Technical Papers

Unequal Distribution of Diffusible Nonelectrolytes Across a Membrane [1]

CLARA L. DEASY

Temple University School of Medicine, Philadelphia

The Donnan equilibrium is a concept which explains thermodynamically the unequal distribution on the two sides of a membrane of diffusible ions in an equilibrated system containing a nondiffusible ion. By an extension of ideas first presented by Wall (*2*), who was interested in the determination of molecular weights by osmotic pressure measurements using mixed solvents, it is possible to show thermodynamically that the presence of a nondiffusible substance on one side of a membrane (or in different concentrations on the two sides) in general affects the distribution of a diffusible nonelectrolyte in the system; *i.e.* the concentrations of diffusible *nonelectrolyte* will be unequal on the two sides of the membrane.

It can readily be shown by a derivation similar to that developed by Wall that at equilibrium the following relationship holds:

$$\frac{1}{\overline{V}_L} \ln \frac{a^1_L}{a_L} = \frac{1}{\overline{V}_D} \ln \frac{a^1_D}{a_D}, \qquad (1)$$

where $\overline{V}_L$ and $\overline{V}_D$ are the partial molar volumes of liquid and of diffusible nonelectrolyte, respectively, on the side of the membrane containing nondiffusible solute, a_L and a_D are the activities on the side of the membrane containing nondiffusible solute, and a^1_L and a^1_D are the activities on the side not containing nondiffusible solute.

Since, in general, the partial molar volumes of liquid and of diffusible solute on the side of the membrane containing nondiffusible solute are not equal, then $\frac{a^1_L}{a^1_D} \neq \frac{a_L}{a_D}$.

For solutions which are very dilute or which approach ideality, concentration terms may be used in place of activities in (1).

It can also be readily shown that (1) is applicable to systems containing different concentrations of nondiffusible solute on the two sides of the membrane.

Qualitative deductions regarding the type of concentration changes to be expected in any particular system can be derived from (1). If the partial molar volume of the liquid, $\overline{V}_L$, is greater than that of the solid, $\overline{V}_D$, then $\frac{a^1_L}{a_L} > \frac{a^1_D}{a_D}$. If $\overline{V}_L$ is less than $\overline{V}_D$, then $\frac{a^1_L}{a_L} < \frac{a^1_D}{a_D}$.

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If the diffusible nonelectrolyte present in the system is a gas, and if the solutions are ideal to the extent that Raoult's law can be applied to the liquid and Henry's law to the gas, Equation (1) becomes

$$\frac{1}{\overline{V}_L} \ln \frac{x^1_L}{x_L} = \frac{1}{\overline{V}_G} \ln \frac{x^1_G}{x_G}, \qquad (2)$$

where x_L and x_G are the mole fractions of liquid and of gas, respectively, on the side of the membrane containing nondiffusible solute, and x^1_L and x^1_G are mole fractions of liquid and of gas on the other side of the membrane.

Quantitative data which show the approximate magnitude of concentration changes to be expected from the effect are calculated from (2) for several gases in aqueous systems (Table 1).

TABLE 1*

Gas	$\overline{V}_G$	$\frac{\overline{V}_G}{\overline{V}_L}$	x_N	x_L	$\frac{x_G}{x^1_G}$
O_2	28	1.6	0.05	0.95	0.92
"	"	"	0.10	0.90	0.84
N_2	35	2.0	0.05	0.95	0.90
"	"	"	0.10	0.90	0.81
CO_2	40	2.2	0.05	0.95	0.89
"	"	"	0.10	0.90	0.79

* For all calculations it was assumed that $\overline{V}_L = 18$ and $x^1_L = 1$.

In the calculations the partial molar volume of water in the solution was assumed to be its molar volume; the partial molar volume of the gas in the solution was assumed to be its molar volume as a liquid; all temperature effects were neglected. The mole fractions of water in both solutions were calculated neglecting the small concentration of the gas.

If the effect discussed in this paper were neglected, and if the experimentally determined partial pressures of the gas on each side of the membrane were assumed equal at equilibrium (an assumption frequently made, *1*), then the ratio $\frac{x_G}{x^1_G}$ should be 1 for all cases. If it were assumed that the concentrations $\left(\frac{\text{moles of gas}}{\text{moles of liquid}}\right)$ are the same at equilibrium on the two sides of the membrane, then the ratio should be 0.95 (if $x_N = 0.05$) and 0.90 (if $x_N = 0.10$), where x_N is the mole fraction of nondiffusible solute. The actual values (Column 6) indicate that the ratio may vary from 0.79 to 0.92.

With the present lack of necessary data on biological systems, it is impossible to assess quantitatively the importance of this phenomenon in the dis-

tribution of nonelectrolytes across living membranes. However, it is suggested that the phenomenon might be of importance wherever a diffusible nonelectrolyte passes through a membrane with different concentrations of nondiffusible solute on the two sides as, for example, in the passage of oxygen and carbon dioxide through the lung membrane, of gases and of diffusible solids from the blood to the body tissues, and of gases and of diffusible solute through plant membranes, and in the formation of urine and other body fluids.

References

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The Use of Cytochrome C in Combating Tissue Anoxia[1]

SAMUEL PROGER and D. DEKANEAS
Joseph H. Pratt Diagnostic Hospital and *Tufts College Medical School, Boston, Massachusetts*

We have been interested in attacking the problem of tissue anoxia by attempting to improve the efficiency of the tissue utilization of oxygen. In this connection it is important to recall that even under conditions of anoxia there is considerable oxygen in the venous blood. Thus, under conditions of anoxia which are barely compatible with consciousness (about 10 per cent oxygen) there may still be in the venous blood returning to the heart some 4–6 vol. per cent oxygen. Under such conditions there is therefore an available supply of oxygen which needs only to be utilized. Theoretically, respiratory enzymes might favor such utilization.

Our earlier studies (*5*) were with the group of C_4 dicarboxylic acid substances in the so-called Krebs cycle, particularly succinic acid. These are probably among the less important of the respiratory catalysts, but our studies with these substances served to demonstrate that one could, by means of such substances, apparently improve the tissue utilization of oxygen in the living animal under conditions of anoxia.

Among the most important of the substances which promote tissue oxidation are the cytochromes. Of these, only cytochrome C can be readily prepared (*2*). We have used beef heart as the source of cytochrome C and have found that, by reprecipitation and passage through a Seitz filter, it is apparently nontoxic. The fact that it has been demonstrated to be not only nontoxic but stable makes it clinically utilizable. Despite the fact that cytochrome C is a protein (iron porphyrin protein), it appears to be nonantigenic.

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Certain facts make cytochrome C potentially useful. Among these is the fact that its organ content can be significantly increased by parenteral injection (*8, 9*). Also, the organs normally contain considerably more cytochrome oxidase than can be activated by the cytochrome C present (*4, 11*). Hence, if additional cytochrome C can be supplied to the organs, additional cytochrome oxidase is present for activation for the final linkage with molecular oxygen. Also, we find that the magnitude of increase of cytochrome C which can be produced in organs by parenteral injection is such as to produce *in vitro* an increase of oxygen consumption of 50–100 per cent (*9*). The final fact of importance in this connection is, as indicated above, that there is even under conditions of anoxia considerable unused oxygen in the venous blood returning to the heart, so that if the tissue uptake of oxygen can be increased, there is an available supply of oxygen.

On the basis of these considerations it might have been anticipated that conditions associated with anoxia *in vivo* might be benefited by the injection of cytochrome C. This seems, in fact, to be the case, as indicated by the following observations.

The easily hydrolyzable phosphorus fraction, particularly adenylpyrophosphate, is thought to play an important role in the tissue transfer of energy (*3*). This compound continuously donates phosphoric acid radical to other metabolites and hence requires continuous resynthesis. The continual release of this so-called "phosphate-bond" energy is only possible through a continual supply of the energy released by the cell oxidations which are catalyzed by the cytochrome-cytochrome oxidase system and which serve to resynthesize the adenylpyrophosphates. We have demonstrated in rats that the organ content of these phosphates is markedly diminished under conditions of severe anoxia (*10*). When, however, some additional cytochrome C has been supplied to the rats beforehand, the anoxia produces little or no change in the organ content of the phosphates concerned (*10*). If such important biochemical effects of anoxia can be largely prevented by cytochrome C, it is not unlikely that other effects, in addition to those associated with phosphorylation, can also be favorably influenced.

The effects of anoxia on the living heart can be easily studied by means of the electrocardiogram. We have found that such effects on the electrocardiogram as can be produced by moderately severe anoxia (10 per cent oxygen and 90 per cent nitrogen) can be regularly prevented by the previous intravenous injection of cytochrome C (*6*). Also, those patients who experienced subjective distress with such anoxia were free from the distress under the anoxia when they had been injected previously.

These facts naturally lead to some observations on