being supplied by the metabolic processes within the cells. Green and Davis (14) have discovered that the catalysts of the entry of certain carboxylic acids and sugars into the metabolic systems of E. coli resemble enzyme systems in being inducible, and that the induction

can be blocked by certain inhibitors of protein synthesis. These kinetic observations lend further support to the idea that transport carriers may be normal enzyme and catalytic carrier systems.

The active transport processes require the expenditure of energy. For a carrier E and substrate (permeating molecule) S, the following equation may be written: $S + E \longleftrightarrow ES \longrightarrow E + P$, in which ES represents the enzyme substrate complex, P the product. Ghosh (12) suggests that the energy transfer is similar to those involved in other synthetic reactions in the cell.



The enzyme ATPase is necessary for the breakdown of ATP to ADP. ATPase has been reported in the plasmalemma of erythrocytes and Streptococcus faecalis (30).

As yet, no unambigious criteria exist for distinguishing between these mechanism, and there is no firm knowledge as to how these postulated transport mechanisms indeed act.

3. Electro-osmosis: When an aqueous solution is separated from another by a membrane possessing charged pores, and a potential difference exists between the two solutions, ions move through the pores along an electrochemical

potential gradient. In doing so, they carry water molecules with them. This type of movement is called electro-osmosis. The pores through which electro-osmosis may occur in plant membranes are thought to have negatively charged walls (1). This is in agreement with the fact that under normal conditions of pH the walls usually are negatively charged due to the presence of proteins. D. S. Fensom (9) has stated his hypothesis that electro-osmosis may be a motivating factor for phloem transport, as well as for the entry of water into the vacuole of many plant cells.

4. Ion exchange: If two solutions containing different salts are separated from one another by either a cation-permeable/anion impermeable or cation impermeable/anion permeable membrane, one of the two ion species in each case is free to move across in exchange for an ion of like charge. Equilibrium is established when the ratio of the two cations (in the first case) or the two anions (in the second case) is equal on the two sides. At equilibrium, exchange does not stop but equal relative amounts of the two cations or anions move in each direction, so that the ratio remains the same. The total concentration of salt on either side is not affected by exchange, even when a concentration gradient exists, because movement of one ion species, and therefore diffusion of salt, is prevented. Exchange involves equivalent electrical charges so that two univalent ions exchange for one bivalent, three for one trivalent ion, and so on.

The Donnan equilibrium is a more complex ion-exchange system. In this system, the membrane separates a solution containing ions to which the membrane is permeable, from another containing in addition to mobile ions, either cations or anions to which it is impermeable.

Assuming the concentration of the single cation, sodium, on the two sides of a membrane is initially equal (Fig. 3a), the concentration of diffusible anions, chlorides, on side 2 will be lower than on side 1, since part of the cations are balanced by phosphate, which is indiffusible. The proportion of sodium ions as a percent of the diffusible ions on side 1 is less than on side 2. The proportion of chloride ions as percent of the diffusible ions on side 1 is greater than on side 2. Since the concentration of diffusible positive and negative ions is different, chloride ions will tend to move by diffusion from side 1 to side 2, sodium ions will move in the opposite direction. However, as the movement of ions progresses, electrostatic forces will be developed, the effect of which will be to oppose such a separation of electrical charges. Ultimately a condition of equilibrium will result. At equilibrium the electrochemical potentials of the two sides of the membrane are equal, but $\lfloor Na^+ \rfloor_1 > \lfloor Na^+ \rfloor_2$ and $\lfloor Cl^- \rfloor_1 < \lfloor Cl^- \rfloor_2$.

Since the concentrations of mobile ions are unequal on the two sides of the membrane in a Donnan system at

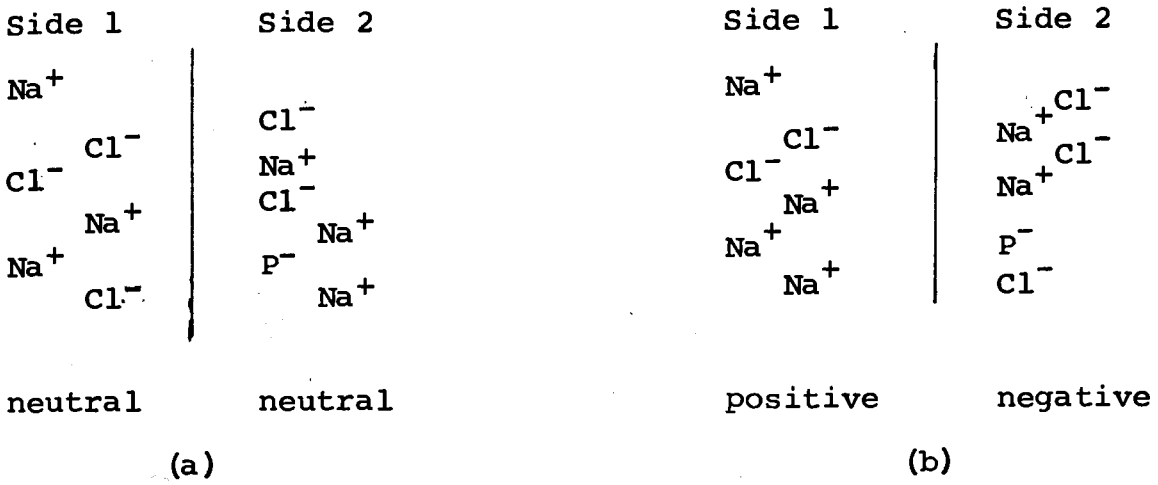


Fig. 3. Diagram to demonstrate the formation of a Donnan Equilibrium

equilibrium while the electrochemical potentials are equal, it follows that there is an electrical potential difference between the two sides. This is sometimes called the Donnan membrane potential.

5. Exchange Diffusion: Ussing in 1947 (24) observed in frog skin a one for one exchange of sodium ions which was greater in magnitude than the net movement. This he called exchange diffusion. It is suggested that in this process the ions cross the membrane in combination with carriers which do not readily cross the membrane in the uncombined state.

The best known example of such an exchange diffusion comes from the work of Mitchell and Moyle (24) on phosphate exchange across the membrane of Staphylococcus aureus. When the bacteria were put into a phosphate-free medium no phosphate left the cells. But on addition of isotope-labelled phosphate to the medium there was a rapid exchange of intra and extra-cellular phosphate without any net movement.

Permeation experiments have been performed using a wide variety of techniques and a wide variety of plant and animal materials. The permeation of materials into plant cells can sometimes be detected by microscopic observations. One method involves measurement of the rate of recovery of a vacuolated cell after it has been plasmolysed in a hypertonic solution. The entry of dyes into cells can sometimes be followed by direct microscopic

observation. Penetration of colorless molecules or ions can occasionally be detected as a result of formation of colored complexes with cell constituents; iron for example, gives a blue color in the presence of tannins. If a cell contains soluble carbonates or oxalates, the entry of calcium ions is indicated by the appearance of crystals of insoluble calcium salts.

The entry of permeants into whole plants, or their parts, can be determined quantitively by analysis of samples of either the cells (or tissues) or the immersion medium. In practice, analysis of the medium is often preferred, since it is then possible to measure uptake after several consecutive time intervals with the same plant material. Analysis of the tissue is often less reliable than that of the medium owing to the presence of a greater number of substances which may interfere with the determination. Also, difficulty may be encountered in extracting salt quantitively, especially if some has been incorporated into organic cell constituents.

A change in the total concentration of ions in a solution can be estimated from the change in electrical conductivity. However, if the uptake of one ion is accompanied by release into the medium of a different ion with the same electrical charge, little or no change in conductivity may be detected even though an appreciable amount of a particular ion may be removed from the solution.

The estimation of some cations, particularly sodium and potassium ions is determined by flame photometry. The apparatus is inexpensive, the method is rapid and reasonably accurate. A more modern and accurate instrument is the atomic absorption spectrophotometer.

The introduction of isotopically labelled compounds into permeability studies made possible experiments which were beyond the reach of early investigators. Radioactive compounds can be detected in extremely small amounts, and their passage across cell membranes can therefore be followed over very brief intervals of time. This permits measurements of the penetration of a compound before there is appreciable metabolism. Furthermore, if such metabolic conversion does occur, it can easily be detected even if the cell already contains large amounts of the same compound. Whereas previously, the movement of a particular substance could be measured only if it resulted in change of intracellular or extracellular concentration, it can now be measured in the absence of any concentration change. Thus a study of the exchange of potassium ions for potassium ions or chloride ions for chloride ions is possible. Ussing (24), using a radioactive sodium nuclide, discovered the mechanism of exchange diffusion across cell membranes. The method in combination with autoradiography has been used to show the uptake by root tips of phosphate and sulphate, and to demonstrate their intracellular location.

Cell permeability has been studied in a wide variety of botanical materials. Many investigators have favored the use of excised roots in permeation studies because roots are the major absorbing organs in most angiosperms. The system is less complex than is a whole plant, inasmuch as any influences of the shoot on uptake by the root may be removed, and it is somewhat less variable in behaviour.

DeVries (3) in 1871 used thin sections of red beet (Beta vulgaris) in an investigation of the permeability of plasmolysed protoplasts. Since then, storage tissue parenchyma has been widely used in permeation studies. Storage organs which have been used include potato (Solanum tuberosum) and artichoke (Helianthus tuberosus) tubers and red beet and carrot (Daucus carota) roots. The material is used in the form of thin disks, not exceeding 1 mm in thickness.

Multicellular algae have been used extensively in permeation studies. Giant coenocytic vesicles of the marine algae Valonia and Halicystis allow extraction of uncontaminated vesicle sap for detailed chemical analysis. Even a perfusion of the living vesicle with solution of known composition is possible. For many purposes, the smaller but more easily obtainable single cells of different members of the Characeae such as Chara, Nitellopsis, or Nitella, are equally suitable.

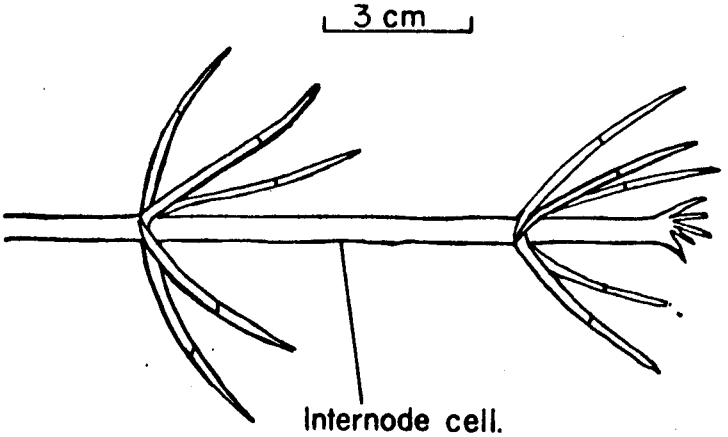
An enormous amount of work on permeation problems has been carried out with animal cells. Since erythrocytes are easy to isolate, float freely in their natural environment and are not attached to other structures in the body, they have been used by many investigators. Because of their large size, and ease with which they can be manipulated, muscle cells and nerve cells have been used a great deal in permeation studies.

Organic molecules of various molecular weights and various electrical charges have been used to examine permeation processes (3). Recently it has been possible to label such molecules with ^{14}C to produce very high specific activities. The high specific activities in conjunction with the highly sensitive liquid scintillation assay techniques make it possible to re-examine permeation in single cells at very low concentrations.

The isotope technique in collaboration with the giant cells of Nitella is a powerful combination for studying permeation of a single membrane.

Nitella and other members of the Characeae are distinguished by their large internode cells which are often more than 10 cm. long and up to 2 mm in diameter. The internode cells are separated from each other at the nodes by smaller cells from which branches develop (Fig. 4). The large internode cell has a large central vacuole surrounded

Fig.4a. A plant of Nitella flexilis (Characeae) showing a large single internode cell.



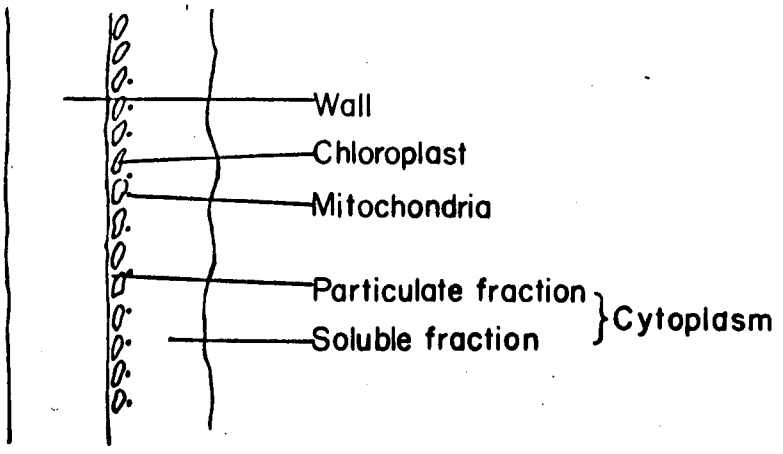


Fig 4b. Schematic section of a Nitella cell.

by cytoplasm containing many small ellipsoidal chloroplasts. One feature of the cytoplasm is its streaming motion spirally around the long axis. The ellipsoidal chloroplasts are readily identified and may be carried in the streaming cytoplasm, but generally they adhere to the walls. The streaming cytoplasm is sensitive to slight changes in the internal and external environment and particularly to changes in the osmotic potential of the medium. Hence, one is able to assess the physiological condition of the Nitella during the course of an experiment.

These cells can be cut open at one end with a razor and the "vacuolar" contents collected quite easily on absorbent paper for these flow freely from the cut end of the cell. The "cytoplasmic" fraction can be recovered on absorbent paper by squeezing the remaining contents away from the cell wall with a beaded glass rod. Although there may be some contamination between the two fractions, microscopic examination shows that all the chloroplasts remain in the "cytoplasmic" fraction.

Statement of Problem

High specific activity ^{14}C-labelled compounds that are now available can be used to study permeation processes in single large Nitella cells. For the investigation reported in

this thesis a series of three neutral amino acids, glycine, L-alanine and L-valine was used. The permeation of these compounds was compared to that of a basic amino acid, L-arginine, an acidic amino acid, L-aspartate, and two neutral sugars, D-glucose and sucrose.

The data on permeation of all these compounds was analysed to produce a description of the Nitella cell membrane, which fits at least two mechanisms.

Materials and Methods

Nitella flexilis used for these experiments was obtained from the Northwest Biological Supply House, Victoria, B.C. It was maintained in aquaria containing guppies for several weeks without apparent injury.

Permeation experiments were carried out using ^{14}C-labelled amino acids and sugars (Table 1). The amino acids were obtained from the New England Nuclear Corporation, the sugars from the Radioisotope Laboratory, Queen's University.

In a typical experiment to measure permeation of a ^{14}C-labelled amino acid, or sugar, twenty Nitella cells were trimmed from neighboring cells and placed separately in a petri dish containing 10 ml. of distilled water. In the trimming of cells, the neighboring cells were cut off with a razor blade. A small stump of nodal cells left at each end was convenient for the handling of cells with forceps.

The distilled water was carefully decanted and 10 ml. of 1×10^{-7}M arginine-^{14}C was added to the petri dish. At 30 minute intervals a cell was removed, washed by dipping into a series of three changes of distilled water, and excess water was removed with absorbent paper. One end of the cell was cut with a razor and the "vacuolar" and "cytoplasmic" fractions collected separately for assay. The fractions were counted with a liquid-scintillation spectrometer, Packard Tri-Carb Model 3003.

Table 1. The ^{14}C-labelled compounds used in permeation experiments

Compound	Molecular weight	Specific activity	Electrical behavior
Amino Acids			
Glycine	75	74.4 mc/mM	electrically neutral
L-alanine	89	111.0 mc/mM	electrically neutral
L-valine	117	208.5 mc/mM	electrically neutral
L-aspartate	133	167.0 mc/mM	negative (-1)
L-arginine	174	250.0 mc/mM	positive (+1)
Sugars			
D-glucose	180	14.2 mc/g	neutral
Sucrose	342	28.5 mc/g	neutral

The absorbent paper containing the extract was placed in a vial containing scintillation fluid consisting of 3.0g PPO[1], and 0.1g dimethyl POPOP[2] in one litre of toluene. In order to correct for quench and to convert from cpm to dpm a 10 µl aliquot of ^{14}C standard containing 4240 dpm was pipetted onto a square of absorbent paper, placed into a vial of scintillation fluid and counted. The ratio of the actual count rate for the spike to the absolute radioactivity of the spike gives the counting efficiency for the isotope. Counting efficiency depends on the amount of quench and the width of the window on each channel of the spectrometer. The channels were set to count ^{14}C with an efficiency of from 68 to 77%.

Each extract was counted for three five-minute periods. The average of these counts was taken as the count rate. This was corrected for background and converted to dpm which in turn were converted to micrograms. The technique is illustrated in Table 2.

The amount of material recovered from each of the two fractions was plotted against time and the data was tested by regression line analysis. The slope of this line was a measure of the rate of uptake of the material offered to the Nitella cells.

[1] PPO : 2, 5-diphenyloxazole

[2] Dimethyl POPOP : 1, 4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene

Table 2. Example of primary data and calculations of uptake of Arginine-^{14}C by 12 Nitella cells with increasing time from 0.5 to 6.0 hours. Trial one of six trials.

Length of time in Arginine-^{14}C	Count rate "Vacuole"	Count rate "Cytoplasm"	Count rate less background "Vacuole"	Count rate less background "Cytoplasm"	Absolute count rate "Vacuole"	Absolute count rate "Cytoplasm"	Amount recovered "Vacuole"	Amount recovered "Cytoplasm"
hrs	cpm	cpm	cpm	cpm	dpm	dpm	$\mu g \times 10^{-5}$	$\mu g \times 10^{-5}$
.5		291	56	276	72	356	2.2	11.1
1.0	214	248	199	233	257	301	8.0	9.4
1.5	287	507	272	492	351	634	10.9	19.7
2.0	271	467	256	452	330	583	10.3	18.1
2.5	415	542	400	527	516	680	16.0	21.1
3.0	508	658	573	643	739	829	23.0	25.7
3.5	407	835	392	820	506	1058	15.7	32.9
4.0	763	675	748	660	965	851	30.0	26.4
4.5	376	1161	361	1146	466	1478	14.5	45.9
5.0	685	2303	670	2288	864	2952	26.8	91.7
5.5	848	1033	833	1018	1075	1313	33.4	40.8
6.0	687	1347	672	1332	867	1718	26.9	53.3

Background: 15 cpm

Correction for quench:

Absolute radioactivity of spike - 4240 dpm

Actual count of spike - 3275 cpm

$$\text{Efficiency} = \frac{3275}{4240} = 0.77$$

Correction factor - cpm x 1.29 = dpm

Conversion of dpm to micrograms:

Specific activity of Arginine-^{14}C offered

$2.2.10^6$ dpm = 0.69 μg

$$1 \text{ dpm} = \frac{0.69}{2.2\ 10^6} = 3.14\ 10^{-7}\ \mu g$$

Correction factor - dpm x $3.14\ 10^{-7}$ = μg

Channel Setting Gain - 8.58% A^{50} to B^{1000}

For statistical analysis of a bivariate population two similar methods are appropriate, regression and correlation. If the two variables under consideration are designated X and Y, Y may be either a consequence of X (through heredity, for instance) or merely a concurrent variate. If X and Y are suspected of having a cause-effect relationship, regression is the better method. If X and Y are independent of each other correlation is the appropriate method to use.

The linear regression equation is of the general form $\hat{Y} = bX$ in which $\hat{Y}$ is called "estimated Y" or "regression Y," and represents a value of Y based on all the values of X. The quantity "b" is known as the regression coefficient. It is also the slope of the regression line or the rate of change in Y with unit change in X.

When the regression line does not pass through the origin of X's and Y's - as is usually the case with biological data - the regression equation takes the form $\hat{Y} = a + bX$ where "a" is the intercept of the regression line on the Y axis. The statistical analysis is illustrated in Table 3.

Six trials were performed with each of the compounds and the data analyzed as above. The mean uptake by the "cytoplasmic" and "vacuolar" fraction at each time interval was calculated and plotted against time and the slope determined by regression analysis. The regression lines for the six trials

Table 3. Statistical analysis of uptake of Arginine-^{14}C by 12 Nitella cells shown in Table 2.

Length of time in Arginine-^{14}C hrs.	Amount recovered "Vacuole" $\mu g \times 10^{-5}$	Amount recovered "Cytoplasm" $\mu g \times 10^{-5}$
0.5	2.2	11.1
1.0	8.0	9.4
1.5	10.9	19.7
2.0	10.3	18.1
2.5	16.0	21.1
3.0	23.0	25.7
3.5	15.7	32.9
4.0	30.0	26.4
4.5	14.5	45.9
5.0	26.8	91.7
5.5	33.4	40.8
6.0	26.9	53.3

Vacuole

$\Sigma X = 39.0$ $\Sigma Y = 217.6$ $n = 12$ $b = \Sigma xy/\Sigma x^2$

$\bar{x} = 3.2$ $\bar{y} = 18.1$ $= 4.7$

$\Sigma X^2 = 162.5$ $\Sigma Y^2 = 4986.9$ $\Sigma XY = 874.1$ $Y - \bar{y} = b(X-\bar{x})$

$(\Sigma X)^2/n = 126.8$ $\Sigma Y)^2/n = 3945.8$ $(\Sigma X)(\Sigma Y)/n = 707.3$ $Y = 15.0+4.7X$

$\Sigma x = 35.7$ $\Sigma y = 1041.1$ $\Sigma xy = 166.8$

Cytoplasm

$\Sigma Y = 395.9$ $n = 12$ $b = \Sigma xy/\Sigma x^2$

$\bar{y} = 33.0$ $= 11.3$

$\Sigma Y^2 = 18844.2$ $\Sigma XY = 1690.2$ $Y - \bar{y} = b(X-\bar{x})$

$(\Sigma Y)^2/n = 13081.2$ $(\Sigma X)(\Sigma Y)/n = 1287.0$ $Y = -2.2+11.3$

$\Sigma y = 5763.0$ $\Sigma xy = 403.1$

The regression for this series of cells is:

$$Y = -2.2 + 11.3X$$

where the rate of uptake is 11.3×10^{-5} $\mu g\ hr^{-1}$

were analyzed to determine whether the line $\hat{y} = a + bx$ was significantly better than $\hat{y} = bx$ or to put it in other words to determine whether the line passed through the origin. An example of this statistical analysis is illustrated in Table 4.

The uptake for six trials of each ^{14}C-labelled compound into the "vacuolar" and "cytoplasmic" fraction of Nitella cells was analyzed, using this statistical model. The "F" values obtained indicate that for each compound, there was no lag in uptake into either "fraction" of the cell.

In a different experiment the permeation of L-arginine-^{14}C into Nitella was determined for a series of concentrations ranging from 1.1×10^{-8}M to 1.1×10^{-7}M. Cells were removed from the solution and determinations made on the "cytoplasmic" and "vacuolar" fractions at 20 minute intervals for 150 minutes. Similar experiments were performed with D-glucose-^{14}C and sucrose-^{14}C using concentrations ranging from 10^{-6}M to 10^{-5}M and L-aspartate-^{14}C using concentrations ranging from 1.5×10^{-8} to 1.5×10^{-7}M. Cells were removed from the sugar solution and extracted at 30 minute intervals for three hours.

A second set of experiments involving concentration gradients was carried out using D-glucose and sucrose. Four sets of fifteen Nitella cells were placed in petri dishes containing 10 ml. of cold glucose or sucrose ranging from 10^{-1} to 10^{-4}M plus 10^{-5}M D-glucose-^{14}C or sucrose-^{14}C. A fifth set

Table 4. Statistical analysis of six trials to determine the presence or absence of a lag in the uptake of arginine-^{14}C into the "vacuole" of Nitella cells.

Trial number	1	2	3	4	5	6	Total	Q
Degrees of freedom (k-2)	4	4	4	4	4	4	(k-2).n =24	
Residual ss for (a,b) line* $\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$	727.29	263.32	505.49	209.77	2587.44	2203.86	T1= 6493.17	T1/(k-2) n=270.5
(a,b) line vs (b) line (by subtraction)	106.45	23.35	24.43	5.67	27.62	258.94	T2= 717.00	T2/n= 119.5
Residual SS for (b) line** $\Sigma Y^2 - (\Sigma XY)^2/\Sigma X^2$	833.74	286.67	529.92	211.44	2615.06	2462.80		

$$FL\ 4L = \frac{T2/n}{T1/(k-2).n} = 0.09$$

If F >> 1 then (a,b) line is significantly better than b line.

F = 0.09 indicates that the (b) line originating at the origin gives a good fit to the data.

Conclude: There was no lag in the uptake of arginine-^{14}C into the "vacuole" of Nitella cells.

* (a,b) line : $\hat{y} = a + bx$.

**(b) line : $\hat{y} = bx$.

of Nitella cells was placed into 10 ml. of 10^{-5}M D-glucose-^{14}C or sucrose-^{14}C.

The purity of D-glucose-^{14}C and sucrose-^{14}C solutions were determined by two dimensional paper chromatography on Whatman 3 MM paper. The solvents were phenol: water: ammonium hydroxide (267:37:1) and n-propanol: ethyl acetate: water (7:1:2).

The radioactive compounds were located by autoradiography using Ilford Ilfex x-ray film. Spots corresponding to the positions of the sugars were cut from the chromatogram. These spots were placed in vials containing the same scintillation fluid as used to count the two cell fractions. The counting of the spots gave the relative activity of each compound formed on the chromatogram. The background was taken from a vial containing a piece of non-radioactive paper which had been run through the chromatogram solutions. The validity of this method has been established, (27).

To determine if sucrose-^{14}C was entering the cell a solution containing 50 mg. each of sucrose, glucose and fructose was prepared. 25µl of this solution was spotted on four sheets of chromatogram paper. As a control, extracts of 5 Nitella cells taken from the aquarium were spotted on one chromatogram. Extracts of five Nitella cells taken from a solution of 2.5 x 10^{-5}M sucrose-^{14}C after three hours were spotted on each of the other three chromatograms. The position of the labelled compound could not be identified by autoradio-

graphy as the amount of ^{14}C in the cell was too small. One chromatogram was tested for sugar using a solution consisting of 0.5 ml. benzidine, 10 ml. acetic acid, 10 ml. 40% (w/v) trichloroacetic acid, and 80 ml. of 90% ethanol sprayed on the chromatogram. The chromatogram was then heated at 100-110°C for 5 to 10 minutes. Spots corresponding to the position of the sugars on the other two chromatograms were cut out, placed in vials and counted.

A similar experiment was performed with L-aspartate-^{14}C. A solution containing the three sugars and several amino acids was prepared. 25µl of this solution was spotted on each of five sheets of chromatogram paper. As a control, the extracts of five Nitella cells were spotted on one chromatogram. Extracts of five Nitella cells taken from a solution of 1.5×10^{-7}M L-aspartate-^{14}C after three hours was spotted on each of the other four chromatograms. Replicate chromatograms were tested for amino acids and sugars. The sugars were located as above, the amino acids were located with ninhydrin (18). Spots corresponding to the positions of the amino acids and sugars on the unsprayed chromatograms were cut out, placed in vials and counted.

Molecular models of each compound were constructed using the Fisher-Heischfelder-Taylor system and photographed using kodacolor-X, speed 64 ASA film.

Several variable factors had to be considered in this investigation using the experimental model. These included light, temperature, O_2 and CO_2 concentration, pH, age, size and volume of cells. Experiments were performed in the same light environment, about 50 ft-c of white fluorescent light. Room temperature was 23°C ± 0.5°C. No attempts were made to control the concentration of O_2 or CO_2 produced by physiological processes in the cell. The medium was not buffered because the buffers required to maintain pH in the range 6 - 8 are quite inefficient. Also, with introduction, these compounds may interact with the permeant to cause deviation in the permeation behaviour. Because cytoplasmic streaming is very sensitive to small physiological changes or changes in pH, the presence of streaming is the best indication that constant experimental conditions were being maintained throughout the experiment. In all experiments cells were constantly checked for cytoplasmic streaming and were not used unless this criterion was met.

Age of the cells could not be determined. Cells were harvested at random and were of different sizes. No attempt was made to measure the volumes of individuals to correct for this difference in size. The uptake data were quite variable, however application of statistical analysis of the many replicates and trials used allowed conclusions to be drawn.

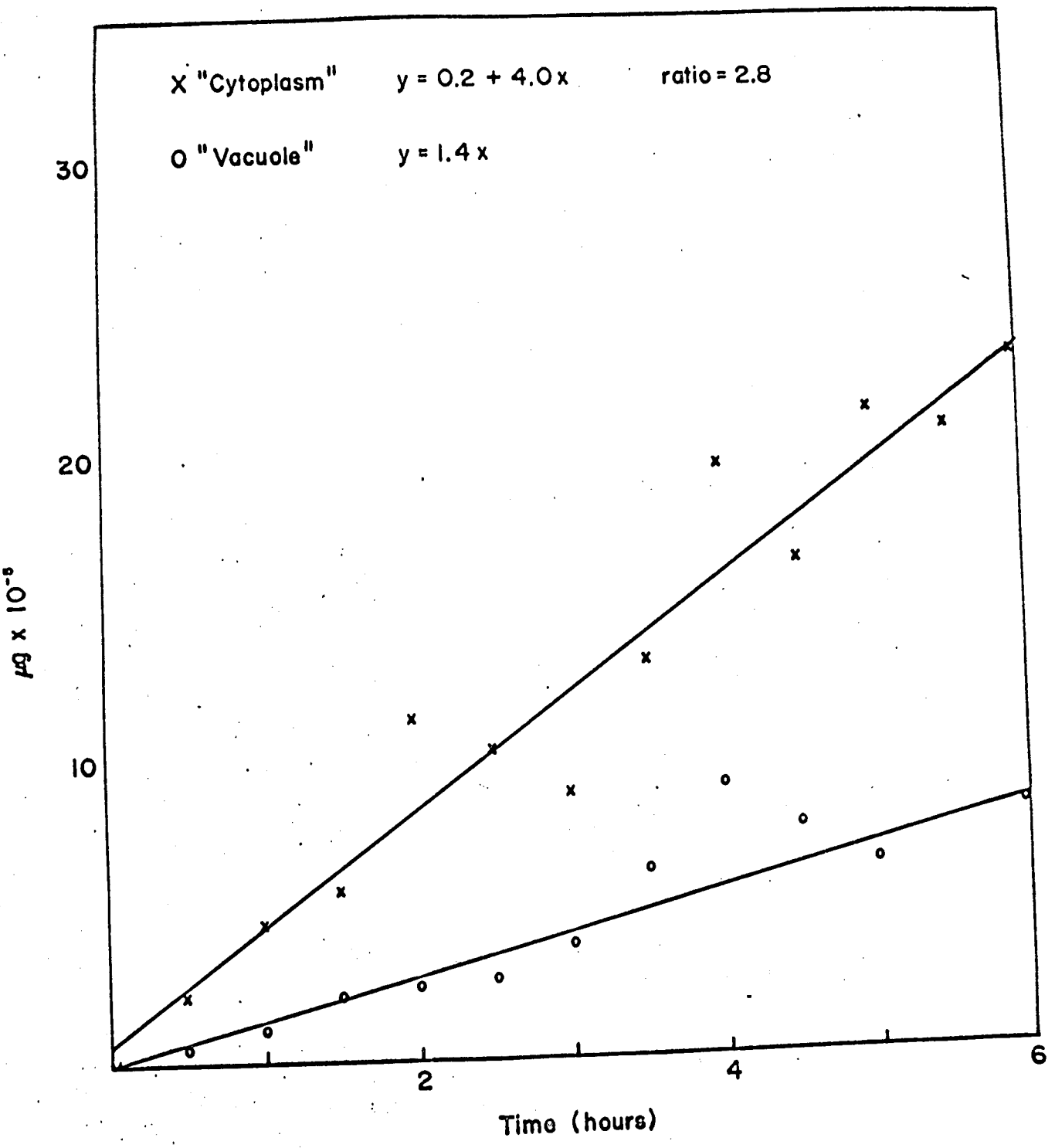
Results

Permeation of Neutral Amino Acids

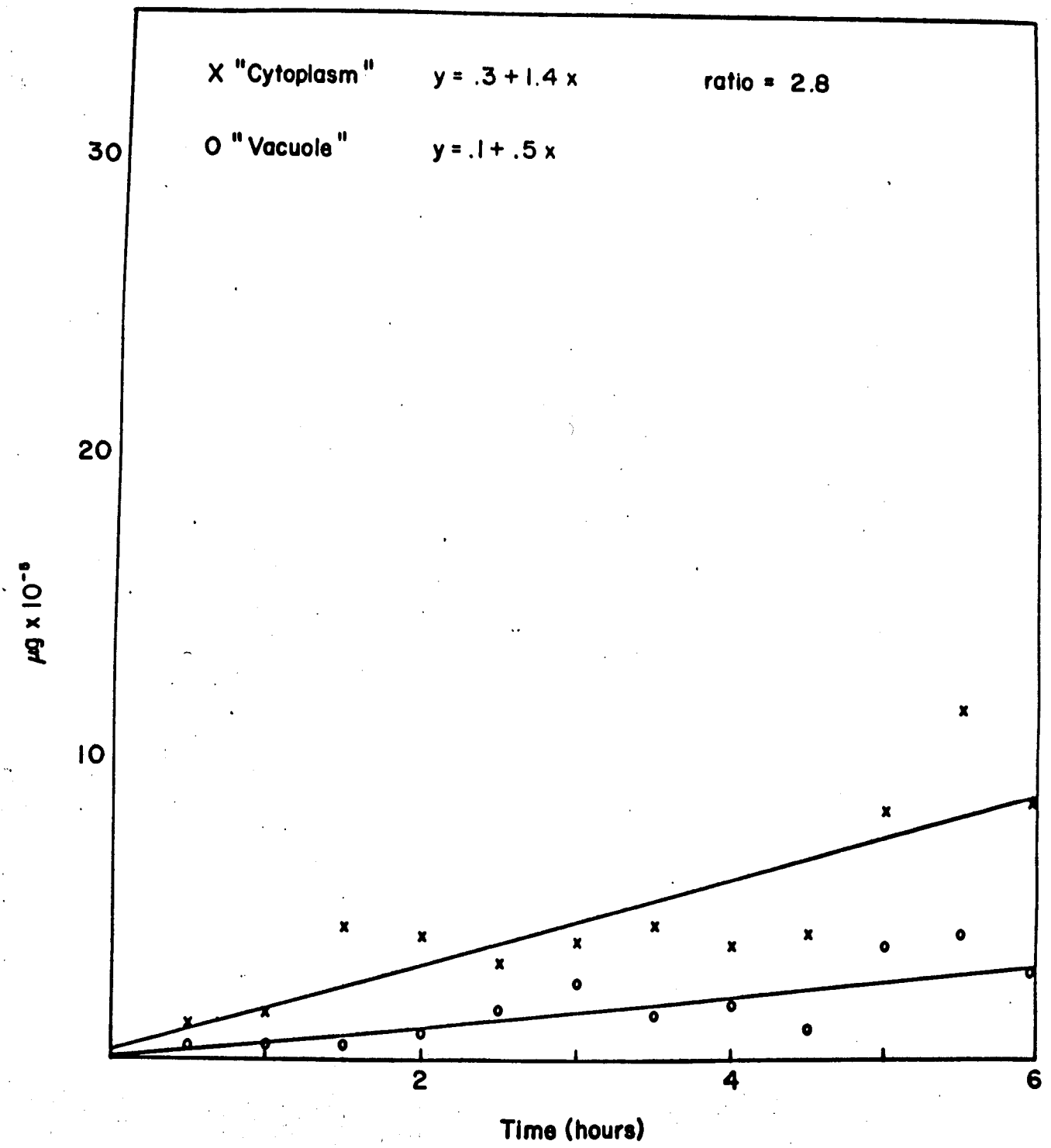
The first experiment examined the permeation of glycine-^{14}C into Nitella. Twenty cells were harvested and placed in a petri dish containing 10 ml. of distilled water. The distilled water was decanted and 10 ml. of 3×10^{-7}M glycine-^{14}C was added to the petri dish containing the cells. At 30 minute intervals over six hours, a cell was selected at random and washed by dipping into a series of three changes of distilled water. The excess water was removed with absorbent paper. One end of the cell was cut with a razor and the "vacuolar" and "cytoplasmic" fractions collected separately for assay. At intervals during the six-hour experiment, a cell was removed and examined microscopically for mechanical damage. If mechanical damage resulted in cessation of the protoplasmic streaming, then the trial was discontinued and repeated. There was no damage to any cells for the first six hours. By 24 hours protoplasmic streaming had stopped and all the cells were plasmolyzed. Six trials were used on a total of 120 cells.

Using identical techniques, experiments were performed to examine the permeation of 1×10^{-7}M L-alanine-^{14}C and 1×10^{-7}M L-valine-^{14}C. Fig. 5 summarizes the permeation of glycine-^{14}C, L-alanine-^{14}C and L-valine-^{14}C into the Nitella cell.

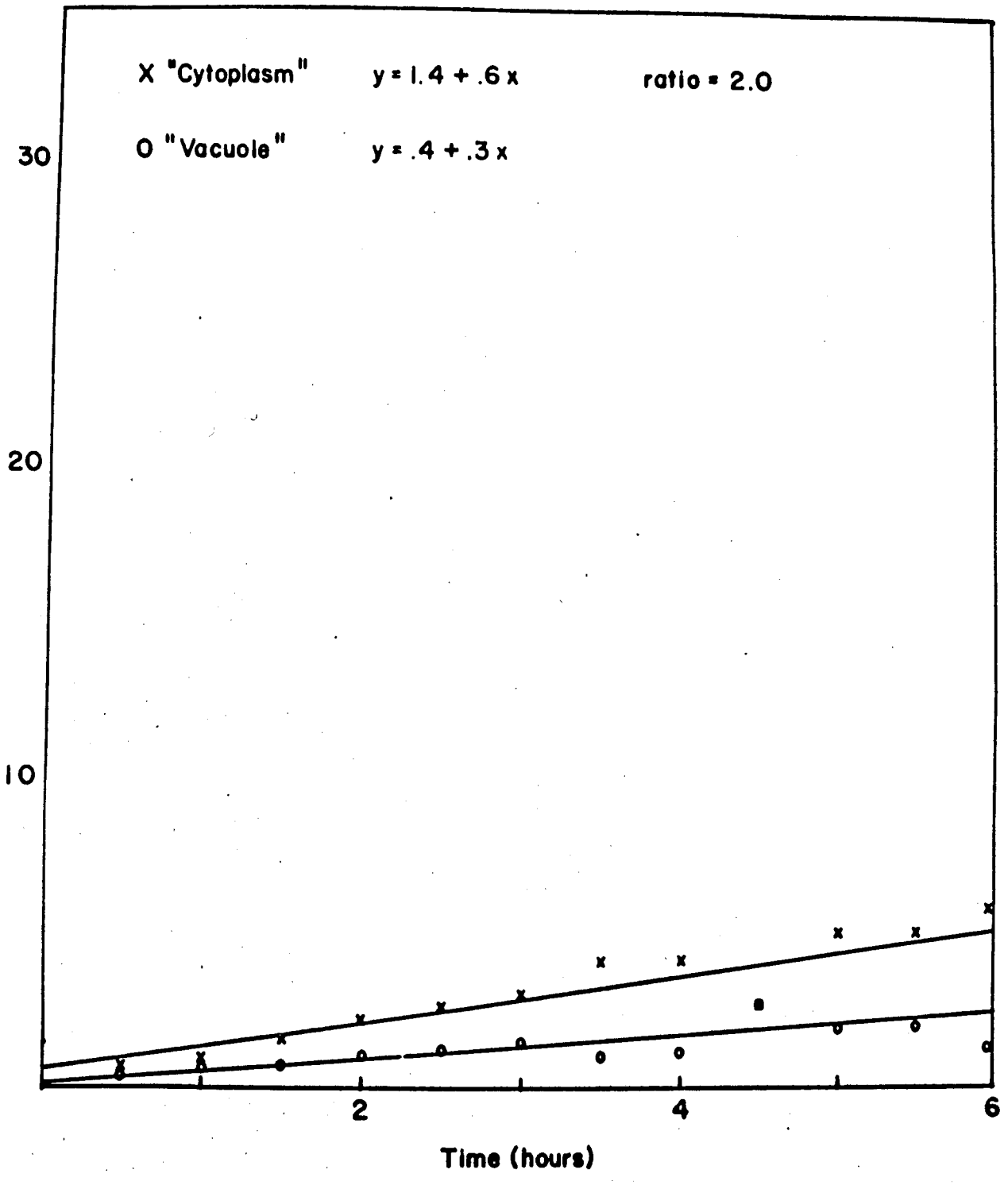
Fig. 5. The permeation of neutral amino acids into the "cytoplasmic" and "vacuolar" fractions of *Nitella flexilis*.



A Glycine-^{14}C



B L-alanine-^{14}C



C L-valine-^{14}C

The permeation of each of the neutral amino acids is significant. The quantity of amino acids entering the cell is directly proportional to the length of time in which the cell was in the amino acid solution. Also, there was no indication of any lag in uptake of the amino acids. Since the ^{14}C in the "vacuolar" fraction also increases in a linear manner with time, it is probable that the amino acids were able to penetrate both the plasmalemma and the tonoplast.

The slope "b" for the permeation of glycine-^{14}C into the "cytoplasm" is 4.0; "b" for the permeation of the glycine into the "vacuole" is 1.4. Hence, the permeation rate of glycine into the "cytoplasm" is greater than the permeation rate into the "vacuole." Similarly the permeation of L-alanine-^{14}C and L-valine-^{14}C was at a greater rate into the "cytoplasm" than into the "vacuole."

The rates of permeation into the "cytoplasm," expressed as $\mu g\ hr^{-1}$, were 4.0×10^{-5} for glycine-^{14}C, 1.4×10^{-5} for L-alanine-^{14}C, and 0.6×10^{-5} for L-valine-^{14}C.

That there was no lag in uptake was confirmed by applying the statistical analysis outlined in Table 4 to determine the "goodness of fit" of a line originating at the origin in each of these graphs. The results of this analysis are presented in Table 5. For each of the neutral amino acids, the "F" value is less than one. Thus, the slope passes through the origin, i.e. there is no initial lag period or initial period of rapid uptake.

Table 5. "F" values which determine the "goodness of fit" of a line through the origin to the data obtained for each compound

Compound	"F" Value	
	Vacuole	Cytoplasm
Glycine-^{14}C	.008	.017
Alanine-^{14}C	.13	.20
Valine-^{14}C	.021	.22
Aspartate-^{14}C	.55	.37
Arginine-^{14}C	.44	.09
Glucose-^{14}C	.07	.49
Sucrose-^{14}C	.17	.24

"F" < 1.0 indicates that the line passes through the origin and there is no lag to uptake.

The permeation of these compounds into a Nitella cell increases linearly with time, within the limits of the time interval used.

It has been reported that in the case of both plant and animal cells (3,29), an increase in length of the non polar portion of amino acids as well as an increase in the degree of methylation results in an increase in lipid solubility. Christensen (29) has demonstrated that the permeation of neutral amino acids into human erythrocytes is directly related to the length of the hydrocarbon chain.

To visualize the size and shape of the various compounds at the molecular level models were constructed of each compound using the Fisher-Heischfelder-Taylor system, Table II. These models are constructed to the scale 1 cm = 1 Å. The atoms are represented by spherical balls with flat faces where covalent bonds may be formed. The radius of the spheres represent, to scale, the van der Waals radius of the atom, or the radius to which the electron orbitals of the atoms extend with appreciable density. With the models, "covalent bonds" are formed between the "atoms," by means of small metal fasteners at the centers of the flat faces of the "atoms," and these couplings are so designed that the resulting "bond" between two "atoms" represent the correct interatomic distances between their centers. Likewise, the angles between the faces are such as to form "bonds" at the correct angles. Of course, single,

double, and triple bonds differ in bond length and angle, so different model "atoms" are required to represent different bond configurations of a given element. These models are extremely useful tools in visualizing and comparing various compounds used in permeation experiments.

Molecular size, as well as the degree of methylation decreases in the order L-valine>L-alanine>glycine. At the concentrations used in this series of experiments, the micromolar as compared to the millimolar level, the permeation of these three amino acids is opposite to that reported in the literature for erythrocytes. Glycine penetrates the quickest, the order of penetration being glycine>L-alanine>L-valine.

Fig. 5 summarizes the ratio of the permeation of each compound into the cytoplasm of a Nitella cell to the permeation of the compound into the vacuole. As seen above, the permeation rate differs for each of the neutral amino acids, decreasing in the order glycine>L-alanine>L-valine. However, the permeation ratios, cytoplasm:vacuole, of each of these amino acids are very close to the same value, ranging from 2.0 to 2.8.

The permeation rate into a Nitella cell decreases with increasing methylation. The proportion of the amino acid crossing the tonoplast to that crossing the plasmalemma is similar for each compound. This suggests that the plasmalemma is the limiting membrane for permeation from the external environment into the "vacuole."

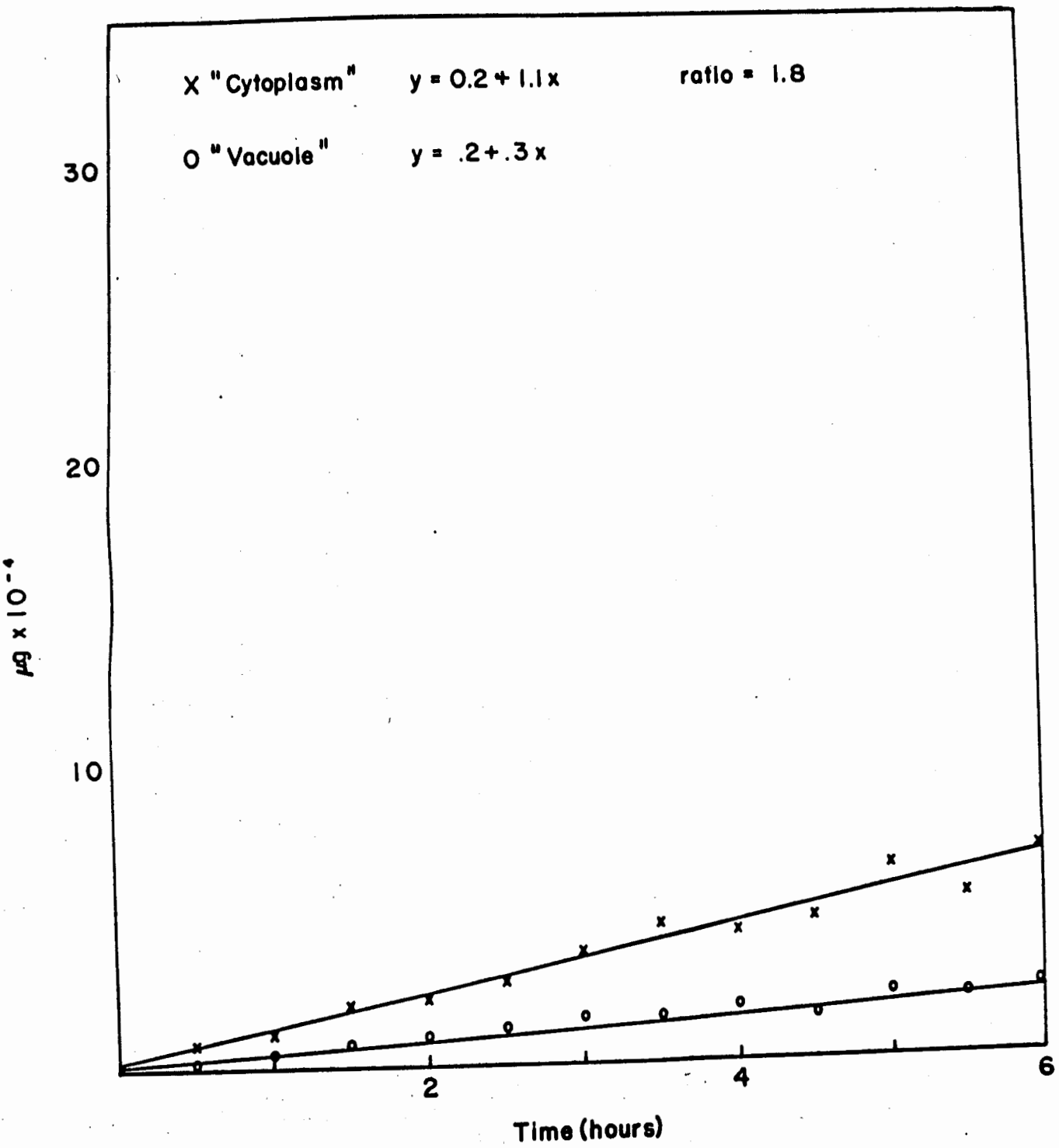
The Permeation of Basic and Acidic Compounds

The permeation of certain basic and acidic compounds into Nitella cells was examined to compare any influences the electrically charged molecules may have on permeation with that of the neutral amino acids. Identical techniques to that used with the neutral amino acids were applied to the permeation of a basic amino acid, L-arginine-^{14}C. The results of six trials are summarized in Fig. 6A.

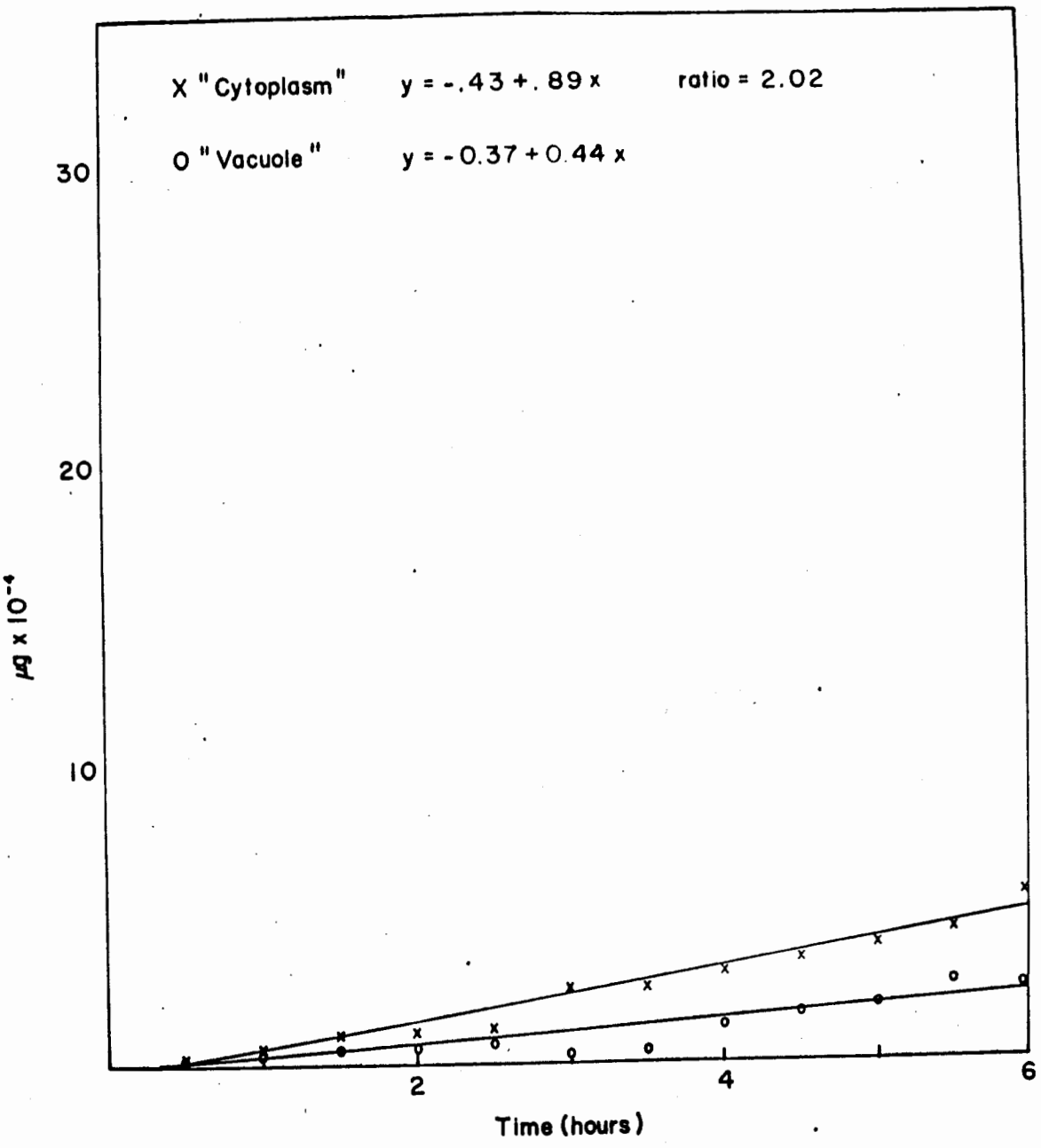
The permeation of L-arginine is significant. The quantity of L-arginine entering the cell is proportional to the length of time in which the cell was in the solution of L-arginine. The ^{14}C in the "vacuolar" fraction also increases in a linear manner with time. Hence, it is probable that L-arginine was able to penetrate both the plasmalemma and tonoplast.

The permeation rate of L-arginine into the "cytoplasm" is 1.1×10^{-4} $\mu g\ hr^{-1}$ into the "vacuole," 0.3×10^{-4} $\mu g\ hr^{-1}$. Hence the permeation of L-arginine into the cytoplasm is faster than into the vacuole. The "F" value of the entry of arginine into the cytoplasm and into the vacuole is less than one. Therefore, there is neither an initial lag period or period of rapid uptake. The quantity of this compound entering the Nitella cell increases linearly with time, within the experimental interval.

Fig. 6. The permeation of basic and acidic amino acids into the "cytoplasmic" and "vacuolar" fractions of *Nitella flexilis*.



A L-arginine-^{14}C



B L-aspartate-^{14}C

Comparison of Figs. 5 and 6 shows that the rate of permeation of L-arginine is an order of magnitude greater than the permeation of L-valine into a Nitella cell. If the mechanism in the plasmalemma for the permeation of L-arginine into a Nitella cell was the same as that for the neutral amino acids, the permeation of the much larger molecule L-arginine should be slower than the permeation of L-valine.

L-arginine, unlike L-valine, has a single unbalanced positive charge in solution. This influences the permeation of the compound into the cell. The mechanism for uptake of arginine is apparently different from the mechanism for the uptake of the electrically neutral amino acids.

As a comparison to the permeation of the basic compound, the permeation of an acidic compound, L-aspartate-^{14}C, into Nitella cells was examined using the same technique. The results of six trials are summarized in Fig. 6B.

The rate of permeation of L-aspartate into the "cytoplasm" is 0.9×10^{-4} $\mu g\ hr^{-1}$ and into the "vacuole" it is 0.4×10 $\mu g\ hr^{-1}$. Hence the permeation of aspartic acid into the cytoplasm is faster than into the vacuole of a Nitella cell. Also, the "F" values of permeation of L-aspartate-^{14}C into the "cytoplasmic" and "vacuolar" fractions of a Nitella cell are less than one, Table 6. Therefore, there is neither an initial lag period of permeation nor an initial period of rapid uptake.

The permeation of L-aspartate into these cells increases linearly with time, within the limits of the time interval used.

L-aspartate enters the Nitella cell at the same rate as L-arginine. Its molecular size is greater, and degree of methylation is less than L-valine. Since it is entering the cell at a much faster rate than the neutral amino acids, a different permeation mechanism must be present in the cell membrane.

Both arginine and aspartic acid permeate ten times faster than the neutral amino acids. Arginine has a positive charge and aspartate a negative charge. It is hard to visualize how these two molecules can be taken up by the same mechanism. Certainly if pores in the membrane are involved, then the negative charges that are known to surround the pores should impede one molecule while increasing the uptake of the other. Also, if a carrier mechanism is postulated, a different carrier should exist for the two differently charged molecules.

Kinetic analysis of uptake of these two oppositely charged molecules should supply information about mechanisms. Such experiments were undertaken and one is reported in the next section.

Kinetic Analysis of Permeation of Amino Acids

The permeation experiments performed with the amino acids indicate that there are differences in mechanisms. A carrier mechanism is one which has been proposed. The existence of such a mechanism can be demonstrated by applying enzyme kinetic analysis to uptake data.

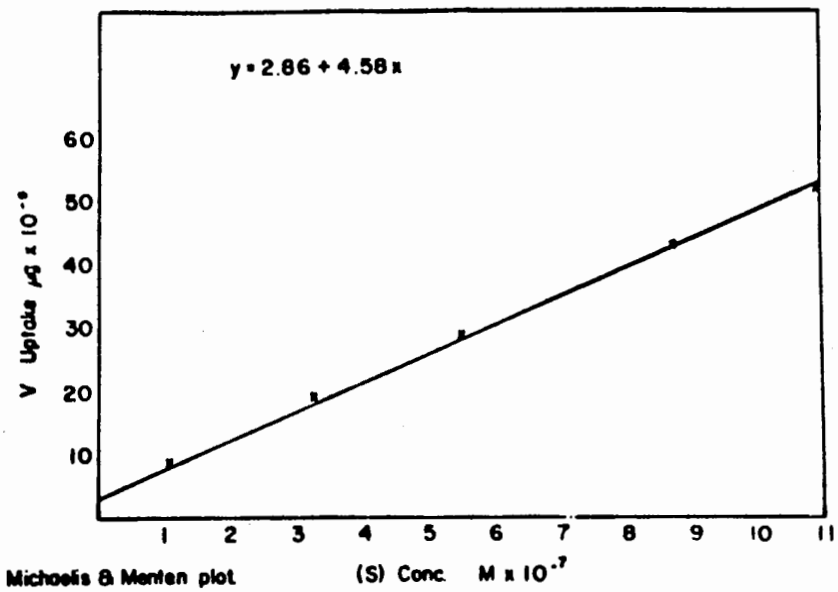
The permeation of L-arginine-^{14}C into Nitella was determined for a series of five different concentrations ranging from 1.1×10^{-8} to 1.1×10^{-7}M, Table 6A. Cells were removed from the solution at each concentration and the uptake was determined for the combined "cytoplasmic" and "vacuolar" fractions of the cell, at 20 minute intervals for 150 minutes.

The mean value of permeation of L-arginine was determined for each concentration used. This data was analyzed using the Michaelis-Menten model for the formation of an enzyme-substrate complex. Also, the mathematical method of Lineweaver and Burk, was used on the data. The results are illustrated in Fig. 7A and Table 6A.

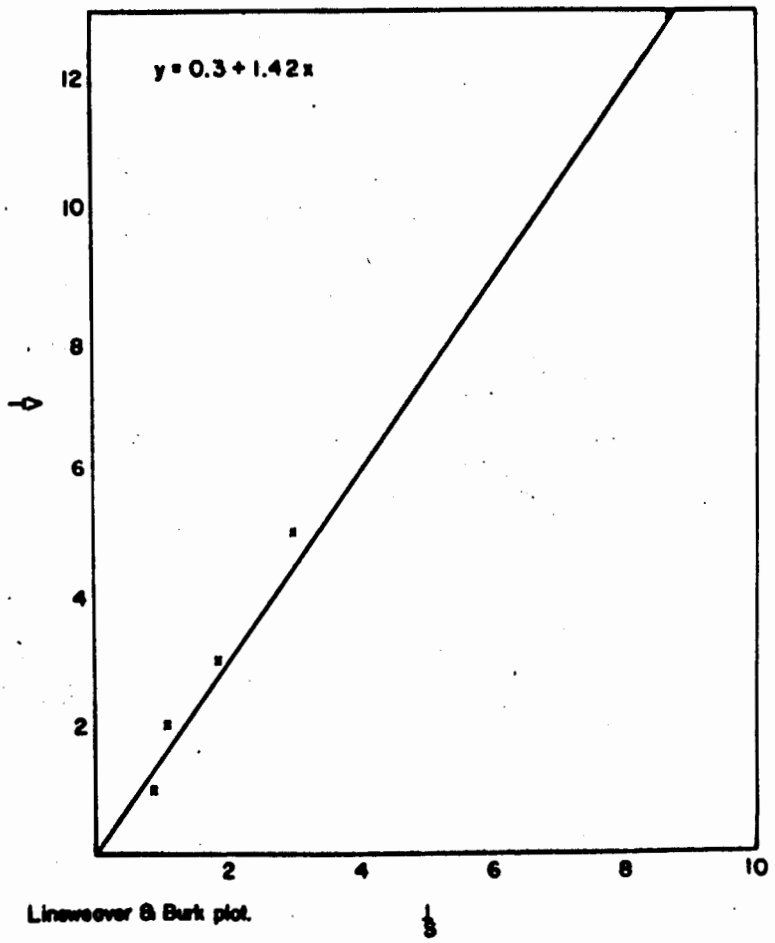
The rate of permeation of L-arginine is directly proportional to the concentration gradient. It does not exhibit kinetic features similar to those of enzyme and carrier systems.

A similar experiment was performed with L-aspartate-^{14}C. A series of five concentrations ranging from 1.5×10^{-8} to $1.5 \times 10^{-7}_{m}$ were used, Table 6B. Cells were removed from the solution at each concentration and uptake determinations made on the

Fig. 7. Kinetic analysis of uptake of basic and acidic amino acids into *Nitella flexilis*.

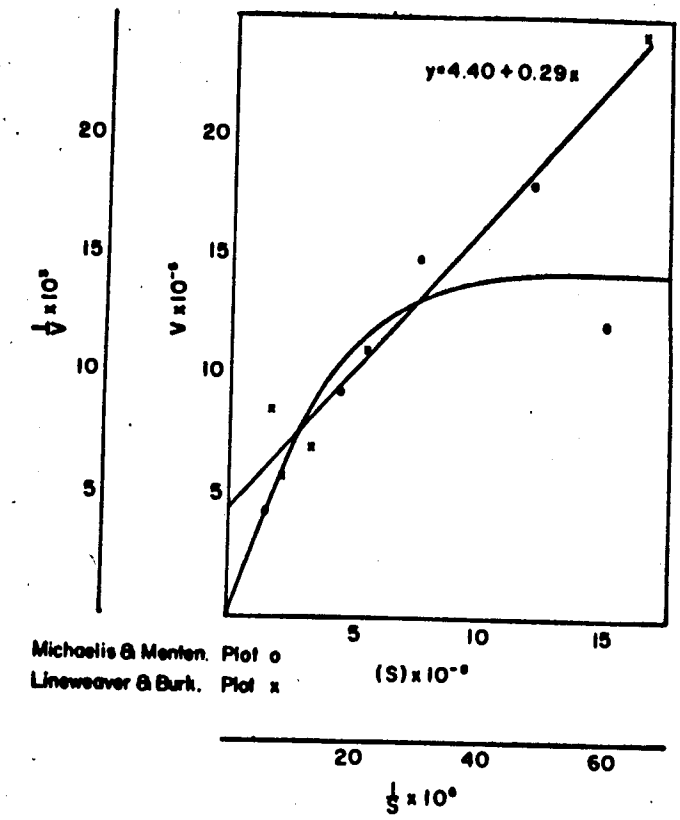


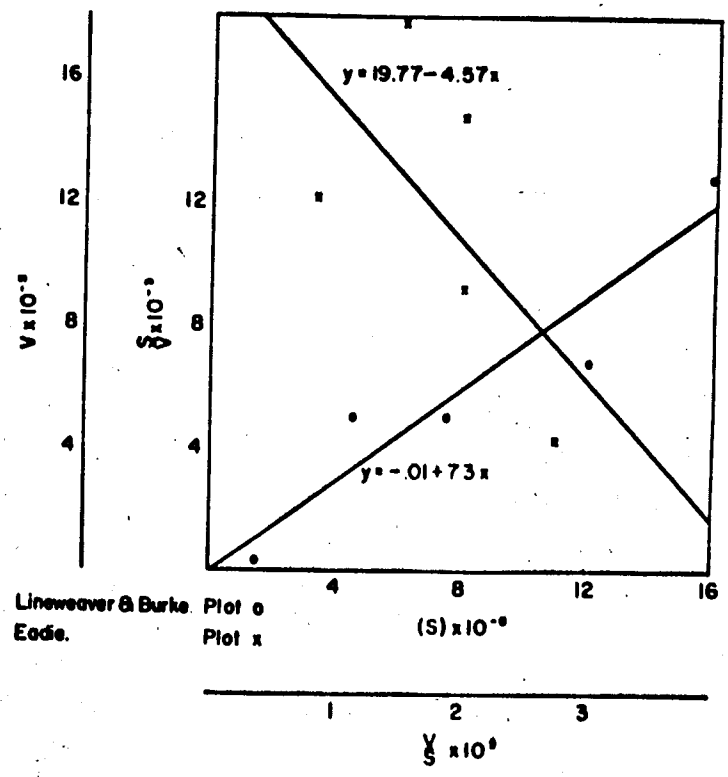
Michaelis & Menten plot



Lineweaver & Burk plot.

A L-arginine-^{14}C





B L-aspartate-^{14}C

Table 6. Kinetic analysis of permeation of ^{14}C-labelled basic and acidic amino acids into Nitella flexilis.

A. L-Arginine-^{14}C

Concentration $\lfloor s \rfloor$ moles/liter x 10^{-8}	Uptake V micrograms x 10^{-5}	$\frac{1}{V}$ x 10^{3}	$\frac{1}{\lfloor s \rfloor}$ x 10^{7}
1.1	7.4	13.5	9.0
3.3	18.2	5.4	3.0
5.5	28.6	3.5	1.8
8.8	43.2	2.3	1.1
11.0	52.9	1.8	0.9

B. L-Aspartate-^{14}C

Concentration $\lfloor s \rfloor$ Moles/liter x 10^{-8}	Uptake V micrograms x 10^{-5}	$\frac{1}{V}$ x 10^{3}	$\frac{1}{\lfloor s \rfloor}$ x 10^{6}	$\lfloor s \rfloor / V$ x 10^{-2}	$V / \lfloor s \rfloor$ x 10^{3}
1.5	4.1	66.7	24.4	3.6	2.7
4.6	9.1	21.8	10.9	5.0	2.0
7.5	14.7	13.3	6.7	5.1	1.9
12.0	17.9	8.3	5.6	6.7	1.5
15.0	12.1	6.7	8.3	12.3	0.8

combined cell fractions at 30 minute intervals for 180 minutes.

The mean value of permeation for each concentration used was determined. This data was then analyzed using the Michaelis-Menten model, the mathematical model of Lineweaver and Burk and of Eadie. The results are illustrated in Table 6B and Fig. 7B.

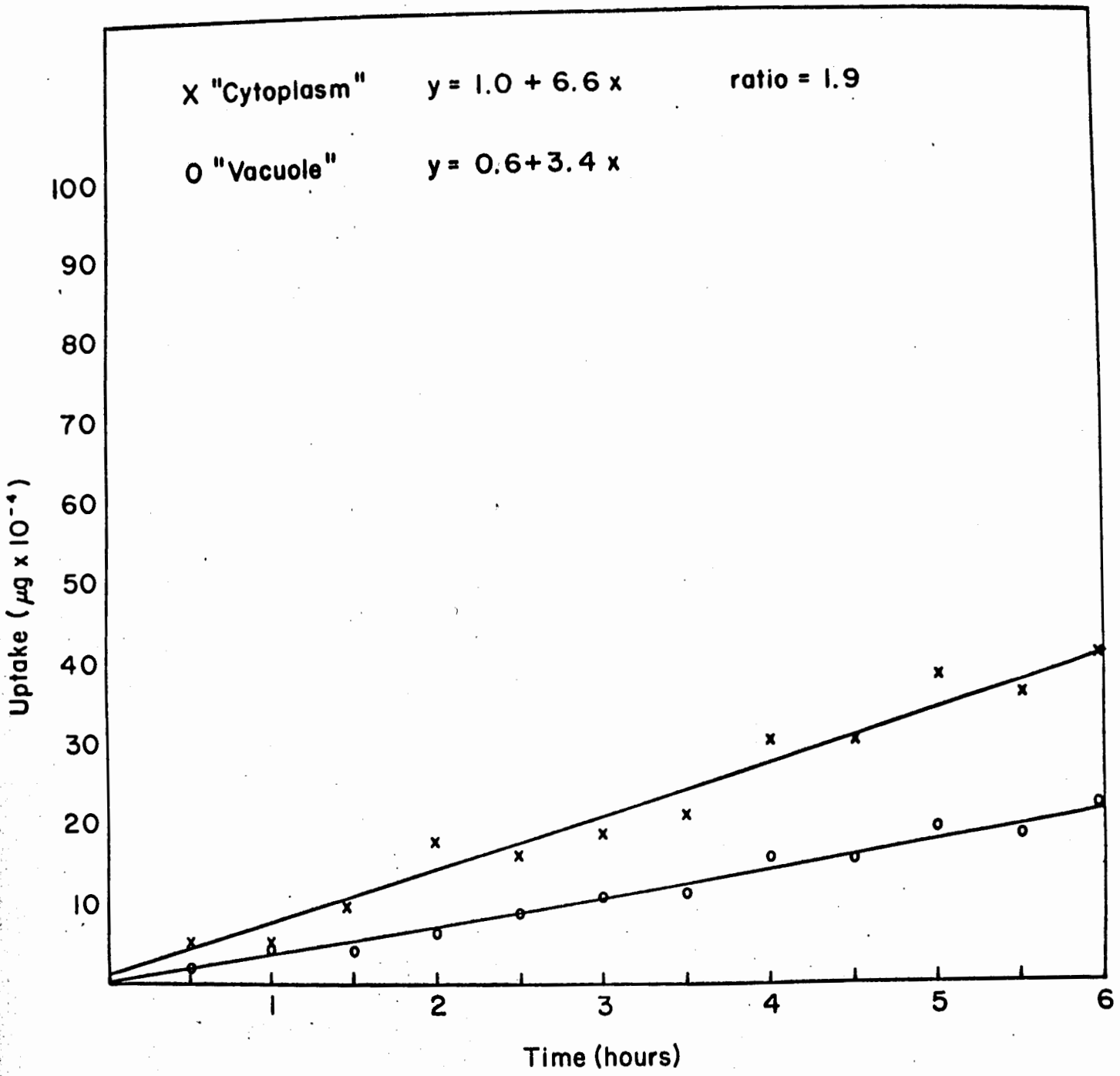
The rate of permeation of L-aspartate-^{14}C is not directly proportional to the concentration gradient, it satisfies enzyme-carrier kinetics. This means that at least two mechanisms are involved in the permeation of amino acids into Nitella. The carrier mechanism is possible for L-aspartate but there must be another mechanism for L-arginine.

Permeation of Sugars into Nitella

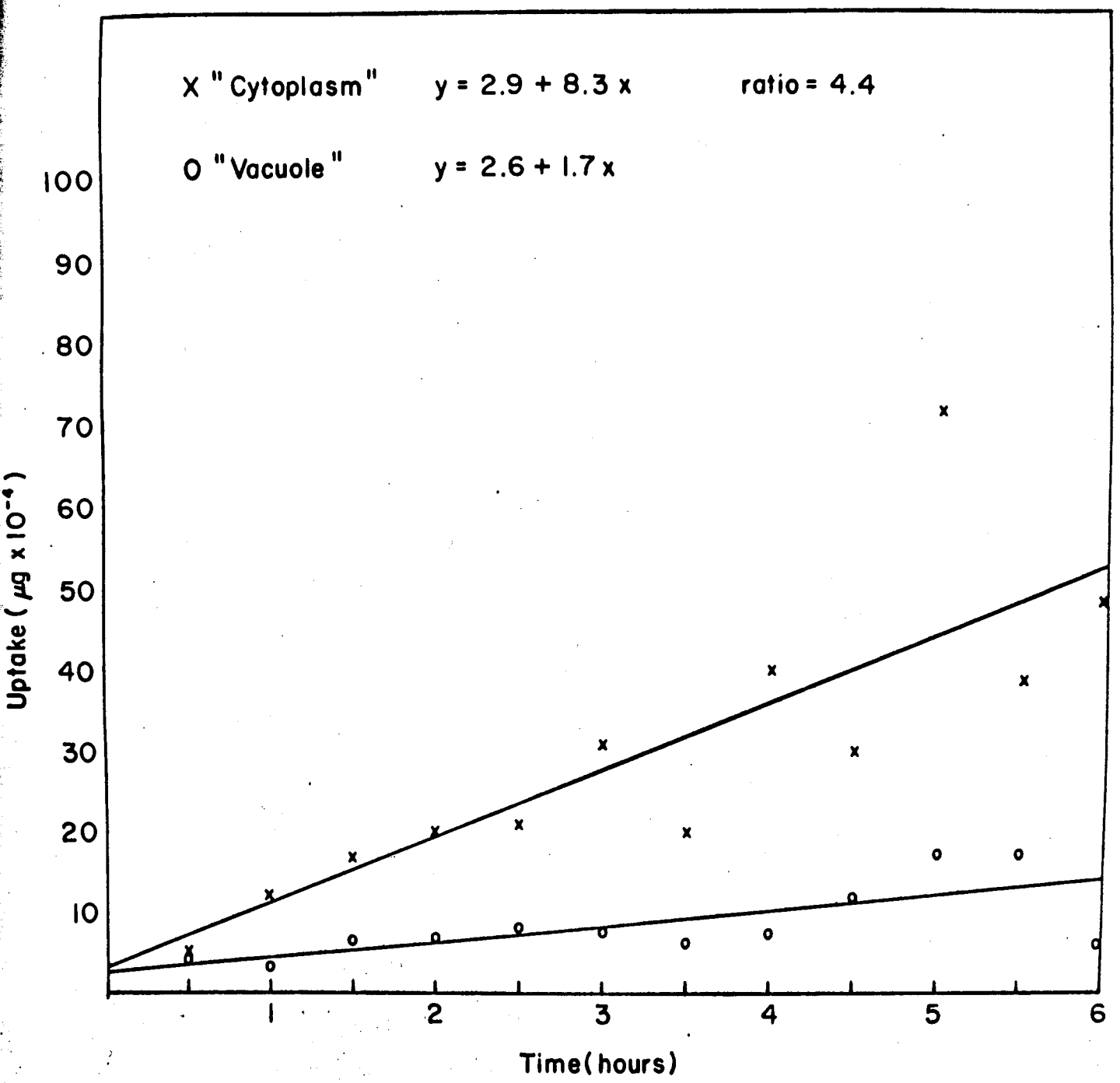
The neutral amino acids in solution are electrically neutral, they possess an equal number of acidic carboxyl and basic amino groups. Experiments investigating the permeation of D-glucose-^{14}C and sucrose-^{14}C were performed to compare the permeability properties of the membrane to the two types of neutral compounds.

Experiments to examine the permeation of D-glucose-^{14}C and sucrose-^{14}C into Nitella cells were performed with the techniques used on the ^{14}C-labelled amino acids. For each sugar, six trials were performed, on a total of 120 cells. The concentration of the sugars used in the experiments was 1×10^{-6}M. Fig. 8 summarizes the permeation of glucose-^{14}C and sucrose-^{14}C into the cells.

Fig. 8. The permeation of ^{14}C-labelled sugars into *Nitella flexilis*.


X "Cytoplasm" y = 1.0 + 6.6 x ratio = 1.9
O "Vacuole" y = 0.6+3.4 x
Uptake (μg x 10⁻⁴)
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
Time (hours)

A D-glucose


X "Cytoplasm" y = 2.9 + 8.3 x ratio = 4.4
O "Vacuole" y = 2.6 + 1.7 x
Uptake (μg x 10⁻⁴)
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
Time(hours)

B Sucrose

The permeation rate of D-glucose-^{14}C into the "cytoplasm" is 7.7×10^{-4} $\mu g\ hr^{-1}$, into the "vacuole" 3.7×10^{-4} $\mu g\ hr^{-1}$. The rate of entry of sucrose-^{14}C into the "cytoplasm" is 8.3×10^{-4} $\mu g\ hr^{-1}$, and into the "vacuole" it is 1.7×10^{-4} $\mu g\ hr^{-1}$.

For each of the sugars, the "F" value of permeation into the "cytoplasm" and "vacuole" is less than one. Therefore, as is the case for the amino acids, the amount of these two compounds in the cell increases linearly with time, within the experimental interval. Since the ^{14}C in the "vacuolar" fraction is directly proportional to the length of time in which the cell was in the sugar solutions, it is probable that D-glucose-^{14}C and sucrose-^{14}C were able to penetrate both the plasmalemma and the tonoplast.

The results suggest that D-glucose and sucrose permeate the cell membrane and that they move across the plasmalemma at the same rate, but that the tonoplast is slightly less permeable to sucrose than D-glucose.

If the main mechanism of permeation is by diffusion through pores in the cell membranes, sucrose, a considerably larger molecule would exhibit a slower rate of permeation than D-glucose. One or both sugars may cross the membrane as an enzyme carrier complex. To examine this possibility both sugars were examined kinetically.

Kinetic Analysis of Permeation of Sugars

The permeation of D-glucose-^{14}C into Nitella was determined for a series of concentrations ranging from 4×10^{-5} to 1×10^{-6}M, Table 7A. Cells were removed and the uptake determined at each concentration, for the combined "cytoplasmic" and "vacuolar" fractions of the cell, at 30 minute intervals for 180 minutes.

The mean value of permeation was determined for each concentration used. This data was analyzed using the Michaelis-Menten model, the mathematical method of Lineweaver and Burk and of Eadie. The results are illustrated in Fig. 9A and Table 7A.

By the same method, the permeation of sucrose-^{14}C into Nitella was determined for a series of concentration ranging from 2.5×10^{-5} to 1.0×10^{-6}M. The data was analyzed kinetically as for D-glucose-^{14}C, Fig. 9B and Table 7B.

The rate of permeation of both D-glucose-^{14}C and sucrose-^{14}C is not directly proportional to the concentration gradient, it satisfies enzyme-carrier kinetics. This suggests that D-glucose and sucrose may penetrate the cell membrane by means of an enzyme-carrier mechanism.

To determine the permeation of sugar across a wider range of concentrations, a second set of experiments were carried out. Cold D-glucose was added to solutions of 1×10^{-5}M D-glucose-^{14}C to prepare four 10 ml. solutions of D-glucose, the concentrations ranging from 1×10^{-4} to 1×10^{-1}M. D-glucose-^{14}C was used as a

Table 7. Kinetic analysis of permeation of ^{14}C-labelled sugars into Nitella flexilis

A. D-glucose

Concentration $\lfloor s \rfloor$	Uptake v	$v/\lfloor s \rfloor$	$\lfloor s \rfloor / v$	1/v	$1/\lfloor s \rfloor$
Moles/liter x 10^{-6}	Micrograms x 10^{-3}	10^2	10^{-3}		10^4
40	8.7	2.1	4.6	110	2.5
20	9.6	4.8	2.1	100	5.0
4	7.7	19.0	0.5	120	25.0
2	3.2	16.0	0.6	310	50.0
1	4.0	40.0	0.3	250	100.0

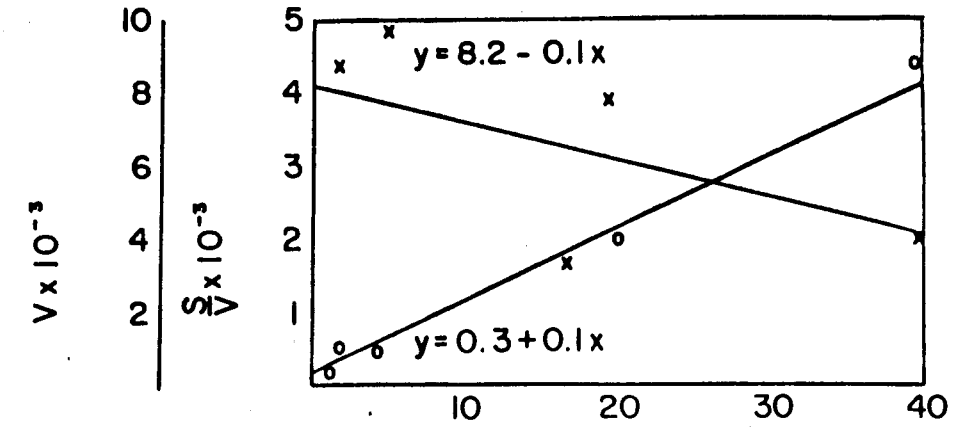
Vmax. (calculated) = 0.0089
Km (calculated) = 0.0160

B. Sucrose

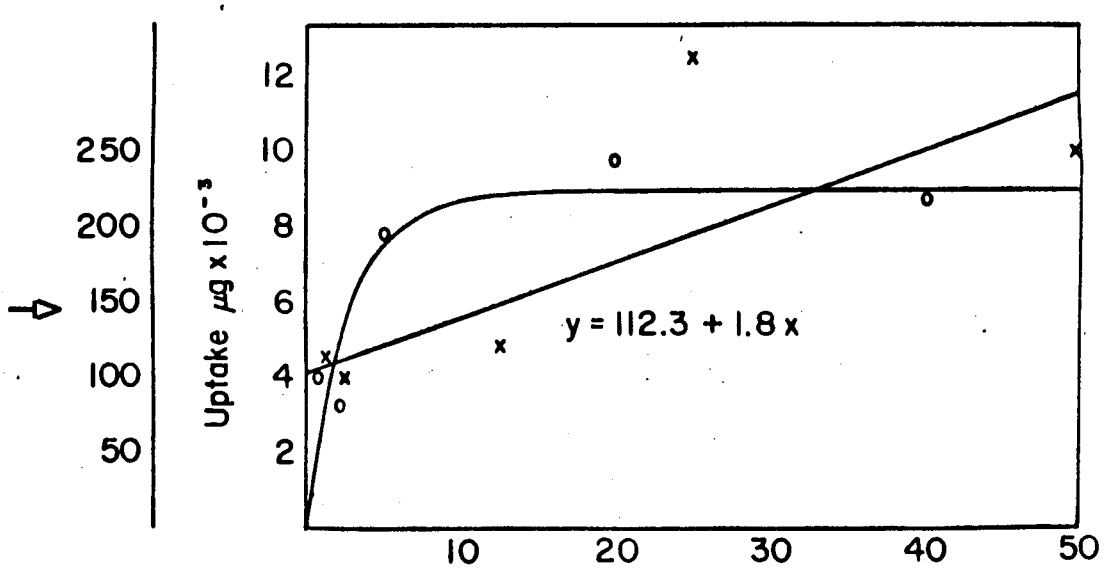
$\lfloor s \rfloor$	Uptake	$v/\lfloor s \rfloor$	$\lfloor s \rfloor / v$	1/v	$1/\lfloor s \rfloor$
Moles/liter x 10^{-6}	Micrograms x 10^{-3}	10^2	10^{-3}		10^4
25.0	7.4	2.9	3.4	130	4
10.0	9.8	9.8	1.0	100	10
5.0	7.3	14.5	0.7	140	20
3.3	5.1	15.3	0.6	200	30
1.0	4.1	40.9	0.2	240	100

Vmax (calculated) = .0083
Km (calculated) = .0108

Fig. 9. Kinetic analysis of uptake of ^{14}C-labelled sugars by *Nitella flexilis* using concentrations ranging 4×10^{-5}M to 1×10^{-6}M.

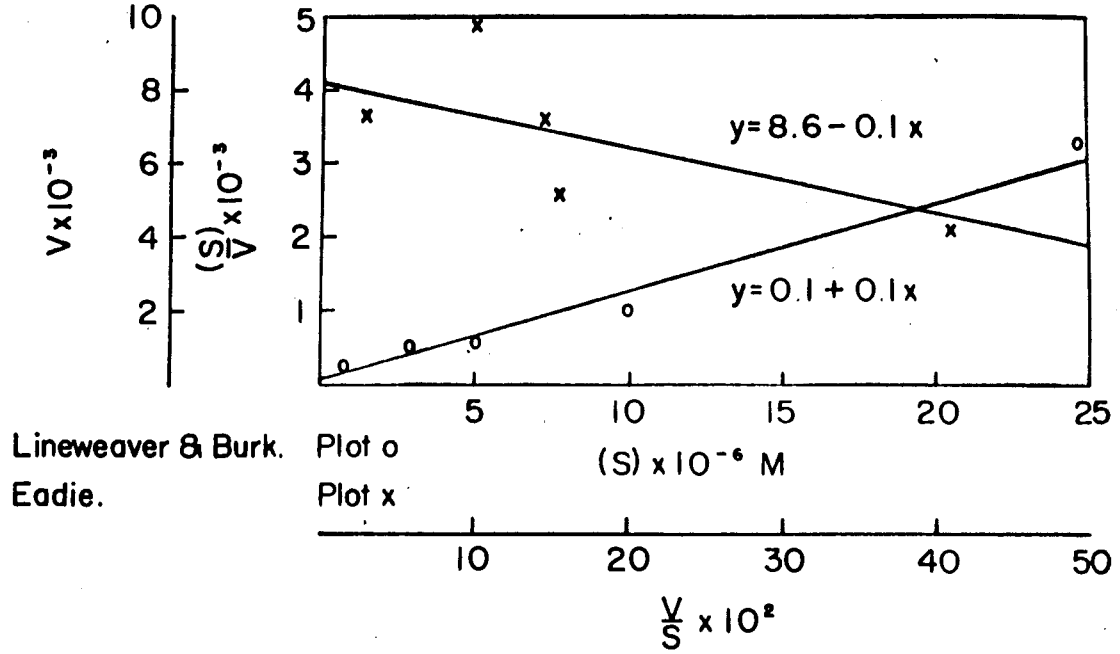


Lineweaver & Burk. Plot o
Eadie. Plot x



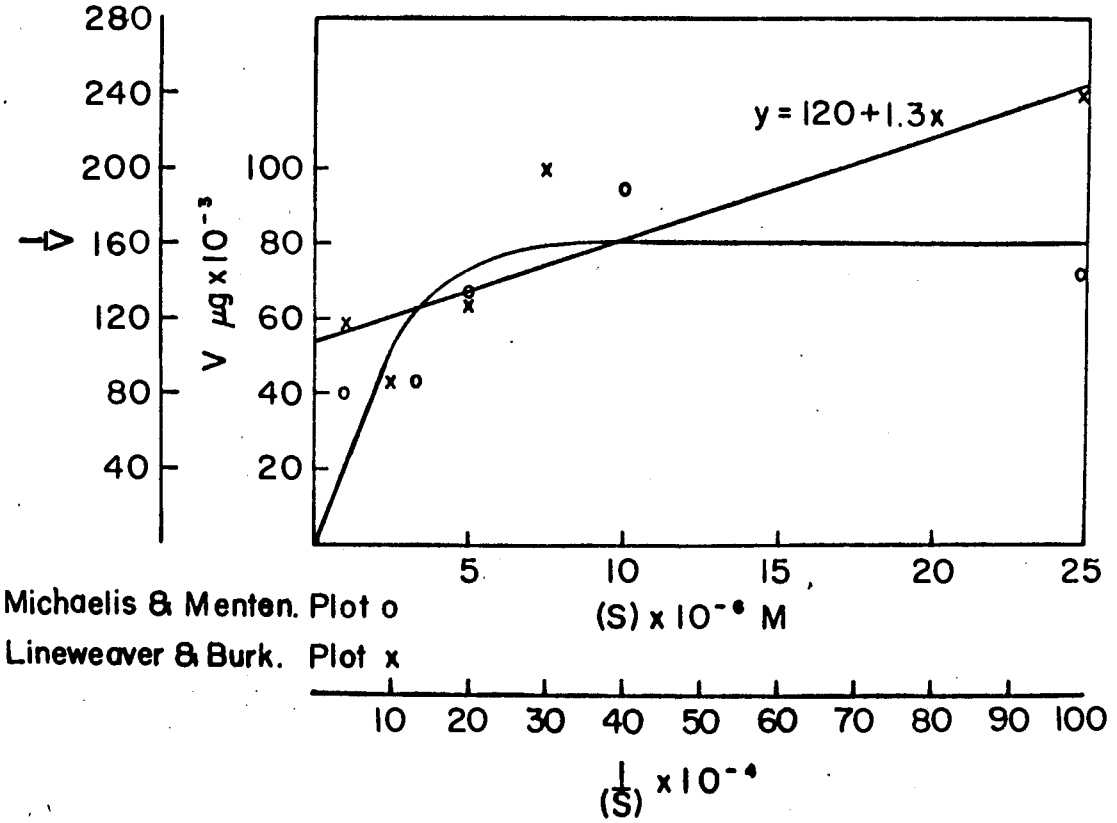
Michaelis & Menten. Plot o
Lineweaver & Burk. Plot x

A D-glucose



Lineweaver & Burk. Plot o

Eadie. Plot x



Michaelis & Menten. Plot o

Lineweaver & Burk. Plot x

B Sucrose

tracer in the experiment. A fifth 10 ml. solution of D-glucose-^{14}C ($1 \times 10^{-5}M$) was also prepared. Cells were removed at 30 minute intervals from each of the solutions for 180 minutes and kinetic analysis made on the data for total cell uptake, Fig. 10A.

A similar experiment was performed using sucrose, the concentrations ranging from $1 \times 10^{-5}M$ to $1 \times 10^{-1}M$, prepared in the same manner. Fig. 10B illustrates the results.

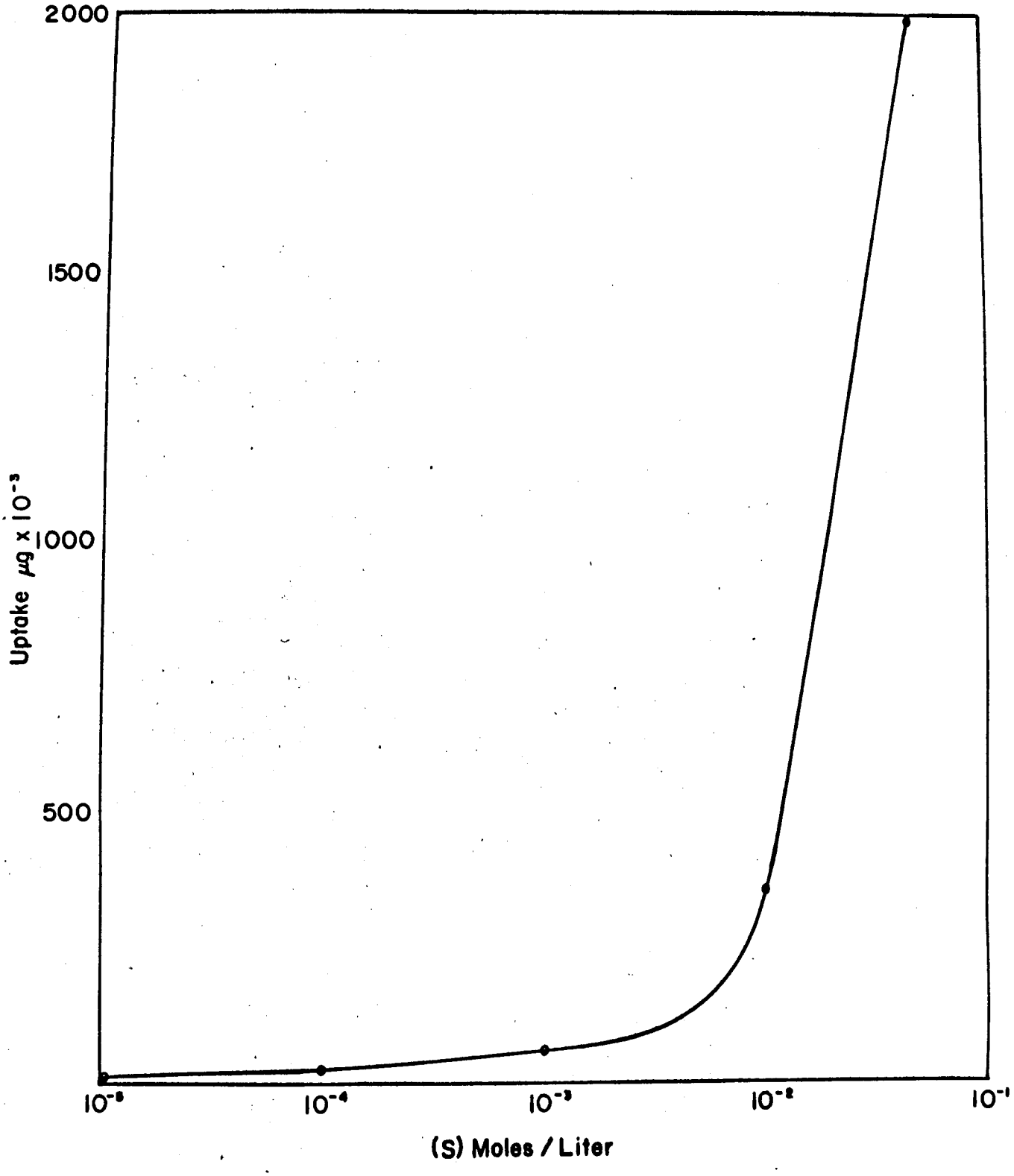
The kinetic experiments reveal a common over-all pattern of concentration dependence for uptake, which can be assumed to result from the operation of two types of processes. One of these can be described in terms of Michaelis-Menton kinetics; the other, up to the highest levels studied, fails to saturate and hence follows diffusion kinetics.

An investigation into the form in which compounds permeate

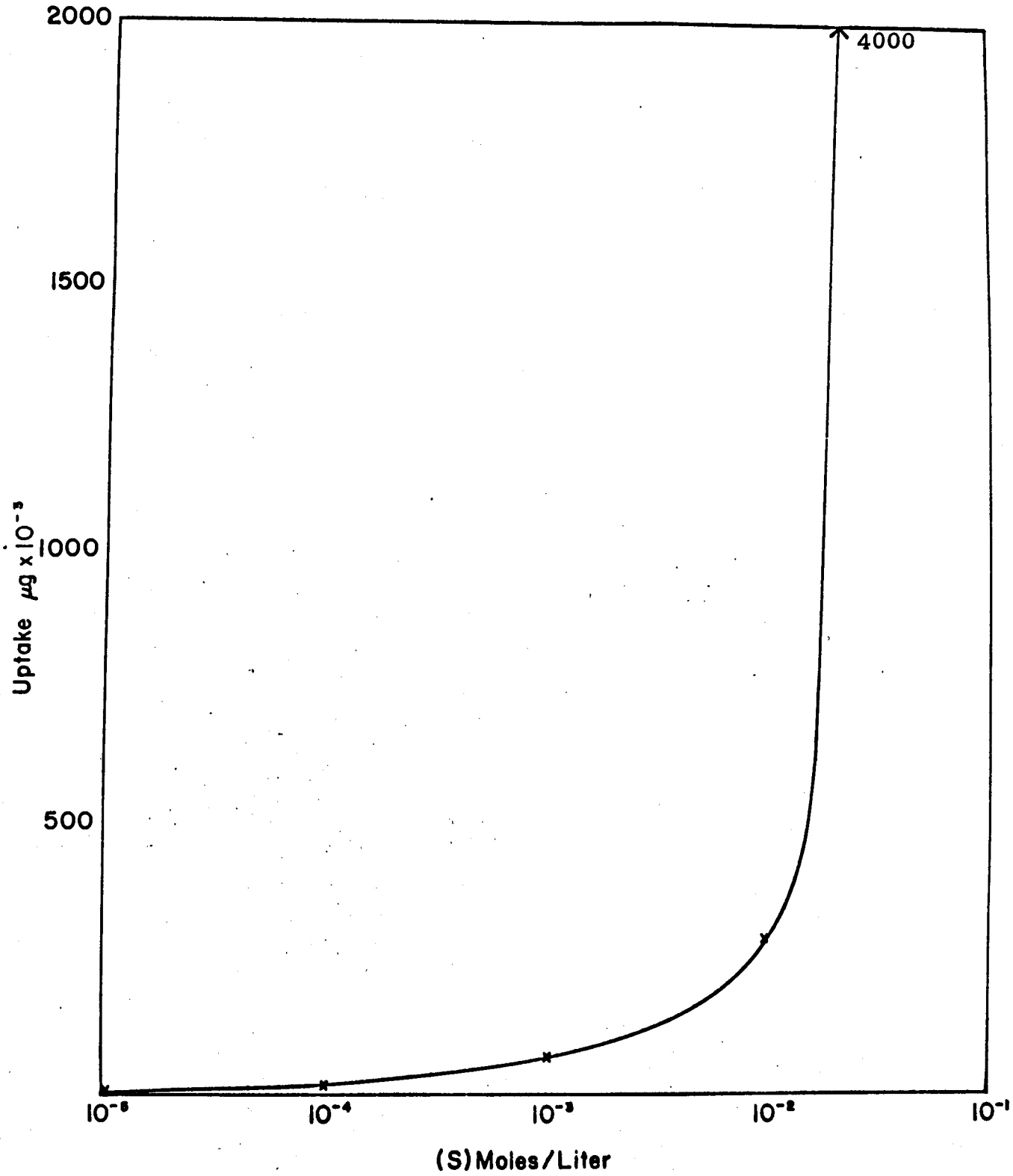
Ursino (28) in 1964 performed permeation experiments with ^{14}C-labelled D-glucose and sucrose. Under his experimental conditions, sucrose-^{14}C did not permeate the plasmalemma of Nitella flexilis for at least two and one-half hours. Other workers (6) have also reported the impermeability of the Nitella plasmalemma to sucrose. In the experiments described in this thesis, sucrose-^{14}C appears to permeate into Nitella cell fractions, this permeation being directly proportional to time.

The detection technique using the scintillation counter provides information about the amount and location of ^{14}C-labelled

Fig. 10. Kinetic analysis of uptake of sugars by *Nitella flexilis* using concentrations ranging from 1×10^{-5}M to 1×10^{-1}M.


2000
1500
1000
500
Uptake μg x 10⁻³
10⁻⁵
10⁻⁴
10⁻³
10⁻²
10⁻¹
(S) Moles / Liter

A D-glucose


2000
1500
1000
500
4000
Uptake μg x 10⁻³
10⁻⁵
10⁻⁴
10⁻³
10⁻²
10⁻¹
(S) Moles/Liter

B Sucrose

compounds but does not provide information about the nature of such compounds. Since, the results differed from the results of previous workers and since the results were similar to those for D-glucose, the following question is raised. Did sucrose-^{14}C permeate the cell membrane or is it hydrolysed to D-glucose-^{14}C and D-fructose-^{14}C by some mechanism at the outer side of the plasmalemma, D-glucose-^{14}C and D-fructose-^{14}C then permeating through the plasmalemma?

In an attempt to answer this question, the compounds in the combined cell fractions were separated by two dimensional paper chromatography after a three hour feeding in 2.5×10^{-5}M sucrose-^{14}C. The ^{14}C-labelled sugars cannot be identified by autoradiography, the amount of labelled compound in the cell being minimal. The sugars can be identified by two dimensional paper chromatography by using a cold solution of D-glucose, D-fructose and sucrose as tracers. This solution is spotted on the chromatogram as described previously, along with the cell extract. The concentration of unlabelled sugars is sufficient to produce colour reaction with the benzidine spray. The spots are then cut out from unsprayed chromatograms and counted in the liquid scintillation counter. The results are summarized in Table 8.

To examine the distribution of these three sugars after cells were treated for three hours in D-glucose-^{14}C, a similar experiment was performed using 2.5×10^{-5}M D-glucose-^{14}C. The results are summarized in Table 9..

Table 8. Distribution of ^{14}C-labelled D-glucose, D-fructose and sucrose in Nitella flexilis after treatment for three hours in Sucrose-^{14}C.

Compound	Chromatogram I cpm	Chromatogram II cpm	Chromatogram III cpm	%
Sucrose	1074	3162	2179	60.67
D-glucose	336	1626	1945	36.95
D-fructose	56	120	78	2.38

Concentration of sucrose-^{14}C ..... $2.5 \times 10^{-5}M$

The data represents five cells extracted on each chromatogram.

Table 9. Distribution of ^{14}C-labelled D-glucose, D-fructose and sucrose in *Nitella flexilis* after treatment for three hours in Glucose-^{14}C.

Compound	Chromatogram I	Chromatogram II	Chromatogram III	%
	cpm	cpm	cpm	
Sucrose	162	381	466	44.5
D-glucose	410	238	592	54.7
D-fructose	3	9	7	0.8

Concentration of D-Glucose-^{14}C ..... 2.5×10^{-5}

The data represents five cells extracted on each chromatogram.

The data, Table 8, indicate the presence of sucrose-^{14}C in the cell, after the cells were placed for three hours in sucrose-^{14}C. The amount of sucrose-^{14}C present is greater than that of glucose-^{14}C. However, when the cells were placed in a medium containing D-glucose-^{14}C for three hours, sucrose-^{14}C again was present as a major fraction of the three sugars in the cell, Table 9. One can suggest from these results that perhaps sucrose-^{14}C is hydrolysed at the plasmalemma converting it to its constituent hexose sugars. These compounds then permeated across the plasmalemma into the cell.

To resolve the problem of the form in which compounds permeate the plasmalemma and tonoplast, an experiment was performed to investigate the possibility of separating and identifying amino acids and sugars in the cellular fractions. For this investigation L-aspartate-^{14}C was used as the permeating molecule.

The distribution of the ^{14}C-labelled compounds is listed in Table 10. The ratio of ^{14}C in the "cytoplasmic" fraction to that in the "vacuolar" fraction is 1.8, the ratio of L-aspartate-^{14}C is 2.0. These values agree with the values obtained in the initial permeation experiment.

The cells are actively metabolizing. A large number of compounds, exhibiting a wide range of molecular weights and degree of methylation, have been labelled with ^{14}C. With the exception of D-fructose-^{14}C, the percent of each ^{14}C-labelled

compound in the two fractions is similar. This appears to suggest that these diverse compounds are permeating across the tonoplast into the vacuole at the same rate. The investigations in this thesis and the reports of other workers show that this is not the case. Molecules exhibiting such diversity in molecular weight, electrical properties and degree of methylation do not permeate at the same rate.

The technique of separating the two fractions of Nitella cells and of analyzing the compounds present in each of the two fractions after cells have been immersed in a ^{14}C-labelled solution has not been reported in the literature. It was adopted from the method developed by Thompson (27) to detect low concentrations of ^{3}H. The technique is a valid one. ^{14}C-labelled compounds which are present in concentrations too low to be detected by autoradiography can be detected by two dimensional paper chromatography. This is done by using cold compounds as tracers to locate each present on the chromatogram. These spots can then be assayed by the scintillation counter to detect radioactivity. This technique offers some exciting possibilities in the field of permeation investigations using Nitella cells.

Table 10. Distribution of ^{14}C-labelled compounds in the "vacuole" and "cytoplasm of Nitella flexilis after three hours in L-aspartate-^{14}C.

Compound	Chromatogram I				Chromatogram II			
	"Vacuole"		"Cytoplasm"		"Vacuole"		"Cytoplasm	
	cpm	%	cpm	%	cpm	%	cpm	%
L-lysine	7	1.7	9	1.2	5	0.8	9	0.8
L-asparagine	2	0.4	4	0.5	10	1.6	22	2.0
L-aspartate	43	10.6	87	12.1	59	9.8	113	10.6
L-glutamate	13	3.3	31	4.3	38	6.3	96	9.0
L-proline	0	0	0	0	0	0	0	0
L-valine	0	0	7	0.9	5	0.8	6	0.5
L-glycine	133	32.7	184	25.6	189	31.3	287	27.1
D-sucrose	3	0.7	7	0.9	3	0.4	8	0.7
D-glucose	197	48.5	323	45.0	281	46.6	434	40.9
D-fructose	8	1.9	65	9.0	12	1.9	84	7.9
Total	406		717		602		1059	
Ratio, "vacuole"/ "cytoplasm"		1.8				1.8		
Radio, "vacuole"/ "cytoplasm" for L-aspartate		1.9				2.0		

Concentration of aspartate-^{14}C ..... $1.5 \times 10^{-7}M$

The data represents 5 cells extracted on each chromatogram.

Discussion

The single Nitella cell was used for permeation studies of ^{14}C-labelled solutes. It was chosen because it contains two well defined membrane systems, the plasmalemma and the tonoplast, separating the cell into two main compartments. The plasmalemma separates the cell from the external environment, the tonoplast separates the vacuole from the cytoplasm. It was the aim of the investigation, using this model, to examine the permeability properties of the two membranes. This was done by assay of the two compartments to determine the rate of permeation of ^{14}C-labelled compounds across these membranes.

Permeation of solutes into the cell was based on the assumption that the cell wall does not effect permeation. Cellulose and polyuronic acids in the cell wall are arranged to form large pores with electrical properties (1). These pores in the cell wall are considered to be inert. They do not interfere with diffusion of low molecular weight permeants to the surface of the plasmalemma. Hence, any lag in uptake into the "cytoplasmic" fraction would be due to the behaviour of the plasmalemma.

One technique described in the literature for differentiation of the membranes involved in permeation in Nitella, is the cutting off of one end of the cell. It has been assumed that the watery fraction which flows quite easily from the cell is the vacuolar contents. Also, it is assumed that the fraction that is squeezed away from the cell wall is the cytoplasmic fraction. Because all the chloroplasts are in the latter fraction, it has been throught that this is a valid technique.

However, two pieces of evidence indicate that this is not so. Laites (17) found that the watery fraction contained some protein indicating that it did not consist of vacuolar contents alone.

The second piece of evidence is shown in Table 10. For the model used in this investigation, there are three compartments involved, the external environment, the cytoplasm and the vacuole. Because of the partitioning of these fractions one would not expect the distribution of the compounds in the "cytoplasmic" and "vacuolar" fractions to be similar. However, all of the ten compounds with the possible exception of fructose are partitioned equally between the two fractions. We must conclude, therefore, that the technique does not separate the cell into the two fractions bounded by the two membranes. Rather, the data indicates that the

watery fraction flowing from the cut end of the cell contains part of the cytoplasm as well as the vacuole. We might speculate that the fractions are separated at the interface of the sol-gel phases, that can be seen in the cytoplasm. The deviation of D-fructose-^{14}C leads to some speculation. D-fructose-^{14}C may be concentrated in the gel phase of the cytoplasm by being preferentially adsorbed or taken up by the chloroplasts.

The model used in these experiments, therefore, involves permeation across a single membrane, the outer cell bounding plasmalemma. We are however, dealing with two compartments, the "cytoplasmic" fraction consisting of the gel layer along the plasmalemma. Also, the "vacuolar" contents consisting of the watery or sol layer of the cytoplasm as well as the vacuolar sap.

The permeability of the cell is a function of the character both of the plasmalemma and of the penetrating molecule. Important characteristics involved are molecular weight and diameter of the penetrating molecule, their polar or non polar nature and the substituents attached to the basic carbon skeleton.

Several types of mechanisms have been proposed as possibly being involved in the permeation of compounds through the plasmalemma into the plant or animal cell. The results of this investigation into the permeability properties of the

plasmalemma of Nitella cells suggest that more than one of these mechanisms is operating.

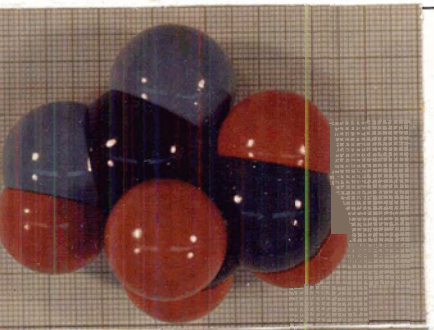
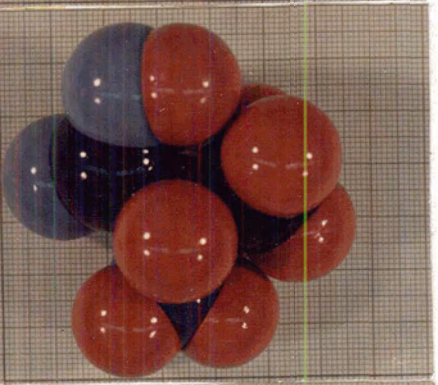
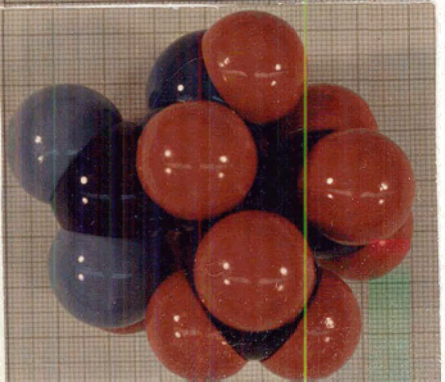
One mechanism involved in the entry of compounds into cells is active transport. Most hypotheses advanced to explain active transport involve binding of the penetrating ion or molecule to some constituent of a membrane which acts as a carrier. Active transport may be treated kinetically using the enzyme analysis of Michaelis and Menton, and the mathematical models of Lineweaver and Burk and of Eadie.

The data for aspartic acid fit the Michaelis-Menten model for the formation of an enzyme substrate complex ie. a carrier. It is concluded that the entry of L-aspartate into Nitella flexilis is an active transport process. L-aspartate-^{14}C penetrated the plasmalemma at a rate ten-fold greater than L-valine, at the same concentration. Comparison of the Fisher-Heischfelder-Taylor models of L-aspartate and L-valine (Fig. 11) indicated that the two molecules are approximately the same size. However, L-aspartate has two carboxyl groups, as a result, in aqueous solution it acquires a single negative charge.

L-aspartate is insoluble in organic compounds. Therefore, it will not dissolve in the lipid component of the plasmalemma. Also, L-aspartate ions will likely be unable to permeate through pores in the membrane owing to electrostatic

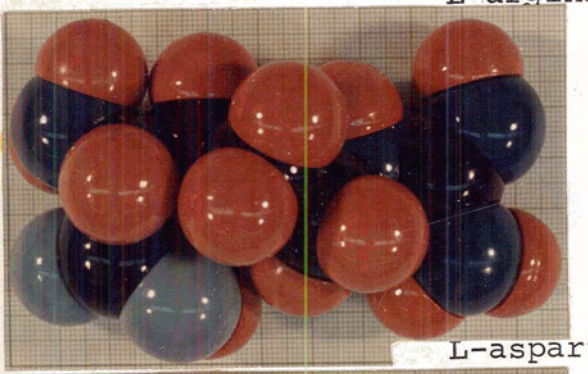
Table 11. Summary of compounds used for permeation investigations of Nitella flexilis.

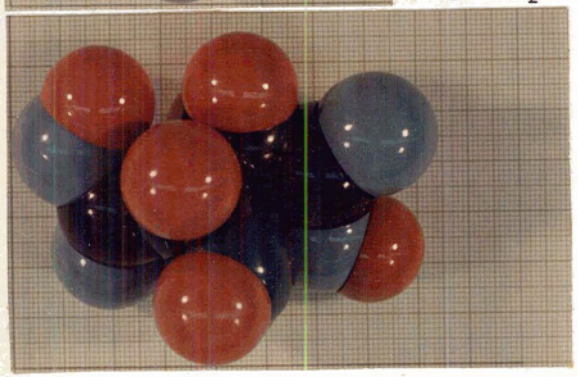
A Neutral Amino Acids

	Compound	Molecular weight	No. of carbon atoms	No. of CH_3 groups	Charge	Rate of Permeation "Cytoplasm" $\mu g\ hr^{-1}$	Rate of Permeation "Vacuole" $\mu g\ hr^{-1}$	Postulated mechanism
	Glycine	75	2	0	±	4.0×10^{-5}	1.4×10^{-5}	molecular sieve
	L-alanine	89	3	1	±	1.4×10^{-5}	0.5×10^{-5}	molecular sieve
	L-valine	117	5	2	±	0.6×10^{-5}	0.3×10^{-5}	molecular sieve

B Basic and Acidic Amino Acids

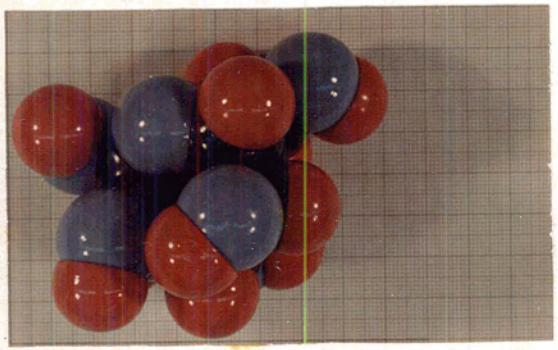
Compound	Molecular weight	No. of carbon atoms	No. of CH groups	Charge	Rate of Permeation "Cytoplasm" μg hr^{-1}	Rate of Permeation "Vacuole" μg hr^{-1}	Postulated mechanism
L-arginine	174	6	0	+	1.1×10^{-4}	0.3×10^{-4}	electro-osmosis
L-aspartate	133	4	0	−	0.9×10^{-4}	0.4×10^{-4}	carrier

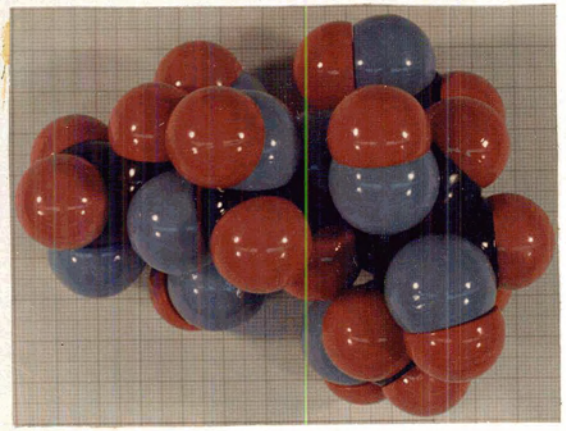




C Sugars

Compound	Molecular weight	No. of carbon atoms	No. of CH_3 groups	Charge	Rate of Permeation "Cytoplasm" $\mu g\ hr^{-1}$	Rate of Permeation "Vacuole" $\mu g\ hr^{-1}$	Postulated mechanism
D-glucose	180	6	0	0	6.6×10^{-4}	3.4×10^{-4}	carrier and molecular seive
Sucrose	342	12	0	0	8.3×10^{-4}	1.7×10^{-4}	hydrolysis to glucose and fructose before permeation





repulsion exercised by the negative charges in the pore walls.

Gayle and his co-workers (3) found that if cells of Staphylococcus aureus are suspended in L-glutamate and glucose, rapid uptake of glutamic acid takes place into the cells. This accumulation is abolished by inhibitors which prevent glucose metabolism. Also, the rate of uptake is independent of external concentration, except for very low values. This, then, indicates that glutamic acid uptake is the result of active transport. L-glutamate and L-aspartate are very similar in structure, both are dicarboxylic amino acids, acquiring a single negative charge in aqueous solution. It is interesting to note that with respect to the dicarboxylic amino acid the algal and bacterial membranes are similar.

The permeation of D-glucose in this investigation fits an active transport mechanism only at very low concentrations of substrate. Ursino (28) suggested that D-glucose permeates into Nitella flexilis through pores in the plasmalemma. It has been shown by Conway and Downey (4) among others that D-glucose enters the yeast cell by an active transport system. Henderson (15) has stated that the uptake of D-glucose into rat muscle cells measured as a function of the extracellular concentration seems to follow Michaelis-Menten kinetics, suggesting that the uptake of D-glucose is associated with some enzyme-carrier mechanism in the plasmalemma.

The kinetic experiments carried out in this investigation indicate that at low concentrations, below 1×10^{-5} M, the entry of D-glucose into Nitella flexilis is by some enzyme-carrier mechanism. The uptake of D-glucose at higher concentrations is not by a carrier mechanism and is discussed later.

Another model, which has been proposed for entry of substances into the cell, is the lipid solubility principle (3). Lipid soluble compounds diffuse across the plasmalemma by dissolving in the lipid component of this membrane.

Amino groups, hydroxyl groups, carboxyl groups and keto groups decrease the lipid solubility and hence, the permeation power. As a result, amino acids and sugars are the most lipid insoluble compounds that occur as natural constituents of cells. The plasmalemma of plant and animal cells has proved to be more or less impermeable to them. This applies even to a relatively small compound such as glycine. What uptake there is, is thought to be due either to diffusion through pores or due to processes of active transport. This fits Danielli's concept (2), that the plasmalemma is not a homogeneous lipoprotein layer but that specialized areas constitute a small fraction of the membrane and play a vital role in some permeability processes.

Christensen (29) in his investigations on amino acid permeation into erythrocytes has demonstrated a dominant effect of the size of the apolar group of an amino acid on the rate of permeation. The longer the apolar chain the greater the rate of entry. He attributes this, in part, to an increase in lipid solubility. A simple increase in lipid solubility with lengthening apolar chain cannot account for the increased rate of entry alone. One cannot visualize the long chain amino acids passing through a lipid barrier phase without some means of masking their hydrophilic groups. These amino acids do not permeate by simple diffusion through pores because the rate of entry increased with molecular size. Kinetic experiments which Christensen performed led to his suggestion that the permeation of these amino acids was due in part to lipid solubility and in part it was due to some non specific enzyme-carrier complex.

The molecular-sieve principle has undergone considerable controversy. Pores, if present in membranes, are considered to be too small to be detected by electron microscopy (23), thus, it has been suggested that the membrane pore is less than 10 Å in diameter. Solomon has suggested a pore diameter in erythrocytes of 8.4 Å. Kavanau (16) has stated that pores are wider dynamic structures which are

capable of changing their structure from an open type configuration to a closed type configuration in which pores are obliterated.

One aim of this investigation was to investigate the possibility of permeation of molecules through pores in the plasmalemma using a series of neutral amino acids of increasing molecular size.

In this investigation with neutral amino acids, the rate of permeation decreases with increasing molecular size and degree of methylation. In this investigation then, the results are opposite to those obtained by Christensen using erythrocytes. The size of the apolar group or an increase in the degree of methylation of the amino acids is not influencing the rate of uptake. Therefore, amino acids do not permeate by dissolving in the lipid component of the plasmalemma and diffusing across to enter the cytoplasm. This is in agreement with Overton's hypothesis.

An examination of the molecular models (Fig. 11) of these compounds reveals their relative size. Molecular size decreases in the order L-valine>L-alanine>glycine. The molecular size does not decrease in a linear manner, L-valine is a branched-chain amino acid, the other two compounds are straight-chain amino acids.

The results of this investigation show that the permeation rate is correlated with molecular size. Therefore, from this data the conclusion is reached that these compounds are permeating through pores in the plasmalemma into the cytoplasm. Permeation is passive, the driving force being due to the chemical potential gradient. This investigation with amino acids supports Solomon's conclusion that the plasmalemma has a pore diameter of 8.4 Å.

L-alanine is one methyl group larger than glycine, L-valine contains two methyl groups. The ratio of permeation, glycine: L-alanine is 2.9, the ratio, L-alanine: L-valine is 2.3. L-valine, in addition to two methyl groups, is considerably larger than L-alanine because it is a branched-chain amino acid, Table 11. One would expect the second ratio to be greater than 2.9, since the size of L-valine relative to L-alanine is greater than the size of L-alanine relative to glycine. L-valine is very slightly soluble in organic solvents. One may speculate that this compound also permeates, to a certain extent, by diffusion through the lipid component of the plasmalemma. If this is so, it is interesting that two different mechanisms are involved in the permeation of a single compound across the plasmalemma.

The erythrocyte cell is an atypical cell, lacking both a nucleus and vacuoles. Therefore, it is not surprising that the permeability characteristics of the plasmalemma of the erythrocytes towards neutral amino acids is not the same as the characteristics of the Nitella flexilis plasmalemma.

Other evidence has been obtained in this investigation to support the molecular sieve principle. One such piece of evidence was derived from permeation experiements using L-arginine-^{14}C.

The permeation rate of L-arginine-^{14}C is greater by a factor of ten, than that of the neutral amino acids. From examination of the molecular model (Fig. 11) it is seen that L-arginine is larger than L-valine. Unlike L-valine it has no methyl groups, but several amino groups. It differs from the neutral amino acids in that in aqueous solution the molecule acquires a positive charge.

The fact that the rate of permeation of L-arginine into Nitella cells was so much faster than the permeation rate of L-valine suggests, at first glance, that this compound did not enter the cell through pores in the plasmalemma. Also, because the compound is insoluble in organic solvents, the number of amino groups and the carboxyl group completely masking any effect of the methylene groups, L-arginine does not dissolve in the lipid component of the plasmalemma.

Analysis of kinetic studies performed on this compound indicated that L-arginine did not enter Nitella by means of an enzyme-carrier mechanism. The fact that L-arginine does not permeate into the cell by an enzyme-carrier mechanism and is very lipophobic leads to some speculation about the mechanism involved. It is probable that L-arginine permeates into the cell through pores in the membrane. However, it does not fit into the permeation pattern of the neutral amino acids, their permeation rate decreasing with increasing molecular size. It may be that the positive charge carried by L-arginine is a critical factor in the permeability of the plasmalemma to this ion.

Briggs, Hope and Robertson (1) have suggested that the pores of Nitella have negatively charged walls. MacRobbie (18) has shown that the electric potential of Nitella translucens is negative with respect to the outer solution. L-arginine ions diffusing passively into the sphere of influence of the negative charged walls of the pores would be attracted by these charges. These cations then move by electrical attraction along an electrochemical gradient into the cell.

One may speculate that the permeation of L-arginine is due to electro-osmosis. The cations move into the cell, through pores with negatively charged walls, in response

to a bioelectrical potential difference between the cell interior and the environment. The permeation characteristics of the neutral amino acids and L-arginine into Nitella cells supports the molecular sieve principle. The electro-osmotic mechanism is a variation of the principle, permeation is through pores, but, the charge carried by the compound influences its permeation characteristics.

The unbalanced charged atom on the arginine ion is located at the end opposite the amino group. Hence, one can speculate that L-arginine would enter the pore and move through it length-wise. The width of L-arginine lies within the limits of the pore size suggested by Solomon (23).

As stated earlier the entry of D-glucose into Nitella flexilis at very low concentrations is by means of some enzyme-carrier mechanism. However, since the uptake of D-glucose appeared to reach a maximum at a very low concentration and since Ursino (28) demonstrated that glucose permeation into Nitella flexilis occurs at much higher concentrations a second kinetic analysis was made covering a wider range of concentrations. The results indicated that a second process is involved at higher concentrations which failed to saturate up to the highest concentration used and hence follows diffusion kinetics.

D-glucose is a hydrophilic non-electrolyte and

is very lipid-insoluble. Hence, it does not dissolve in the lipid component of the plasmalemma. Permeation then, of D-glucose at higher concentration does not fit the lipid solubility theory. One can conclude from this data, that the diffusion of D-glucose into the cell is through pores in the plasmalemma. Since D-glucose is slightly under 10A° one must modify the pore size, as established by Solomon for erythrocytes when dealing with the plasmalemma of Nitella flexilis.

These experiments using radioactive glucose, arginine and neutral amino acids confirm those of Collander (3), who used non-radioactive sugars and other neutral molecules to determine the relative pore size in plant cell membranes. They are interpreted to mean that pores are present in the Nitella cell plasmalemma, and that these membrane pores are sufficiently large to allow the easy passage of glucose into the cell.

The permeation of sucrose into Nitella flexilis leads to considerable speculation. In the initial permeation experiments with D-glucose and sucrose, the rates of uptake of the two sugars were the same. The km values derived from kinetic analysis were similar. It is highly unlikely that sucrose (fig. 11) which is roughly twice the size of glucose would enter the cell at the same rate. It is possible

to postulate a carrier which may attach to the glucose moiety of sucrose and transport it across the membrane at the same rate as D-glucose. However, this does not explain the diffusion of sucrose through pores being as fast as that of D-glucose.

Another similarity is observed when the data obtained by paper chromatography on the distribution of ^{14}C in the cells following immersion in D-glucose-^{14}C or sucrose -^{14}C is examined. The ^{14}C is distributed roughly the same, between glucose and sucrose in the two groups of cells. Because of the very great similarity between the uptake of D-glucose and sucrose in Nitella flexilis, the conclusion is reached that sucrose is hydrolysed at the plasmalemma and then enters the cell as its constituent hexose sugars, D-glucose and D-fructose.

Summary

1. The single large internode cells of Nitella flexilis can be used for studying permeation of neutral, basic and acidic amino acids and sugars.
2. The technique of separating the protoplasmic contents into two compartments bounded by the plasmalemma and tonoplast is not valid. The "cytoplasmic" fraction contains only part of the cytoplasm, probably the gel phase, with its chloroplasts, that is next to the plasmalemma. The "vacuolar" fraction contains the vacuolar sap as well as some cytoplasm, probably the sol phase next to the tonoplast.
3. More than one mechanism is involved in permeation processes. The operating mechanism is determined by the characteristics of the permeating molecules.
4. The permeation of the neutral amino acids fits the molecular-sieve principle. It has been speculated that permeation of L-valine is enhanced by a slight solubility of this compound in the lipid component of the membrane.
5. The permeation of L-arginine also appears to be through pores. However, permeation of L-arginine is not as simple as that of the neutral amino acids. It resembles electro-osmosis, permeation of ions through pores with charged walls.

6. By contrast, kinetic analysis suggests that the uptake of L-aspartate is due to a carrier system.
7. Active transport via a carrier of D-glucose appears to occur at very low concentrations. At higher concentrations, the permeation of D-glucose appears to be by diffusion through pores in the plasmalemma.
8. It is suggested that sucrose does not permeate across the Nitella plasmalemma. Rather, sucrose is hydrolysed and permeates as its constituent hexose sugars.

References

1. Briggs, G. E., A. B. Hope and R. N. Robertson. 1961. Electrolytes and plant cells. Blackwell Scientific Publications, Oxford.

2. Brown, F. and J. F. Danielli. 1964. The cell surface and cell physiology. In: Cytology and Cell Physiology. G. H. Bourne, ed. Academic Press, New York and London, p. 150-182.

3. Collander, R. 1959. Cell membranes. In: Plant Physiology, Vol. II. F. C. Steward, ed. Academic Press, New York and London, p. 3-102.

4. Conway, E. J. and M. Downey. 1950. An outer metabolic region of the yeast cell. J. Biochem. 47: 347-355.

5. Dainty, J., P. C. Croghan and D. S. Fensom. 1963. Electro-osmosis with some applications to plant physiology. Can. J. Botany 41: 953-966.

6. Dainty, J. and B. Z. Ginzburg. 1963. The permeability of the cell membranes of Nitella translucens to urea and the effect of high concentrations of sucrose on this permeability. Biochim. et Biophys. Acta, 79: 112-121.

7. Danielli, J. F. 1952. Surface chemistry and cell membranes. In: The Permeability of Natural Membranes. H. Davson and J. F. Danielli, eds. Cambridge Univ. Press, London and New York.

8. Fensom, D. S. and J. Dainty. 1963. Electro-osmosis in Nitella. Can. J. Botany, 41: 685-690.

9. Fensom, D. S. 1963. The bio-electric potentials of plants and their functional significance. Can. J. Botany, 41: 831-851.

10. Fensom, D. S., D. J. Ursino and C. D. Nelson. 1966. Determinations of relative pore size in living membranes of Nitella using the techniques of electro-osmosis and radioactive tracers.

11. Frey-Wyssling, A. and K. Mühlethaler. 1965. Ultrastructural Plant Cytology. Elsevier Publishing Co. New York.

12. Ghosh, N. R. 1963. Active transport and transport energy. Nature, 200: 175-176.

13. Goldacre, R. J. and I. J. Lorch. 1950. Folding and unfolding of protein molecules in relation to protoplasmic steaming, amoeboid movement and osmotic work. Nature, 166: 479-499.

14. Green, H. and B. D. Davis. 1956. In: Enzymes: units of biological structure and function. O. H. Gaebler, ed. p. 509-521. Academic Press, New York.

15. Henderson, M. J. 1964. The uptake of glucose into cells and the role of insulin in glucose transport. Can. J. Biochem. 42: 933-944.

16. Kavanau, J. L. 1963. Structure and function of biological membranes. Nature, 198: 525-530.

17. Laites, G. 1966. Personal Communication.

18. MacRobbie, A. C. 1962. Ionic relations of Nitella translucens. J. Gen. Physiol, 45: 861-878.

19. Mitchell, P. 1960. Approaches to the analysis of specific membrane transport. In: Biological Structure and Function. Proc. Int. Symp. 1st Stockholm. 581-608.

20. Mitchell, P. 1957. A general theory of membrane transport from studies of bacteria. Nature 180: 134-136.

21. Nelson, C. D. 1965. Membrane transport of electrolytes and sugars in single cells of Nitella. Trans. Royal Soc. Can. 3: 201-212.

22. Sjostrand, F. S. 1960. Morphology of ordered biological structures. Rad. Res. Supp. 2. 349-386.

23. Solomon, A. K. 1960. Pores in the cell membrane. Scientific American, 203: 146-153.

24. Stein, W. D. and J. F. Danielli. 1956. Structure and Function in red cell permeability. Disc. Far. Soc. 21. 238-251.

25. Stern, H. and D. L. Nanney. 1965. The Biology of Cells. J. Wiley & Sons Inc., New York.

26. Thompson, J. F. and F. C. Steward. 1951. Investigations of nitrogen compounds and nitrogen metabolism in plants. Variables in 2-directional paper chromatography of nitrogen compounds: a quantitative procedure. Plant Physiol. 421-440.

27. Thompson, R. G. 1966. Studies on translocations of photosynthetic products in young soybean plants using $^{14}CO_2$ and $^{3}H_2O$. Thesis. Simon Fraser University, Burnaby.

28. Ursino, D. J. 1964. Studies on permeation using electro-osmosis. M.Sc. Thesis, Queen's University, Kingston.

29. Winters, C. G. and H. N. Christensen. 1963. Migration of amino acids across the membrane of the human erythrocyte. J. Biol. Chem. 239(3): 872-878.

30. Wolfe, L. S. 1964. Cell membrane constituents concerned with transport mechanisms. Can. J. Biochem. 42: 971-988.

Curriculum Vitae

Name:	Donald George Wallen
Place & Year of Birth:	Perth Road, Ont., 1933
Education:	University of King's College, 1957-1961
	B.Sc. (Biology) 1961
	Dalhousie University, 1961-1962
	B.Ed. 1962
	Queen's University Summer School, Summers 1963, 1964
	School of Graduate Studies, Simon Fraser University, 1965-1966
Experience:	Fisheries Research Board, St. Andrews, N.B. Summers 1960, 1961
	Secondary School Teacher, Ontario 1962-1965
	Teaching Associate, Department of Biological Sciences, Simon Fraser University, 1965-1966.