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Note on the Penetration of Electrolytes.

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When a weak electrolyte penetrates a living cell the distribution at equilibrium[1] between the inside and outside does not tell us whether it enters the cell in ionic or molecular form, for the equilibrium is the same in both cases, being determined by the fact that the ionic activity product (or chemical potential) must be the same inside and outside. Assuming that the dissociation constants are the same inside and outside and that the activity coefficients equal 1 we may write for a weak acid, HA,

$$\frac{(H_i)\,(A_i)}{M_i} = K = \frac{(H_o)\,(A_o)}{M_o}$$

where the subscripts o and i denote the external and internal concentrations respectively, and M denotes undissociated molecules. Since the ionic product inside must be equal to that outside we have $(H_i)\,(A_i) = (H_o)\,(A_o)$ and therefore $M_i = M_o$.[2]

For convenience we may consider a cylindrical plant cell in contact with a solution at one of its end surfaces, the content of its central vacuole being well stirred (which may sometimes happen as

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[1] This was mentioned in a lecture before the Mayo Clinic in 1927 (*cf.* Osterhout, W. J. V., Some aspects of cellular physiology, in Mayo Lectures, 1928) and differs from the view expressed in earlier papers.

When there is no Donnan equilibrium there is no true equilibrium as long as there is a difference between the pH value of the sap and the external solution, but a weak acid may very quickly come to an apparent equilibrium inside and outside as has been found experimentally (*e. g.*, in the case of CO_2 and H_2S, *cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1925-28, viii, 131; Osterhout, W. J. V., and Dorcas, M. J., *J. Gen. Physiol.*, 1925-26, ix, 255).

[2] For the purposes of this paper we need not consider the internal concentration of ions but it can always be found if we know the pH value of the sap, M_i and K.

the result of protoplasmic motion) : we may assume that the external solution is likewise stirred and has a large volume so that it remains practically constant during penetration. For convenience we shall assume that the pH value is the same inside and outside and remains practically constant during the experiment.

If molecules alone enter and the quantity passing in be called x it is evident that the observed rate $\frac{dx}{dt}$ will be proportional to the net rate of passage of molecules through the protoplasm,[3] D_M, to the permeability of the protoplasm to molecules, P_M, and to the difference between the external concentration of molecules, M_o, and the internal concentration found in the vacuole, M_i. Hence we may write $\frac{dx}{dt} = D_M P_M (M_o — M_i)$. This is similar to the formula employed by Northrop for diffusion through collodion membranes in contact with solutions.[4] Putting $D_M P_M = V_M$ (the apparent velocity constant of the process) we have[5]

Let us now consider what will happen if ions alone enter. Unless there is an exchange of ions going in opposite directions[6] H^+ cannot enter without A^- so that we may speak of the penetration of ion pairs $(H^+ + A^-)$. In order that an ion pair may enter H^+ and A^-

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[3] The cell wall will be left out of consideration in the present discussion.

[4] Northrop, J. H., *J. Gen. Physiol.*, 1928-29, xii, 435. The formula employed by Northrop is $Q = \frac{t\ D\ AS\ (C_1—C_2)}{h}$, where Q is the quantity passing in in the time t, D is the diffusion constant in the collodion, S is the partition coefficient between collodion and water, A is the area, and h the thickness of the collodion membrane and C_1 and C_2 are the external and internal concentrations of molecules. The S of Northrop's formula might be regarded as analogous to the P in our formula but evidently P cannot be regarded simply as a partition coefficient since the protoplasm is not a single phase; we regard P as a proportionality factor representing the number of molecules passing through the protoplasm as compared with the number striking the surface (*e. g.*, if half the molecules striking the surface enter, P will be equal to 0.5). We might regard the permeability of the protoplasm as depending also on D_M but it seems simpler not to do this. D_M may be regarded as the reciprocal of the time required to pass through the protoplasm and hence takes account of its thickness as well as the diffusion constant in its various phases and any other factors (*e. g.*, chemical combinations) which may affect the rate of progress.

[5] The observed curve of penetration may be of the first order, *cf.*, Irwin, M., *J. Gen. Physiol.*, 1925-28, viii, 147.

$$\frac{dx}{dt} = V_M(M_o—M_i \text{ and } V_M = \frac{1}{t}\log\frac{M_o}{M_o—M_i}$$

[6] Since this cannot alter the internal or external concentration in this case (where we assume that H^+ and A^- are the only ions present in quantity) we need not consider it here.

must collide at the surface and the situation is like that in a bimolecular process of the type

$$H_0 + A_0 \underset{\text{outward}}{\overset{\text{inward}}{\rightleftarrows}} H_i + A_i$$

so that the observed rate $\frac{dx}{dt}$ would be equal to the difference between the inward $(\frac{dx}{dt})_i$ and outward $(\frac{dx}{dt})_o$ rates, *i. e.*,

$$\frac{dx}{dt} = \left(\frac{dx}{dt}\right)_i - \left(\frac{dx}{dt}\right)_o$$

If M_o is constant $(\frac{dx}{dt})_i$ is constant and is proportional to the rate of progress of ion pairs through the protoplasm, (D_P), to the permeability of the protoplasm to ion pairs, (P_P), and to the number of collisions of H^+ and A^- at the external surface which in turn is proportional to the product of H^+ by A^-. Hence we may write

$$\left(\frac{dx}{dt}\right)_i = D_P P_P HA$$

According to the mass law $HA = KM_o$. Substituting this value we have

$$\left(\frac{dx}{dt}\right)_i = D_P P_P K M_0$$

In the same way we may write $(\frac{dx}{dt})_o = D_P P_P K M_i$ and the observed rate $\frac{dt}{dx}$ will be equal to $(\frac{dx}{dt})_i—(\frac{dt}{dx})_o = D_P P_P K M_o—D_P P_P K M_i$. Putting $D_P P_P K = V_P$ (the apparent velocity constant of the process) we have

$$\frac{dx}{dt} = V_P(M_0 - M_i) \text{ and } V_P = \frac{1}{t}\log\frac{M_0}{M_0 - M_i}$$

At the beginning (when $M_i = 0$) we may call the rate $(\frac{dx}{dt})_{Pb}$ and write

$$\left(\frac{dx}{dt}\right)_{Pb} = V_P M_0$$

and in the same way the rate at the beginning when molecules alone enter may be called $(\frac{dx}{dt})_{Mb}$ so that we may write $(\frac{dx}{dt})_{Mb} = V_M M_o$. If both molecules and ions enter we have

$$\left(\frac{dx}{dt}\right)_{Mb+Pb} = \left(\frac{dx}{dt}\right)_{Mb} + \left(\frac{dx}{dt}\right)_{Pb} = (V_M + V_P)M_0$$

and the apparent velocity constant of the process $V_{M+P} = V_M + V_P$.[7]

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[7] We assume that the ions and molecules enter without mutual interference, which seems reasonable as long as the pH value is the same in the external solution, the protoplasm and the vacuole. In case the pH value in the vacuole is less than that in the external solution some of the ion pairs will be changed to molecules on entering but the fraction thus changed will be constant as long as the pH value of the vacuole is constant.

Since $V_M = D_M P_M$ and $V_P = D_P P_P K$ it is evident that if $D_M = D_P$ and $P_M = P_P$ we have $V_P = V_M K$. If K is small V_P will be small in comparison[8] with V_M and $(\frac{dx}{dt})_{Pb}$ will be small in comparison with $(\frac{dx}{dt})_{Mb}$. In other words if the protoplasm is permeable to molecules we shall expect very little increase in the rate in case ion pairs enter in addition unless K is large. In general the penetration of strong electrolytes into *Valonia* and *Nitella* appears to be very slow and the electrical resistance of the protoplasm of these cells seems to be high, indicating that the value of D_P or of P_P is small. Thus in the experiments of Dr. Blinks on *Valonia* and *Nitella*,[9] the electrical resistance of the protoplasm in external contact with NH_4Cl is very high but we know that ammonia enters very rapidly and raises the pH value of the sap; presumably it enters as NH_3 or as undissociated NH_4OH.

If K is small we should not expect to find much difference experimentally between the velocity constants at high and low pH values in the case of a weak acid. This view is supported by the results of preliminary experiments (carried out by Mr. Jacques) on the penetration of CO_2 into *Valonia* at high and low pH values.

It is evident that even when ion pairs alone enter, the rate of penetration falls off with decrease of M_o and it must therefore fall off with increase of pH value and of A_o when the total external concentration is kept constant.

These conclusions will apply qualitatively in case the time curve is not of the first order.

In case of an exchange of ions[10] of the same sign going in opposite directions, *e. g.*, exchange of H^+ for Na^+, the total quantity of cations, Q, passing through the membrane in unit time is proportional to the total concentration (H + Na) of cations inside multiplied by that outside so that we may put $Q = R(H_i + Na_i)(H_o + Na_o)$ where R is a proportionality factor and the subscripts *i* and o denote the concentrations inside and outside respectively. The

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[8] In case the curve is not of the first order the velocity constants may be compared (without determining the equation of the curve) as follows: If the velocity constant of two curves (I and II) are V_I and V_{II} we may put $V_I \div V_{II} = T_{II} \div T_I$ where T_I is the time required to bring curve I to a given stage (*e. g.*, half way to equilibrium) and T_{II} is the time required to bring curve II to the same stage.

[9] For experiments on penetration of NH_2 into *Valonia* see Brooks, M. M., *Pub. Health Rep.*, Washington, D. C., 1923, xxxviii, 2074. For experiments on *Nitella* see Irwin, M., *J. Gen. Physiol.*, 1925, ix, 235.

[10] Doubtless the cell can produce sufficient ions (*e. g.*, $H^+ + HCO_3^-$) to ensure adequate exchange.

quantity of H^+ passing out is equal to $\frac{Q\,H_i}{H_i + Na_i}$ and that passing in is equal to $\frac{Q\,H_o}{H_o + Na_o}$ and the net amount passing in is the difference between these expressions: on reducing them to a common denominator we get

Net amount of H passing in $= R[(H_o\,Na_i)—(H_i\,Na_o)]$.

The same reasoning would apply to a strong electrolyte[11] such as KOH since it is permissible for purposes of calculation to assume that a few undissociated molecules are present in the case of strong electrolytes. It seems possible that K^+ and OH^- might combine at the non-aqueous surface of the protoplasm to form a molecule which could pass through and dissociate again on reaching an aqueous medium. We may perhaps use as an analogy a closed system containing 2 aqueous solutions of HCl separated by air. As HCl has some vapor pressure at higher concentrations it is evident that the air must contain some molecules of HCl (which may pre-exist as such in the solution or may result from a combination of H^+ and Cl^- at the surface to form a molecule[12] which passes into the air). The product (H) (Cl) is the same at equilibrium in the 2 aqueous solutions (if we use activities) but in the gas phase equals zero. In this respect the gas phase may resemble the non-aqueous layers of the protoplasm (except that the product in such layers might be greater than zero).

In the case of HCl the vapor pressure increases slowly with the concentration up to a certain point after which it rises rapidly. The same may be true of the non-aqueous layers of the protoplasm. Such a result has been observed,[13] "injury" being mentioned as a possible cause, which may of course be the case.

Summary: if the time curve of penetration is of the first order the rate of penetration of undissociated molecules may be regarded as $(\frac{dx}{dt})_M = D_M P_M (M_o—M_i)$ where D_M is the rate of progress through the protoplasm, P_M is the permeability of the protoplasm to molecules, M_o is the external and M_i the internal concentration of undissociated molecules. The corresponding value, when ion pairs alone enter (in the case of a binary electrolyte with no exchange of ions), is $(\frac{dx}{dt})_P = D_P P_P K (M_o—M_i)$ where K is the dissociation constant. It is evident that if the protoplasm is equally permeable to undissociated molecules and to ion pairs (*i. e.*, if $P_M = P_P$) the penetration of ion pairs will be very small when K is

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[11] Osterhout, W. J. V., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 234.

[12] Since we assume that a molecule of HCl passing from the air to the aqueous surface can dissociate to form ions the reverse process must also be possible.

[13] Irwin, M., *J. Gen. Physiol.*, 1925-28, viii, 147.

small. When K is large the high electrical resistance of the protoplasm in *Nitella* and *Valonia* suggests that either D_P or P_P is small (*i. e.,* when a substance is present largely as ions there is very little penetration).

If the external concentration remains constant while the pH value changes so as to decrease the concentration of undissociated molecules the rate of penetration will diminish even when the protoplasm is permeable to ion pairs only.

These conclusions apply qualitatively when the time curve of penetration is not of the first order.

In case of an exchange of ions the rate will be proportional to the product of the exchanging ions on opposite sides.

The fact that certain weak electrolytes enter rapidly and that the electrical resistance of the protoplasm in contact with strong electrolytes is very high suggests that strong electrolytes may enter largely as undissociated molecules formed at the surface by collision of ions.

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Investigations of Methods in the Study of Anaphylaxis.*

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Comparisons were made of the intraperitoneal, respiratory and Dale methods with the intravenous route to determine which method of inducing anaphylactic shock was most reliable in the evaluation of a given state of hypersensitiveness. To make such a determination, we deviated somewhat from the regular method of a single shock dose and employed what might be termed the double shock method, that is we compared 2 criteria in the same animal.

In the first group we gave intraperitoneal injections to 46 sensitized animals. From one to several hours later these same animals received an intravenous injection of the same material in considerably smaller amounts.

Of these 46 animals, 21 were negative by both methods. Of the remaining, after the intraperitoneal injection 12 were negative, 3 gave a ± reaction,[1] 5 gave a + reaction and 5 gave a +++ reac-

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[1] — no anaphylactic reaction. ± doubtful reaction. + showing dyspnea and