CHAPTER 3

The Structure and Possible Function of the Vacuole

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I. Structure and Origin

Vacuoles are an obvious and characteristic feature of plant cells. In the older, pre-electron microscope literature the size of vacuoles, their distribution among various types of cells and various types of plants, the nature of the contents and inclusions and the pH etc. have been described and discussed, although most of the discussion of function has been and still is almost pure speculation. Some further observations have been made in recent years (Voeller, 1964; de Robertis *et al.*, 1965); these have added something, although not a very great deal, to our total knowledge.

All cells contain what might be called a vacuolar system represented by the endoplasmic reticulum, the Golgi complex and the nuclear envelope. In plant cells this vacuolar system has added to it the plant vacuole(s) to which we usually reserve the name vacuole. All these vacuoles, vesicles, cisternae and so on, seem to be essentially "aqueous inclusions" in the cytoplasm bounded by phospholipid membranes. On the whole, as judged by the evidence gained from electron microscope pictures, these phospholipid membranes, and the plasmalemma, are very similar so-called unit membranes. It is true that some of them may appear fractionally wider than others and some may be "rough" and others "smooth". It may be, too, that some are of the Danielli type of bimolecular layer of lipid, while in others the membrane may be made up of subunits, micelles of lipid coated with protein. However, at present, we would say that they are all basically similar phospholipid membranes. The special name of tonoplast is given to the membrane bounding the large central vacuole of mature plant cells.

There has, of course, been some speculation as to the origin of the characteristic vacuoles of plant cells. Some think of them as originating by being

budded off, as vesicles, from the Golgi complex and subsequently growing and coalescing to produce the final large central vacuole (Marinos, 1963). Others think of a similar origin from the endoplasmic reticulum (Buvat, 1963) or that they arise from the synthesis of a highly hydratable macromolecule in the cytoplasm which "draws" water to it leading to the creation of a little aqueous bubble around this macromolecule and this bubble subsequently acquires a phospholipid membrane as a boundary (Guilliermond, 1941).

These speculations have not had much physico-chemical basis which is not surprising for there is little physico-chemical understanding of the nature of cytoplasm. However, one or two things can be said with respect to this system in which, apparently, a homogeneous aqueous phase or phases can separate out and/or coexist with a complex colloidal phase. If the phases are in thermodynamic equilibrium with respect to water, then the chemical potential of water must be the same in the vacuoles as in the cytoplasm. The chemical potential of water can be written as the sum of three components (plus the standard chemical potential of pure water at atmospheric pressure):

$$\mu_w = \mu_w^0 + RT\ln a_w + P\bar{V}_w + \Gamma$$

In this expression $RT\ln a_w$ (a_w is the activity of the water) expresses the contribution due to dissolved solutes; $P\bar{V}_w$ ($\bar{V}_w$ is the partial molar volume of water) is the effect of hydrostatic pressure (P) and Γ is the so-called matric potential which depends on the interaction of the water with macromolecules and colloidal constituents. The $RT\ln a_w$ and Γ terms are negative. This separation of μ_w into components is probably not thermodynamically justifiable but it forms a useful basis for discussion.

If we assume that the $P\bar{V}_w$ terms are the same in a vacuole and in the cytoplasm (although this may not be true), then only the two negative terms need to be considered. In a large vacuole it seems certain that Γ is negligible compared to its value in the cytoplasm. Thus equality of μ_w can only be achieved by having a higher solute concentration in the vacuole than in the cytoplasm. (If the vacuole is to grow then the vacuolar solute concentration must be higher still to make μ_w (vacuole) less than μ_w (cytoplasm).) Since the cytoplasmic colloids probably carry a net negative charge, the Donnan effect operating on ionized solutes tends to make the solute concentration in the cytoplasm *higher* than in the vacuole; this, of course, only makes it still more difficult to get a higher solute concentration in the vacuole. Thus it is tentatively concluded that we must expect, at the vacuolar surfaces, inwardly directed solute "pumps" the function of which is to produce and maintain the necessary higher vacuolar solute concentration. There is not much experimental evidence containing the relative solute concentrations in the vacuole and the cytoplasm. What little there is comes from ion analyses on

large coenocytic algae; these are quoted in Table I and it can be seen that the total ionic concentration in the vacuole is, in general, greater than that in the cytoplasm. It could still be that there are other solutes in the cytoplasm which would invalidate this evidence.

From the above thermodynamic considerations about μ_w, the chemical potential of water, a vacuole might start in a small region centred on a particularly hydrophilic (and water structure-ordering) macromolecule because here there could be a large, negative contribution to μ_w from Γ. But such a vacuole could not develop without a fairly rapid increase in the local solute concentration for the Γ contribution would rapidly decrease as the

TABLE I

Ionic concentrations (mM) in "flowing" cytoplasm (i.e. not including the stationary chloroplast layer) and the vacuole of certain giant algal cells.

	Nitella flexilis (Kishimoto and Tazawa, 1965)		*Nitella translucens* (MacRobbie, 1962)		*Nitella translucens* (Spanswick and Williams, 1964; Spanswick *et al.*, 1967)	
	cytoplasm	vacuole	cytoplasm	vacuole	cytoplasm	vacuole
Potassium	125	80	120	79	93	67
Sodium	5	27	54	37	37	73
Chloride	36	135	?	150	65	160

vacuole enlarged. However, there would appear to be nothing against such an origin on thermodynamic grounds. One of the big problems in connection with such an origin is the appearance, presumably at an early stage because of the apparent early necessity for solute pumping systems, of a phospholipid membrane around the incipient vacuole. This implies the synthesis of phospholipid in the cytoplasm and its "adsorption" as a bimolecular layer, or its equivalent, at the surface of the developing vacuole. We seem to know little about the mechanism of such an adsorption of phospholipid. It may be a "passive" process, involving a decrease in free energy, but it seems more likely that metabolic energy is involved in ordering the phospholipid molecules and then subsequently maintaining the structure of the membrane.

Most people are probably more attracted to the idea that vacuoles do not arise *de novo* but are products of a pre-existing vacuolar system, whether Golgi, endoplasmic reticulum or more non-specific vacuoles and vesicles. Again much phospholipid synthesis is needed and the phospholipids have to be "slotted-in" to the pre-existing vacuolar membranes. There are also the same physico-chemical problems of the expansion of vacuoles which requires a lower μ_w in the vacuoles than in the cytoplasm. In addition, a new problem

arises, that of the mechanism of "budding-off" from the pre-existing vacuolar system. This implies, I think, some heterogeneity in the membranes, perhaps of charge, which can allow approach, fusion and separation of the phospholipid systems. Local differences in charge, perhaps due to calcium, and local differences in ion pumping activities may be vital here.

II. Ion Transport

I have so far raised some questions and unsatisfactorily discussed some of the physico-chemical problems associated with the origin and development of vacuoles. It seems that there is little information as yet to resolve the problems involved. A little is known, however, about the permeability properties of the tonoplast, the phospholipid membrane surrounding the large central vacuole of mature plant cells. This information is largely gained from studies on giant coenocytic algal cells and it is possible that it is not completely relevant to the situation in higher plant cells.

The large internodal cells of various members of the Characeae have been most frequently studied and fairly detailed pictures are beginning to emerge, particularly for two species, *Nitella translucens* and *Chara australis*. A kinetic analysis of the exchange of the ions sodium, potassium and chloride between the bathing medium and the cells of *N. translucens*, together with a knowledge of the concentration of these ions in the vacuole and cytoplasm of these cells, shows that the ion fluxes across the tonoplast are up to a hundred times greater than the fluxes across the plasmalemma (MacRobbie, 1962, 1964, 1966). It therefore appears that the tonoplast in these cells is much more permeable to ions than is the plasmalemma. Certain other evidence supports this conclusion. By inserting suitable microelectrodes, the electrical resistance of the plasmalemma and tonoplast can be separately measured; it turns out that the resistance of the tonoplast is at least ten times smaller than that of the plasmalemma in *C. australis* (Hope and Walker, 1961). Also most of the electrical potential difference between the vacuole and the external medium is across the plasmalemma (about 140 mV, cytoplasm negative, across the plasmalemma and not more than 20 mV, cytoplasm also negative, across the tonoplast) (Spanswick and Williams, 1964; Hope and Walker, 1961). The tonoplast does not seem so selective, with respect to cation permeability at least, as the plasmalemma. Thus, in the two most thoroughly investigated species, the tonoplast seems to be a fairly permeable, non-selective, membrane as compared with the plasmalemma. (Although the tonoplast of *N. translucens* and *C. australis* is one or two orders more permeable to ions than the plasmalemma, it is still only of the same order of permeability as a typical animal cell plasma membrane.)

The information obtained with other plant cells is quite dubious. Four

marine or brackish water algae have been examined and no clear idea can be obtained from the results as to whether the tonoplast is more or less permeable to sodium, potassium and chloride ions than the plasmalemma (Gutknecht and Dainty, 1968). This is clearly an area where intensive work is needed.

One curious feature of the tonoplast fluxes in *N. translucens* has been observed by MacRobbie (1966). She finds a correlation between the influxes of potassium and chloride across the tonoplast and the influx of chloride across the plasmalemma. The correlation is such that, for every chloride ion crossing the plasmalemma, about 60 molecules of potassium (plus sodium ?) chloride cross the tonoplast; sometimes it is a small integral number times 60 molecules.

I have previously argued that the origin, growth and existence of vacuoles implies a solute pump, located at the tonoplast, and directed towards the vacuole. The most reliable evidence relevant to this comes from the work on *N. translucens*. The ionic concentrations in the "flowing cytoplasm" and in the vacuole are given in Table I. The electrical potential difference across the tonoplast is about 20 mV, with the cytoplasm negative. From these figures the electrochemical potential differences for sodium, potassium and chloride ions between the vacuole and the cytoplasm can be calculated. They show that both potassium and, particularly, sodium are at a higher electrochemical potential in the vacuole, whereas chloride is approximately in electrochemical equilibrium (Spanswick and Williams, 1964; Spanswick *et al.*, 1967). This result means, fairly unequivocally, that both potassium and, particularly, sodium are "pumped" against the electrochemical potential gradient from the cytoplasm to the vacuole. Similar measurements have been made on the marine alga *Valonia ventricosa* by Gutknecht (1966); here it is even more clear that sodium and potassium are pumped from the cytoplasm into the vacuole. Thus, from what little is known, there seem to be inwardly directed cation pumps at the tonoplast of giant algal cells.

Much less is known about the tonoplast in higher plant cells for the experimental difficulties are very much greater. Individual cells are quite small and the cytoplasm in mature cells is often less than one micron thick, hence it has so far proved practically impossible to measure directly ionic concentrations in the cytoplasm and almost impossible to measure the electrical potential difference between cytoplasm and vacuole. Additionally, flux measurements have to be made on tissues with the accompanying complications of extracellular space and heterogeneous populations of cells. Nevertheless, a few hints have been obtained of the possible situation at the tonoplast. The electrical potential difference between the cytoplasm and vacuole has been measured in root hair cells (Etherton and Higinbotham, 1960); it was found to be small, similar to the situation in the giant algal cells. Some ion flux

measurements have been made on carrot root storage tissue and on excised barley roots (Pitman and Saddler, 1967) which indicate that the tonoplast permeability is fairly high, although the ratio of tonoplast permeability to plasmalemma permeability does not seem so high as in the giant algal cells which have been studied. However, because of the difficulties of a kinetic analysis of flux data from plant tissue, I do not feel that too much reliance can be placed on these permeability statements yet. Thus there is a suggestion, but only this so far, that higher plant cells are not too different from algal cells from the ionic relations point of view. One thing which is clearly different is that the major anion in the vacuole of higher plant cells is an organic acid anion, most commonly malate. Whether this has to be pumped across the tonoplast into the vacuole, as I suspect, or whether it moves passively in response to an electrical potential difference generated by cation pumps are questions which as yet cannot be resolved.

III. Function

One can at present only guess the function of vacuoles. It is clearly recognized that membranes and membrane-bounded organelles are a very important feature of all cells. It is also fairly certain that many of the chemical reactions of the cell takes place on or are closely associated with membranes; it is only necessary to think of photosynthesis, the Krebs cycle and oxidative phosphorylation, protein synthesis and active transport to see that this is so. Thus, phospholipid membrane-bounded compartments must have originated at a very early stage of evolution and have subsequently evolved to have various functions. One can speculate that a very early function of a phospholipid membrane, perhaps the external plasma membrane, was "secretory" or "excretory", being concerned with developing an ion-pumping system to maintain the integrity of primitive protoplasm, i.e. to stop it from being diluted out of existence by the inflow of water. It would appear from the earlier discussion that it is probably necessary for these phospholipo-protein membranes to retain their pumping properties if vacuoles are to form, grow and maintain their identity. Membranes are active transport sites for many solutes other than ions and I therefore envisage the primary function of vacuoles as secretory. It is well known that small vacuoles or vesicles (of, for example, the Golgi apparatus) perform this function with respect to processes such as the transport of cell wall material to and through the plasmalemma or to the site of origin of a new cell wall in a dividing plant cell. Such phospholipid-bounded vesicles would seem well adapted to fusion and subsequent emptying with the plasmalemma; this is a type of inverse pinocytosis. Small vacuoles certainly provide this kind of function in animal cells.

In plant cells, besides the small vacuoles or vesicles in the cytoplasm, the characteristic feature is the large central vacuole. Certainly one can envisage that its major function, or rather the major function of the tonoplast, is secretory, i.e. putting things, usually at a different concentration, into the vacuole. Thus, as is amply documented, the vacuole can act as a subcellular storage organ of useful metabolites or of toxic excretory products. The huge development of the vacuole may be partly associated with the lack of development of an efficient circulatory system in plants and of a kidney or other excretory system. However, it seems plausible to me that the development of the large central plant vacuole is associated with the development of cell walls and the need for turgid cells for skeletal purposes. It is true that the presence of a large vacuole is not essential to produce the high hydrostatic pressure inside plant cells. However, the presence of the vacuole ensures that the hydrostatic pressure is produced with great economy in the production of protoplasm which is now confined to a relatively thin film around the periphery of the cell. Given the primitive highly vacuolated plant cell, the vacuole can be used as a storage and dumping organ and to other advantages as well. The transport from cell to cell via the plasmadesmata becomes more rapid because of the cyclosis of the thin film of protoplasm. The development of special transporting cells (the phloem vessels, the tracheids and xylem vessels) follows fairly naturally from highly vacuolated standard plant cells. Indeed, together with the cell wall and the chloroplast, the large central vacuole is an *essential* feature of plants above the level of microscopic algae.

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