vacuolar surface for some time unaffected, and with its potential more or less completely manifest. On this basis, minimum values of 160 mv., outwardly directed, and 80 to 90 mv., inwardly directed, might be assigned to the outer and vacuolar surfaces, respectively, to account for the observed P.D. and its reversal. The eventual loss of all P.D. could be attributed to alteration of the vacuolar surface, by penetration of the unbalanced solution to it, or to general structural or metabolic changes produced by the unbalanced solution. It is planned to test this by perfusion of the vacuole with unbalanced NaCl.

While this scheme is purely formal, and ad hoc, and explains neither the origin of the individual surface potentials, nor the manner in which they are affected by the unbalanced solution, these results give perhaps the best evidence so far adduced for the existence and considerable independence of such potentials. The two general levels, positive and negative, and the essential all or none nature of these is further so closely paralleled in the results of very different treatments, that some such arrangement or mechanism is indicated.

[1] Blinks, L. R., *J. Gen. Physiol.*, **13**, 223 (1929–30).

[2] Cf. Blinks, L. R., *Ibid.*, **16**, 147 (1932–33).

[3] Blinks, L. R., *Ibid.*, **17**, 109 (1933–34).

[4] Blinks, L. R., *Ibid.*, **18**, (1934–35) in press.

[5] Blinks, L. R., abstr. in Sunti delle communicazione, *VII Int. Physiol. Congr.* Rome (1932). A complete report on the effects of current flow is in preparation.

[6] Osterhout, W. J. V., Damon, E. B., and Jacques, A. G., *J. Gen. Physiol.* **11**, 193 (1927–28); Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.* **11**, 391 (1927–28).

[7] Damon, E. B., *J. Gen. Physiol.*, **13**, 207 (1929–30); **15**, 525 (1931–32).

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## *HOW DO ELECTROLYTES ENTER THE CELL?*

BY W. J. V. OSTERHOUT

LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

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Some facts which may be helpful in this connection were found in experiments on the marine alga *Valonia.*[1]

Experiments with ammonia[2] are very instructive. In the first place it is evident that ammonia does not go in chiefly as $NH_4Cl$ for the rate of entrance falls off when we simultaneously increase the external concentration[3] of $NH_4Cl$ and decrease that of $NH_3$.[4] Furthermore when the concentration of $NH_3 + NH_4^+$ in the cell is rapidly increasing very little change in $Cl^-$ occurs.

In the second place it cannot enter chiefly by ionic exchange (as $NH_4^+$, in exchange for $H^+$ or $K^+$ coming out) since in that case the rate of en-

trance would be approximately proportional[5] to the external concentration of $NH_4^+$ but (as just stated) when this concentration increases and that of $NH_3$ decreases the rate of entrance falls off.

TABLE 1

| pH | MOLAR* CONC. $NH_3 \times 10^4$ | MOLES ($NH_3$ + $NH_4$) ENTERING 1 LITER OF SAP IN 10 MIN. $\times 10^4$ | pH | MOLAR* CONC. $NH_3 \times 10^4$ | MOLES ($NH_3$ + $NH_4$) ENTERING 1 LITER OF SAP IN 10 MIN. $\times 10^4$ |
|---|---|---|---|---|---|
| 6.10 | 0.0216 | 1.4 | 9.05 | 16.30 | 24.0 |
| 6.50 | 0.0550 | 3.2 | 9.25 | 23.60 | 27.8 |
| 6.90 | 0.1380 | 4.5 | 9.40 | 30.40 | 32.7 |
| 7.80 | 1.100 | 8.5 | 9.45 | 32.90 | 34.0 |
| 8.00 | 1.700 | 10.3 | 9.60 | 40.90 | 35.5 |
| 8.10 | 2.100 | 11.5 | 9.70 | 46.50 | 39.0 |
| 8.30 | 3.400 | 10.3 | 9.90 | 58.00 | 39.2 |
| 8.60 | 6.500 | 15.0 | | | |

* $pK_{ab}$ is taken[7] as 9.76.

We may therefore conclude that it enters chiefly as $NH_3$. But if it diffused in as such we should expect the rate of entrance to be approximately[6] proportional to $NH_{3_o} - NH_{3_i}$ (where the subscripts $o$ and $i$ refer to concentrations outside and inside) which is not the case[7] (Fig. 1 and Table 1). How is this to be accounted for?

A very simple explanation (suggested by the study of models[8]) is that electrolytes enter by combining reversibly with a constituent $HX$ of the protoplasm. To illustrate we may write $NH_4OH + HX \rightleftharpoons NH_4X + H_2O$. As the reaction is reversible the concentration of $NH_4X$ at the outer surface of the protoplasm[9] and consequently the rate of entrance[10] will increase more and more slowly as the external concentration of $NH_4OH$ increases, giving a curve of the form shown in figure 1.

The form of the curve may be ascertained by means of the formula

$$(NH_{4_o})(OH_o)(HX_b - NH_4X_e) = k_1(NH_4X_e)$$

where $k_1$ is a constant and the subscripts $b$ and $e$ refer to molar concentrations at the beginning and when the reaction has reached equilibrium, respectively.[11] In this formula $H_2O$ does not appear because its concentration is constant both in the aqueous phase and in the non-aqueous protoplasmic surface which is saturated with water.

Since $(NH_4)(OH) = K(NH_3)$, where $K$ is the ionization constant, we may write

$$(NH_{3_o})(HX_b - NH_4X_e) = k(NH_4X_e).$$

The curve shown in figure 1 is obtained by putting $k = 0.001533$ and $HX_b = 0.005$ M.[12]

The points do not fall closely on the curve: this is not surprising in view of the experimental errors and of the complications in the process of pene-

tration.[13] As the amounts entering were small the determinations were not very accurate (a few readings obviously in error were omitted).

The simplest assumption is that $NH_3$ enters the non-aqueous layer and reacts with $HX$ so that the concentration of $NH_3$ to be used in the equation is that of the non-aqueous layer, but if the partition coefficient of $NH_3$ is

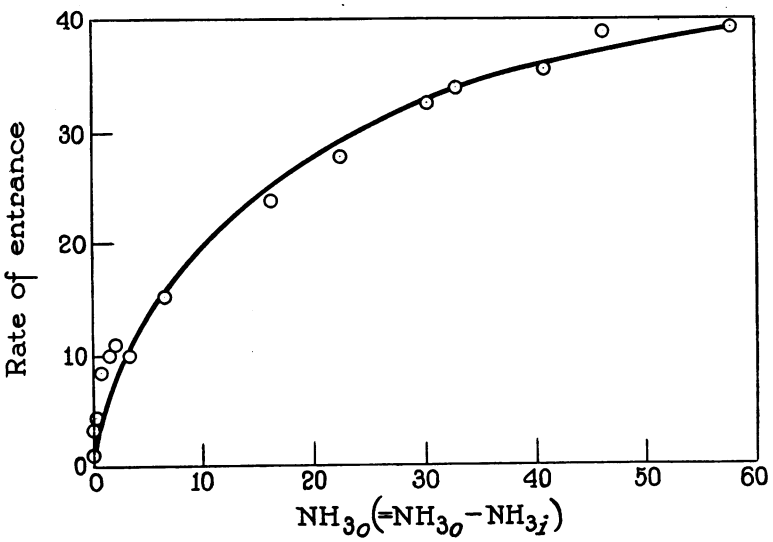


FIGURE 1

The rates of entrance (moles of $NH_3 + NH_4$ entering 1 liter of cells in 10 minutes) are plotted as ordinates and the values of $NH_{3o} = NH_{3o} - NH_{3i}$ as abscissae, where the subscripts $o$ and $i$ refer to concentrations outside and inside, respectively: circles show observed values.

It is assumed that $NH_3$ enters by combining with a constituent of the protoplasm, $HX$, to form $NH_4X$, and that the rate of entrance is directly proportional to the concentration gradient of $NH_4X$. The rate of entrance is taken as proportional to the concentration of $NH_4X$ at the outer surface since in such brief experiments its concentration at the inner surface is negligible.

The curve shows the rate of entrance calculated on the basis of the reaction $NH_3 + HX \rightleftarrows NH_4X$ from the formula $(NH_3)(HX_b - NH_4X_e) = 0.001533\ (NH_4X_e)$ where the subscripts $b$ and $e$ refer to the beginning of the reaction and to equilibrium, respectively ($HX_b$ is taken as 0.005 $M$).

fairly constant (as is presumably the case) it will make no difference in the form of the curve whether we use the concentration of $NH_3$ in the aqueous or in the non-aqueous phase.[14] It seems probable that $HX$ and $NH_4X$ are practically insoluble in water.

Assuming that under the conditions of these experiments the rate of entrance is proportional to $NH_4X$ at the outer surface of the protoplasm (which we may call $NH_4X_o$) we may write as an approximation

$$\text{Rate} = k_2 NH_4 X_o.$$

In calculating the curve in figure 1 it has been assumed for convenience that $k_2 = 1$. If $k_2$ really had this value the fact that $HX_b$ is taken as 0.005 M would be significent but as we do not know the value of $k_2$ the value 0.005 M is of interest only for comparison with experiments on other substances. It is quite possible that $HX$ is not the sole constituent of the non-aqueous layer: it may be merely dissolved in it.

The rise of pH in the sap caused by the entrance of $NH_3$ might cause the curve to flatten out somewhat by increasing $NH_{3i}$, but it could not explain the amount of flattening actually observed. For example, at pH 6.9 in 10 minutes the internal concentration of $NH_3 + NH_4^+$ rose by 0.00045 M which did not suffice to make any detectable rise in the pH of the sap, but the total amount entering was only about half of what would be expected judging from the first two points on the curve and considering the curve to be a straight line.

We may ask whether this could arise from the fact that in the vacuole just inside the inner protoplasmic surface the concentration and pH was greater than the figures given for the sap as a whole. But as such a factor would affect all the reported values in the same sense it might have little effect on the shape of the curve.

It might be thought that the permeability decreases as the external concentration of $NH_3$ increases but the experiments indicate that this is not the case.[15]

Adsorption at the surface might give a curve somewhat like that[16] in figure 1 if adsorption occurred on a micelle at the outer surface and if the micelle moved across the outer non-aqueous protoplasmic surface layer and reacted in the aqueous layer of the protoplasm[17] to form a salt which then passed through this layer, and repeated the process at the inner non-aqueous layer.

It may be added that the curve shown in figure 1 could not be produced in absence of chemical combination by the combined entrance of $NH_3$ entering as such by diffusion and $NH_4^+$ entering by ionic exchange. The rate of entrance of $NH_3$ would be equal to $P_{NH_3}(NH_{3o} - NH_{3i})$, where $P$ is the permeability to $NH_3$, defined as the amount entering under standard conditions[18] when $NH_{3o} - NH_{3i} = 1$. The rate of entrance of $NH_4^+$ passing in by ionic exchange would be equal to $P_{NH_4} = (NH_{4o})(H_i) - (NH_{4i})(H_o)$, the definition of $P_{NH_4}$ being similar to that given for $P_{NH_3}$. If the ratio $P_{NH_3} \div P_{NH_4}$ is constant and $H_o$ and $H_i$ vary but little the rate of entrance of $NH_3 + NH_4$ plotted (as in Fig. 1) against $NH_{3o}$ will give a straight line provided $NH_{3i}$ and $NH_{4i}$ remain relatively small owing to the shortness of the exposure. This line will not pass through the origin and its intercept on the vertical axis will give the value of the ratio[19] $P_{NH_4} \div P_{NH_3}$.

As explained elsewhere[20] the entrance of $NH_4^+$ accompanied by $OH^-$ cannot be distinguished on kinetic grounds from that of $NH_3$. The entrance of $NH_4^+$ accompanied by other anions presents a situation which will be discussed presently: it is ruled out in the present case by the facts stated above.

How do strong electrolytes act? The following possibilities may be considered.

1. Absorption takes place chiefly by ionic exchange, e.g., a cation $M^+$ enters in exchange for $H^+$ coming out. The rate of entrance is then proportional[21] to $M_oH_i - M_i H_o$. When the exposure is so short that relatively little change occurs in $M_i$, $H_i$ and $H_o$, the rate of entrance plotted against $M_o$ should give a straight line. Experiments[22] on *Valonia* and on *Nitella* indicate that ionic exchange plays an unimportant rôle.

2. Both ions enter the non-aqueous layer simultaneously without chemical reaction. If the entering electrolyte is $M$OH the rate of entrance[23] will depend on the partition coefficient of $M$OH between the external solution and the non-aqueous layer for this will determine the concentration gradient of $M$OH across this layer. The rate will therefore be proportional[24] to $(M_o)(OH_o) - (M_i)(OH_i)$. Hence if we plot rate of entrance against $(M_o)(OH_o) - (M_i)(OH_i)$ we should get a straight line. When the time of exposure is short so that $M_i$ and $OH_i$ show relatively little change we should get a straight line by plotting rate against $(M_o)(OH_o)$.

3. The entering electrolyte reacts with a constituent of the protoplasm as in the case of $NH_3$. If the electrolyte is $M$OH, giving the reaction $M$OH $+ HX \rightleftharpoons MX + H_2O$ the case is like that of $NH_4OH$.

If acids give curves like that in figure 1 we may suppose that they unite with a constituent $R$OH of the protoplasm. If salts give similar curves we may consider three possibilities: 1. There are two protoplasmic constituents, $HX$ and $R$OH, the former uniting with cations and the latter with anions. 2. There is an amphoteric substance which unites with both anions and cations. 3. There are present in the protoplasm acids, bases and ampholytes. Such questions must be left to future investigation.

The data in the literature give the impression that, if certain assumptions are made regarding "effective products," curves somewhat like that in figure 1 are obtainable with strong electrolytes. Experiments along this line are now being carried on.

In conclusion let us consider substances which enter against a gradient:[25] this necessitates an expenditure of energy (of which the cell has an abundant supply). As an example let us take the penetration of $M$OH entering when the product $(M)(OH)$ is greater inside than outside. One suggestion for dealing with this situation is to suppose that the process of penetration goes on as it would in the absence of any special expenditure of energy

but with a value of $(M_i)(OH_i)$ smaller than the measured value. If $(M_i)(OH_i) = 10^{-6}$ and $(M_o)(OH_o) = 10^{-8}$, but $M$ neither enters nor leaves the cell, we may say that if no special energy were being expended the expected value of $(M_i)(OH_i)$ would be $10^{-8}$ (0.01) $= 10^{-6}$, i.e., equal to $(M_o)(OH_o)$, and this may be called for convenience the "effective value." This procedure may be regarded as somewhat analogous to that by which concentrations are multiplied by activity coefficients to make their values correspond to their behavior. In the present case the coefficient 0.01 may be called the "efficiency coefficient" and all values of $(M_i)(OH_i)$ may be multiplied by it. Thus if $(M_i)(OH_i) = 10^{-6}$ and the efficiency coefficient is 0.01 the effective product will be $10^{-6}$ (0.01) $= 10^{-4}$ and if $(M_o)(OH_o) = 10^{-6}$ we shall expect $M$OH to enter.

*Summary.*—Experiments on large multinucleate cells of the marine alga *Valonia* indicate that there is little penetration of $NH_4^+$ but that $NH_3$ or $NH_4OH$ enters freely, apparently by combining with a constituent, $HX$, of the protoplasm.

It is not impossible that strong electrolytes also enter by combining with one or more constituents of the protoplasm.

[1] This is well suited to such studies because its multinucleate cells reach the size of a pigeon's egg and the sap can be obtained without contamination.

[2] For a description of the experiments see Cooper, W. C., Jr., and Osterhout, W. J. V., *J. Gen. Physiol.*, **14**, 117 (1930–31).

[3] In sea water to which enough $NH_4Cl$ was added to make its concentration 0.01 M the concentrations of $NH_4^+$ and of $NH_3$ were regulated by changing the pH. There was no sign of injury in these experiments, which lasted only 10 minutes (footnote 2).

[4] The term $NH_3$ as here used includes $NH_4OH$.

[5] Really proportional to $(NH_{4o})(H_i) - (NH_{4i})(H_o)$ but $NH_{4i}$ would be mostly negligible in such brief experiments as those here discussed.

[6] The relation is only approximate since there are a number of complicating factors (cf. Osterhout, W. J. V., *J. Gen. Physiol.*, **16**, 529 (1932–33).

[7] Cf. Osterhout, W. J. V., *J. Gen. Physiol.*, **16**, 529 (1932–33); *Ergebn. Physiol.*, **35**, 967 (1933). In calculating the concentration of $NH_3$ the value of $pK_{ab}$ was taken as 9.76. This is in line with the recent work on $CO_2$ (cf. Buch, K., *J. conseil internat. l'exploration de la mer*, **8**, 309 (1933); *Conseil perm. internat. l'exploration de la mer, rap. et proc. verb. reunions*, **85**, 71 (1933); Buch, K., Harvey, H. W., Wattenberg, H., and Gripenberg, S., *Conseil perm. internat. l'exploration de la mer, rap. et proc. verb. reunions*, **79**, 1 (1932) which indicates that the ionic strength of sea water changes the pK by about 0.5. Taking the $pK'_{ab}$ of $NH_3$ in dilute solutions as 9.26 and adding 0.5 we get 9.76 (it then becomes pK instead of pK' since activities are taken into account). (If we use $pK'_{ab} = 9.26$ for Fig. 1 we can fit the curve approximately by putting $HX_b = 0.005$ and $K = 0.003$). As the exposure lasted only 10 minutes any change in pH due to photosynthesis may be neglected in calculating the concentration of $NH_3$.

[8] Osterhout, W. J. V., *Ergebn. Physiol.*, **35**, 992 (1933).

[9] As in previous papers it is assumed that the outer surface of the protoplasm is a non-aqueous layer and that a very thin film at the outer surface of this layer comes at once into approximate equilibrium with the external aqueous solution (cf. Osterhout, W. J. V., *J. Gen. Physiol.*, **16**, 529 (1932–33)).

It is assumed that the reaction is relatively rapid as compared to the inward diffusion of $NH_4X$ so that the latter determines the time curve.

[10] The rate of entrance will depend on the inward diffusion of $NH_4X$ and since in the present experiments (lasting only 10 minutes) back diffusion may be neglected the rate of diffusion across the outer non-aqueous surface layer of the protoplasm will be proportional to $NH_4X$ at the outer surface of this layer. In longer experiments the back diffusion would have to be taken into account and the rate of entrance would be proportional to $NH_4X_o - NH_4X_i$ where $o$ and $i$ refer to concentrations in the outer and inner surfaces of this non-aqueous layer. (This is illustrated by models: in these only one non-aqueous layer is employed to represent the protoplasm but the principle is essentially the same as when two are employed.) After passing through this non-aqueous layer $NH_4X$ presumably comes in contact with the aqueous layer of the protoplasm and reacts to form salts such as $NH_4Cl$. Since $NH_4^+$ is in equilibrium with $NH_3$ in this aqueous layer an increase in $NH_4^+$ means a corresponding increase in $NH_3$, as long as the pH remains constant. The $NH_3$ will then react with $HX$ in the inner non-aqueous layer of the protoplasm to form $NH_4X$ which will in turn react to form $NH_4Cl$, etc., on reaching the sap. (The sap is more acid than the sea water by about 2 pH units: its average pH is about 5.8 and that of the sea water 8.0.)

It seems quite possible that all these processes are so linked together that the rate of diffusion across the protoplasm will be proportional to the concentration gradient of $NH_4X$ across the protoplasm.

Cf. Osterhout, W. J. V., *J. Gen. Physiol.* **16**, 529 (1932–33); *Ergebn. Physiol.*, **35**, 967 (1933); Osterhout, W. J. V., and Stanley, W. M., *J. Gen. Physiol.*, **15**, 667 (1931–32); Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, **17**, 445, 469 (1933–34).

[11] The reaction is regarded as occurring at the surface of the external non-aqueous protoplasmic layer in such fashion that the value of $NH_4X_o$ is quickly reached (the customary assumption is made that the outer surface of the non-aqueous layer is approximately in equilibrium at all times with the adjoining aqueous phase).

[12] The nature of this curve is such that it is not very easily manipulated by changing constants and in consequence the observed fit is regarded as significant.

[13] Cf. Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, **17**, 445 (1933–34).

[14] But if we employ a substance with a higher partition coefficient than $NH_3$ the concentrations in the non-aqueous layer and consequently the concentration of the compound it forms with $X$ will be greater so that the rate of entrance will be higher.

[15] Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, **14**, 301 (1930–31).

[16] If we plot the logs of ordinates and abscissae of figure 1 we do not get a straight line such as the original adsorption formula demands but certain recent adsorption formulae differ from this.

[17] Osterhout, W. J. V., *Ergebn. Physiol.*, **35**, 1013 (1933).

[18] Osterhout, W. J. V., *Ibid.*, **35**, 990 (1933).

[19] If this ratio is unity the line will be horizontal.

[20] Osterhout, W. J. V., *Biol. Rev.*, **6**, 369 (1931).

[21] Osterhout, W. J. V., *J. Gen. Physiol.*, **14**, 277 (1930–31).

[22] Osterhout, W. J. V., *Ergebn. Physiol.*, **35**, 994 (1933); Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.* **17**, 727 (1933–34).

[23] The electrolyte will be mostly in molecular form in the non-aqueous layer which has a low dielectric constant and hence permits little dissociation. Osterhout, W. J. V., *Ergebn. Physiol.*, **35**, 1009 (1933).

[24] Osterhout, W. J. V., *J. Gen. Physiol.*, **16**, 529 (1932–33); Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, **17**, 445 (1933–34); Osterhout,

W. J. V., *Ergebn. Physiol.*, **35**, 991 (1933). The case where $(M_o)(OH_o) < (M_i)(OH_i)$ will be discussed later on.

[25] Jacques, A. G., and Osterhout, W. J. V., *Proc. Soc. Exp. Biol. and Med.*, **31**, 1121 (1933–34).

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## *DEGENERATION OF XANTHOPHORES IN FUNDULUS MAJALIS*

BY A. A. ABRAMOWITZ

MARINE BIOLOGICAL LABORATORY, WOODS HOLE, AND THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY

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Morphological color change in fishes has been the object of a long series of investigations, but in these studies, with few exceptions, only the melanophores were subjected to experimental tests. Little or no attention has been paid to the xanthophores chiefly because of their lack of distinctness both in the living animal and in histological preparations. It is the purpose of this paper to report some results obtained on the effect of variously colored backgrounds upon the formation and destruction of the yellow-bearing pigment-cells.

While it is well known that changes in the melanin content and in the numbers of melanophores of fishes may be experimentally produced by appropriate backgrounds, few such changes have been recorded for pigment cells other than the melanophores. Hewer ('27) reported an increase in the number of erythrophores per unit area of skin of an English flat-fish following a sojourn upon an orange background. Odiorne ('34) believed that the number of guanophores may be increased after prolonged adaptation to a white background. As regards the yellow pigment, the extensive study of the carotenoid content of four species of fish by Sumner and Fox ('33) constitutes the only work, as far as I am aware, in which positive results have been obtained. They were, however, "concerned very little with the xanthophores themselves" but extracted the yellow pigment from the bodies of many fishes which had been placed over white, black, red or yellow backgrounds for one or two months' duration, and compared these extractions photometrically. In Fundulus parvipinnis, one of the four species employed in their study, no changes in the total amount of xanthophyll were found after a period of about two months over these backgrounds. In the "greenfish," Girella nigricans, however, a long sojourn in a white aquarium was followed by an extensive reduction in the amount of xanthophyll. They conclude "that this reduction, which paralleled changes in the visible coloration of the fishes, was actually due to the optical environment to which they had been subjected, seems altogether pos-