Mechanism of Salt Absorption by Plant Cells

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FEW problems in plant physiology have more general implications than that of the mechanism of salt absorption. The outstanding feature which requires explanation is the means whereby certain salts, which occur in extreme dilution in the medium bathing the cells, attain considerable concentrations in the vacuole, where they are maintained, apparently, in true solution. The obvious analogy between this process and the mechanism of secretion in the animal body is alone adequate justification for again directing attention to this question.

In recent years there have been many physico-chemical speculations which suggest devices whereby living plant cells might evade those simple equilibrium conditions which they rapidly approach after death or injury. The tendency has been to stress mainly the properties (real or hypothetical) of the functional membranes, and to pay but scant attention to the metabolic activities of the living system. One of the most prominent of such theories is still that of Osterhout[1,2]. The principal features of this view and those relevant to this discussion are as follows.

(1) The functional protoplasmic membranes are fluid, lipoidal and non-dissociating.

(2) Salts enter principally, if not exclusively, as undissociated molecules. Cations penetrate only in association with hydroxyl in the form of undissociated free base.

(3) The gradient of 'thermodynamic potential' of free base ([K] × [OH] ; [Na] × [OH]) determines the rate and direction of movement of K and Na respectively.

The theory is largely based upon experiments with the marine cœnocytic green alga, *Valonia macrophysa*, which, like the closely related form *Valonia ventricosa*, occurs in warm seas under highly specialised conditions of light, temperature and aeration.

V. macrophysa occurs at Bermuda and has been investigated mainly in winter, whereas both species are found at Tortugas, either in the specialised environment provided by the moat of Fort Jefferson (mainly *V. macrophysa*) or on the open reef (*V. ventricosa*). The Tortugas material has been examined in the summer months and the criticism has been raised by the workers at Bermuda that it is not 'healthy' at this season. This is not borne out by my own experience, especially in the case of *V. ventricosa*, which is on morphological grounds the most useful species.

In recent years the theoretical views have also been developed by experiments on 'models'[3,4] purporting to represent the living cell, on the implicit assumption that the fundamental postulates are correct. In the light of recent work, one may query this from two points of view, namely :

(1) That the theory is not an adequate explanation even for the special case of *Valonia*.

(2) That it exploits unduly the peculiar features of *Valonia* and its environment in a manner which could not possibly apply to plant cells in general.

Until recently[5], the direct evidence for the theory has rested largely upon experiments in which the *internal* reaction of *Valonia* was modified by the penetration of ammonium hydroxide from ammonium chloride - sea-water[6]. Granted that the theory accounts qualitatively for the *direction* of the subsequent movement of potassium and sodium ions (K out, Na in) it is evident that it cannot account for rates. Contrary to expectations, sodium enters in presence of ammonium chloride much faster than in normal sea-water. Furthermore, in this experiment, one of the most significant of those which test the theory on *living cells*, rather than models, the observed change in sap composition is in the direction of attainment of equality of concentration between sap and external solution. Consequently, potassium and sodium ions each moved *with*, rather than against, the prevalent gradients, not only of potassium hydroxide and sodium hydroxide but also of the respective ions. The crucial test of the theory is its ability to explain and foretell movements in the *reverse* direction. Despite attempts to evade this objection (see ref. 6, p. 310), it seems that the more probable explanation is that the ammonium hydroxide produced changes for which injury is the only, but unsatisfactory, designation. That ammonium chloride in the concentrations employed has such irreversible effects upon *both* species of *Valonia* (*V. macrophysa* and *ventricosa*) as they occur at Tortugas is now quite evident[8, 9]. Unless differences between the Tortugas and Bermuda types of *V. macrophysa* without morphological or taxonomic basis are postulated, it is difficult to believe that such did not apply in the earlier experiments of Osterhout[6]. This is in fact indicated, since some cells were actually so far from normal that they died, and considerable corrections were necessary in the volume data of the ammonium chloride series (see ref. 6, pp. 308, 309). In the light of this the evidence that the remainder were completely uninjured is not convincing.

Recent experiments[10] with potato tissue which imitated the essential features of the above *Valonia* experiments have shown that the effect of ammonium penetration from ammonium salts is to reduce the capacity for the accumulation of *all ions*, namely (K, Na, Cl, Br, phosphate), and this is reflected in decreased water absorption. This occurred although considerable internal concentrations of ammonium produced only a small change of internal pH in the more strongly buffered cell sap of potato. The penetration of ammonium seems to depress the accumulation mechanism. Any other superimposed effects were readily interpreted as simple exchanges involving cations.

Experiments involving ammonium chloride solutions are therefore but a dubious proof of the theory of Osterhout, although from the prominence given to them[1,2] they are apparently regarded as its best confirmation.

The theory in question also demands that changes in external reaction, as well as internal, should affect the distribution of potassium and sodium ions between sap and sea-water. Considerable prominence has been given to the fact that an external pH of 5·5, which causes irreversible injury and death, also induces loss of potassium ions. For reasons already stated, this has little or no value as a confirmation of the mechanism of *accumulation*. Jacques and Osterhout[5] also claim that a recent examination of the effects of external reaction substantiates the theory. It is again apparent that very small concentration changes have been transformed by using volume data to what appear to be considerable differences of *total amount*. In other words, there is no evidence that the internal *concentration* of potassium ions is a function of external *p*H, but rather to the contrary. If the external *p*H really does determine the growth of the cells and hence the total salt content, this is not necessarily a proof of the suggested mechanism of entry and accumulation of potassium ions, but merely another indication of the connexion between the processes of growth and metabolism and that of salt absorption. Unfortunately, the most significant data refer to experiments in the light, where there is considerable uncertainty as to the exact *p*H which prevailed and the nature and extent of its drift[5]. The apparently large magnitude of the suggested difference in absorption of potassium ions (see ref. 5, Fig. 1), which is attributed to the small difference in *p*H between two cultures (one of *p*H 8·8 and the other which "started at 8·2 and rose somewhat but apparently not as high as the other"), strongly suggests to me that some other variable and not the concentration of hydroxyl ions was really the determining factor in this case. This is the more probable when one recalls that, though this organism in its normal habitat may withstand considerable fluctuations (diurnal and otherwise) of certain factors (oxygen concentration, external *p*H, etc.), yet in its growth and distribution it is clearly limited by subtle differences not easy to specify completely. This has been impressed upon me by observations in the moat of Fort Jefferson at Tortugas. Attempts to demonstrate the effect of external *p*H upon the absorption of potassium ions by *Valonia macrophysa* in the dark were inconclusive. One of the reasons suggested may be incorrect since, contrary to the statement that it lacks carbohydrate, *Valonia* may be abundantly supplied with small starch grains[11].

Fortunately, quite apart from its ability to grow conspicuously, *Valonia ventricosa* (not available at Bermuda) will absorb potassium from sea-water enriched with potassium chloride either in the dark or the light. External *p*H's ranging from 9·0 to 5·5 may be produced and maintained by control of the carbon dioxide tension. Under these conditions, it is now apparent[8,12] that an approximately threefold increase of external concentration of potassium ions can produce a definitely significant gain by the cells both of concentration and total amount, and this is but slightly affected by an external *p*H range from 9·0 to a reaction only slightly more alkaline than 5·5. Moreover, a slight but significant maximum occurred not at *p*H 9·0 (as the theory referred to would demand) but at approximately *p*H 7·0. The theory of 'thermodynamic potentials of free base' demands that the tendency for potassium entry, measured by the value of the product [K] × [OH] for external surroundings should be determined equally by the concentration of the potassium and hydroxyl ions. It appears, therefore, that entry of potassium is more closely related to the potassium ion concentration than the hypothetical [KOH]. Similarly, when sea-water is enriched with sodium chloride, some gain of potassium may be observed, which also suggests a closer relation between potassium absorption and [Cl] than [OH], since the [KOH] remained undisturbed[12].

It is submitted that these facts are in accord with other views which show that the rôle of *p*H is not the dominant one which the theory suggests. Hoagland[13], using both *Nitella* and the roots of barley, has shown repeatedly that they can accumulate both ions (for example, potassium and bromide) from solutions more acid than the vacuole. Similarly, I have stressed that the existing *p*H gradients could not explain the behaviour of storage tissues, which accumulate salts from solutions almost identical in reaction with their cell sap. Such facts controvert the theory of Osterhout. In view of its dubious utility in the interpretation of the special case of *Valonia* and the abundant evidence to the contrary, especially where more active systems are concerned, there seems to be little justification for continued emphasis upon the idea that cations (particularly potassium and sodium) penetrate cells in general only in the form of undissociated free base.

The experiments at Tortugas, already referred to, suggest that the obscurity which surrounds the manner of growth of *Valonia* and the extremely low level of its metabolic activity more than outweigh its apparent morphological advantages. That the latter are more apparent than real is suggested by even a casual experience with these organisms. It is an interesting anomaly, both morphologically and physiologically, rather than a typical case from which generalisations may be drawn safely. To my mind, *Valonia* finds its parallel with cells like red blood cells or those storage tissues which have permanently ceased active growth and synthesis, rather than the more vigorously metabolising plant cells like those of rapidly growing roots or certain storage tissues[7] which display greater capacity for salt absorption. In the latter cases, it is still difficult to avoid the view that cells do osmotic work in the simultaneous absorption of both anions and cations by virtue of their metabolism and capacity to grow. Nor does the rôle of growth merely involve cellulose synthesis and wall extension, but rather is the ability for salt accumulation inherently a property of cells still capable of constructive protein metabolism and a turnover of carbohydrate far in excess of that involved in mere cellulose synthesis. Thin discs of potato tissue in dilute aerated solutions of potassium salts may metabolise carbohydrate equivalent to 4·27 kgm. cal. per 45 gm. fresh weight in three days. This is equivalent approximately to one sixth of their original total heat value, and most of this decrease in total energy may be due to heat production. Whether a fraction not yet accounted for is more specifically associated with accumulation of salts is a problem at present under investigation.

Whatever the rôle of carbohydrate metabolism may prove to be, it is difficult to follow Osterhout so far as to believe that, if the necessary metabolic activity were available, the still extensible walls of *Valonia* could not be distended without cellulose synthesis, so that further increment of salt *concentration* (without corresponding water absorption) could be accommodated by an increase of wall pressure. Despite even the evidence of slow cell extension[5] in the recent Bermuda experiments, it seems that the large cells of *Valonia* as commonly used retain the essential properties only in relatively slight degree. Consequently, the rôle of respiration and metabolism as a potential source of energy has been minimised and the chief stress laid upon its possible effect as a determinant of internal reactions. The theoretical manipulations of ionic products (for example, [K] × [OH] ; [Na] × [OH]) are based essentially on equilibrium criteria and create the illusion that rapid metabolism and energy exchanges are unnecessary for salt accumulation *per se*. It is in these operations that undue weight seems to have been given to certain special features, not applicable to cells in general, of what is after all an obscure organism.

Lastly, there seems grave danger that the admittedly ingenious and extensive work devoted to models[3] may divert attention from the fundamental facts that, not only are they far removed from physiological reality, but also that the very principles upon which they are based are such that judgment must be reserved concerning their applicability to the general problem of salt absorption *in vivo*.

[1] Osterhout, W. J. V., *Biol. Rev.*, **6**, 369; 1931.
[2] Osterhout, W. J. V., *Ergeb. Phys. und exp. Pharm.*, **35**, 967; 1933.
[3] Osterhout *et al.*, numerous papers in *J. Gen. Physiol.*, **16** and **17**.
[4] Osterhout, W. J. V., and Stanley, W. M., *J. Gen. Physiol.*, **15**, 667; 1931–32.
[5] Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, **17**, 727; 1934.
[6] Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, **14**, 301; 1930.
[7] Berry, W. E., and Steward, F. C., *Ann. Bot.*, **48**, 395; 1933.
[8] Steward, F. C., and Martin, J. C., Carn. Inst. Wash. Yearbook, 33; 1934.
[9] Blinks (private communication from W. J. V. Osterhout) reports that ammonium in low concentration causes the formation of spores (the so-called aplano spores) by aggregation of the protoplasm. As far as the parent cell is concerned, this represents an irreversible change which is frequently caused by injury—mechanical and otherwise—as emphasised by recent experiments of Kopac at Tortugas (see Carnegie Yearbook, 1933).
[10] Steward, F.C., unpublished data.
[11] Doyle, Wm. L., Contrib. from Tortugas Lab. (In press.)
[12] Steward, F. C., Carn. Inst. Wash. Yearbook, 32, 281; 1933.
[13] Hoagland, D. R., Symposium on Salt Absorption, A.A.A.S. Berkeley, Cal.; 1934.
[14] Steward, F. C., Wright, R., and Berry, W. E., *Protoplasma*, **16**, 576; 1932.
[15] Steward, F. C., *Protoplasma*, **17**, 436; 1932. **18**, 208; 1933.

Conference on Industrial Physics

THE first Conference on Industrial Physics to be held in Great Britain took place in Manchester under the auspices of the Institute of Physics on March 28–30. The subject chosen for the Conference was "Vacuum Devices in Research and Industry", and its chief object was to direct attention to the important part which physics and physicists can and do play in modern industrial life. Nearly six hundred people registered as members of the Conference, of which about a hundred were members of the Institute. The majority of the others were engaged in Government and industrial research laboratories and works, some coming from a considerable distance to attend the meetings. The outstanding success of this new venture of the Institute of Physics has demonstrated beyond all possible doubt that there exists a very large number of men who are engaged in applying physics to the solution of industrial problems, in addition to many who are employing its methods for industrial work of a more routine character. One of the objects of the Institute is to provide facilities for the interchange of ideas among those engaged on industrial physics problems by means of meetings and special journals. Many propositions towards the achievement of these objects will doubtless come to earlier fruition as a direct result of this Conference.

The sessions were held in the University of Manchester. The Conference was formally opened by Sir William Clare Lees and the Vice-Chancellor, and was presided over by Prof. W. L. Bragg. The lectures were all informal in character, and consequently they are not being published; each lecture was followed by a useful discussion. The titles and lecturers were as follows: "Modern Electrical Illuminating Devices" by Mr. J. W. Ryde, of the General Electric Co. Ltd.; "Applications of Photocells" by Mr. T. M. C. Lance, of Messrs. Baird Television Ltd., and Mr. R. C. Walker, of the General Electric Co. Ltd.; "The Cathode Ray Oscillograph in Research and Industry" by Mr. L. H. Bedford, of Messrs. A. C. Cossor Ltd.; "Recent Applications of Mercury Vapour Rectifiers and Thyratrons" by Mr. L. J. Davies and Mr. A. L. Whiteley, of the British Thomson-Houston Co. Ltd.; "High Tension Vacuum Tube Devices in Research and Industry" by Dr. J. D. Cockcroft, of the Cavendish Laboratory, Cambridge; "X-rays in Industry" by Dr. G. Shearer, of the National Physical Laboratory. It was interesting to record that, almost without exception, those who took a leading part in the various activities of the Conference, including the lecturers, were quite young men. The Organising Committee also received considerable commendation for its care in the selection of the lecturers, who were in each instance associated with industrial practice; the Committee preferred to extend the invitations to lecture to such men rather than to distinguished pioneers of the devices who, it was felt, would be more familiar with them in the laboratory stage, as distinct from their adaptation for industrial purposes.

On March 29 the members of the Conference spent the afternoon at the research laboratories and works of Messrs. Metropolitan-Vickers Electrical Co. Ltd., and the next day visits were paid to the Shirley Institute of the British Cotton Industry Research Association, and to the Post Office Telephones.

An exhibition of apparatus, instruments and books cognate to the subject of the Conference was held in the laboratories of the University, in which twenty firms and research organisations took part, and in addition some of the important work which is being carried on in the physical laboratories of the University was demonstrated. A special section intended to exemplify the multifarious uses and utilitarian value of vacuum devices was a feature of the exhibition, and aroused great interest. The specific function of the whole exhibition was to demonstrate that apparatus such as the cathode ray tube and the photocell, for example, are now utilised in devices which are practically fool-proof and can be used as tools in all manner of manufacturing processes. Unlike other exhibitions, it was not so much concerned with demonstrating the underlying physical principles of instruments or details of their mechanism, but rather with the fact that the devices shown were not mere scientific toys that had been brought from the laboratory and disguised, but that these devices were real and necessary industrial tools, which the more enterprising manufacturers are already employing. A limited number of copies of the catalogue of the exhibition is still available from the Institute of Physics, London, S.W.7 (1*s*. 3*d*. post free).

On Saturday morning some 65 parties from local schools visited the exhibition, and in the afternoon and evening it was thrown open to the public. It is estimated that 3,500 people visited the exhibition during the three days.

Prof. W. L. Bragg broadcast a talk about the