with an error of 5 per cent for the larger quantities, the error increasing to 10 per cent for the smallest quantity of 1 mg. The presence of bile salts could not be demonstrated by this method in normal blood, nor in blood drawn from patients without jaundice or liver disease.

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The Behavior of Electrolytes in Valonia.

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The accumulation[1] of strong electrolytes in living cells presents a problem of peculiar interest. An example of this is afforded by *Valonia macrophysa*. The large multinuclear cell of this marine alga consists of a very thin protoplasmic membrane, inside of which is a large central vacuole filled with sap, and outside of which lies a cell wall imbibed with sea water. It is a very striking fact that the sap inside of this very thin film of protoplasm has a concentration of K about forty times as great as that in the sea water which bathes the outside of the film.[2]

This can be explained if we assume that the protoplasm is impermeable[3] (or nearly so) to K^+ and OH^- but that KOH enters in the form of undissociated molecules. This does not require the assumption that molecules of KOH exist as such in the sea water. We assume rather that K^+ and OH^-, striking the surface of the protoplasm, which is presumably non-aqueous, associate to form KOH which then passes through the non-aqueous layer (for the sake of simplicity we will assume for the moment that the entire protoplasm consists of a single non-aqueous layer[4]) and dissociates in the sap inside. Equilibrium will be reached when as many molecules of KOH enter the external surface in unit time as enter the internal surface.

Since the pH value of the sap is about 5.8, while that of the sea water is about 8.2, it is evident that the concentration of OH^- is about 250 times as great in the sea water as in the sap, and that, in consequence, there must be at equilibrium a much higher concentration of K^+ in the sap than in the sea water, to ensure that as many molecules of KOH enter the internal protoplasmic surface, as enter

the external surface. We should therefore expect KOH to enter until the concentration of K^+ in the sap becomes great enough to bring this about; we should then find a much higher concentration of K in the sap than in the sea water, and the accumulation would be explained.[5]

The cell continually produces CO_2, and probably other acids, and if this takes place rapidly as KOH penetrates, the pH of the sap will not rise. We might in that case expect to find $KHCO_3$ and K_2CO_3 accumulating in the sap. Confining the discussion for simplicity to $KHCO_3$, the following suggestion may be made: There may be an exchange of the HCO^-_3 produced inside with Cl^- coming in from the sea water, passing through the protoplasm as ions or as undissociated molecules (CO_2, H_2CO_3, HCl). The distribution of Cl^- might be regulated by the Donnan principle. If K^+ and Na^+ can enter as undissociated molecules which dissociate inside, they are probably to be regarded as diffusible ions in the Donnan sense (since the Donnan principle is not concerned with the manner in which they pass through the membrane, but only with the final result). Even so, if they penetrate very slowly they may be regarded as approximately indiffusible. Since there would appear to be an excess of $K^+ + Na^+ + Ca^{++}$ inside over $K^+ + Na^+ + Mg^{++}$ [6] $+ Ca^{++}$ outside, a constraint may be set up across the protoplasm which will cause an accumulation inside of such anions as can enter more rapidly. If we assume that this applies to Cl^- we should expect to find a higher concentration of Cl^- inside than outside and this is actually the case.[7] It is evident that when Cl^- enters, HCO^-_3 must go out in order to preserve the electrical neutrality of the sap.

It is therefore evident that we can picture a mechanism which will account for the penetration of KOH and its accumulation in the sap as KCl. It is possible that KCl[8] penetrates as such, but this would produce no accumulation, since the penetration would cease as soon as the concentration became equal on the two sides. It would seem that the passage of KCl through the protoplasm is very slow, else it would diffuse out more than it appears to do.

It is evident that the production of CO_2 and the accumulation of KCl will raise the osmotic pressure of the sap and cause the cell to grow by absorbing water.[9] Both growth and accumulation of KCl will then depend on the production of CO_2.[10] The concentration of KCl might be expected to remain approximately the same, as long as the cell is growing (since growth and accumulation are both due to CO_2) and this is actually found to be the case.

We should therefore expect that anything which increases the

production of CO_2 will increase growth and accumulation, *e. g.*, temperature, light, etc. This might explain the results of Hoagland and his associates[11] who find that light increases the rate of accumulation in *Nitella*. It may be added that this is in harmony with the fact that the most rapidly growing cells are those which produce the most CO_2.

If the cell is much less permeable to undissociated molecules of NaOH than to those of KOH, it is evident that as long as it is growing KCl will accumulate inside rather than NaCl, so that the concentration of NaCl in the sap may be much less than that of KCl (even though the reverse is the case in sea water) and much less inside than outside. This is actually found to be the case in *Valonia macrophysa*.[12] In other organisms where a higher proportion of Na is found we must suppose that the protoplasm is more permeable to it.

If NaCl[8] penetrates as undissociated molecules the concentration inside and outside will tend to become equal, but this penetration may be too slow to make any noticeable change as long as the cell is growing. When growth stops, the penetration of NaCl will tend to equalize its inside and outside concentration more rapidly, and the penetration of NaOH will tend to make Na accumulate inside if the pH value is lower than that of the sea water, but both of these processes may be too slow to be noticeable.

This brief outline is intended only to suggest certain possibilities which are being tested in a variety of ways and which will be discussed in later reports. It may be added that they are in harmony with the results of an extensive series of experiments on *Nitella* and *Valonia*.

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[1] The term accumulation is used to denote a greater concentration inside the cell than outside.

[2] The molecular composition of the sap expressed as per cent of the halide in the sap is Cl+Br 100, K 86.24, Na 15.08, Ca 0.288; that of the sea water expressed as per cent of the halide in sea water is Cl+Br 100, K^{-1} 2.15, Na 85.87, Ca 2.05, Mg 9.74, SO_4 6.26. The sap contains about 1.4 parts per thousand organic matter and the halide content is about 0.6 M as compared with 0.58 M in the sea water.

[3] It is not necessary to assume absolute impermeability but only slow penetration. We know, however, that certain ions, such as Mg^{++} and SO_4^{-}, never penetrate into sap from sea water under normal conditions. A considerable variety of evidence indicates that in general undissociated molecules penetrate much more rapidly than ions.

[4] We assume for the sake of simplicity that the protoplasm consists of a single non-aqueous layer but it does not alter the argument if we assume that it consists of an aqueous phase, W, with an outer non-aqueous surface layer, X,

and a similar inner surface layer, Y, since KOH will pass through X and dissociate in W and then associate at the surface of Y, pass through Y, and dissociate in the sap.

[5] The accumulation does not reach equilibrium since as a rule it does not go beyond the point where there is about 40 times as much K in the sap as in the sea water.

[6] This really refers to the Mg in the sea water in excess of SO_4 and takes account of the valence of Mg.

[7] It might be supposed that HCO^-_3 would also be more concentrated inside, but there is a complication due to the fact that the system is not in equilibrium so that the outside is more alkaline and has a higher concentration of HCO^-_3. Osterhout and Dorcas (*J. Gen. Physiol.*, 1925-26, ix, 255) have shown that CO_2 passes rather rapidly through the protoplasm until the concentration of undissociated molecules becomes approximately equal on both sides and that there is more dissociation outside owing to the high pH value of the sea water; hence the higher concentration of HCO^-_3 outside. This complication does not affect HCl which is equally dissociated on both sides. (If the system were in equilibrium with an excess of indiffusible cations inside the pH value inside would be higher than outside and HCO^-_3 would be more concentrated inside, but in both cases at a lower concentration than Cl^-.)

[8] When we speak of the penetration of KCl, NaCl, and NaOH as undissociated molecules we need not assume the existence of such molecules in the sea water, any more than in the case of KOH, since ions striking the surface of the protoplasm may there associate to form molecules and pass as such through the non-aqueous layer.

[9] If the cell wall offers no mechanical resistance we should expect the osmotic pressure inside to be approximately equal to that outside. As a matter of fact there seems to be some mechanical resistance in the case of *Valonia macrophysa* and the osmotic pressure inside is a little higher; the internal osmotic pressure is due almost entirely to KCl and NaCl and the concentration of halide is a little higher inside (see footnote 2).

[10] In case the increase in osmotic pressure and consequent growth is due to such a substance as sugar this will not apply.

[11] Hoagland, D. R., and Davis, A. R., *J. Gen. Physiol.*, 1923-24, vi, 47. Hoagland, D. R., Hibbard, P. L., and Davis, A. R., *J. Gen. Physiol.*, 1926-27, x, 121. We should assume that light acts indirectly in this case by providing for the production of CO_2.

[12] Experiments during the last five years on the penetration of ammonia into *Nitella* and *Valonia* indicate that it penetrates as NH_3 or as undissociated NH_4OH and accumulates as NH_4Cl, as described above for KOH and NaOH, and that it penetrates much more rapidly than KOH and NaOH.