THE TONICITY-VOLUME RELATIONS FOR SYSTEMS CONTAINING HUMAN RED CELLS AND THE CHLORIDES OF MONOVALENT CATIONS

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It has been recognized since the time of the earliest investigations into the osmotic behavior of red cells that different values for volume may be found when the cells are suspended in solutions of different ionic composition but of the same depression of freezing point. The problem as to why this should occur is of historical interest because it was an observation of this type which led Moore and Roaf (Moore and Roaf, 1908; Roaf, 1912) to think of the ion distribution between the red cell and its environment as regulated by ion-binding processes rather than by permeability processes as ordinarily understood; these early observations, however, have been largely discounted, partly because there are serious technical difficulties attached to the determination of the freezing point of plasma and of hemolyzed red cells, and partly because of doubt as to the purity of the salts used and as to the reliability of the methods for measuring volume. Ege (1921) investigated the phenomenon as it presents itself when the solutions are those of salts of the monovalent anions (NaCl, KCl, KNO_3, $NaSO_4$, etc.). He observed differences in equilibrium volume, sometimes as great as 10 per cent, in solutions of the same depression of freezing point; he believed that these differences are best explained by assuming the rate of penetration of the different anions to be different, but was unable to account for the order in which the monovalent anions produce the anomalous effects on volume. Ponder and Saslow (1930, 1931) also noticed discrepancies in the relation between tonicity and volume when rabbit red cells are suspended in hypotonic NaCl, KCl, and LiCl, and attributed the differences to the loss of cell K being greater into some media than into others; this explanation has since been abandoned, but no other has been suggested in its place.

This paper is concerned with the tonicity-volume relations of human red cells in solutions of the salts of the monovalent cations LiCl, NaCl, KCl, RbCl, and CsCl.

1. Red Cell Fragility in Equimolar Solutions of the Chlorides of the Monovalent Cations

Table I gives the tonicity T_0 in which there is just commencing hemolysis and the tonicity T_{50} in which there is 50 per cent hemolysis is systems at 22°C. containing human red cells and hypotonic LiCl, NaCl, KCl, RbCl, and CsCl.

Description of the Hemolytic Systems.—The hemolytic systems in which T_0 and T_{50} are measured are composed of 2 ml. of a series of solutions of the chlorides of the monovalent cations, descending in tonicity from $T = 1.0$ to $T = 0.4$ by steps of $T = 0.05$, to each of which 0.5 ml. of a suspension of washed human red cells is added. The washed cells of 2.5 $(0.4/\rho)$ ml. of heparinized blood are finally suspended in 25 ml. of 1 per cent NaCl to make the suspension. After the addition of the red cells, the tonicities of the descending series become 1.0, 0.96, 0.92, . . . 0.44.

The chlorides of the monovalent cations, after being dried at 60°C. for several days, are freshly prepared in 0.172 M solution in water. Specimens from two sources were used: LiCl, NaCl, KCl, RbCl, and Cs from the Amend Chemical Company, and LiCl, NaCl, and CsCl prepared by Dr. Theodore Shedlovsky for conductivity work. The two sets of preparations have slightly different effects on red cell swelling and hemolysis; the swelling observed with the Amend Chemical Company preparation of

TABLE I

	T_0	T_{50}
LiCl	0.65	0.53
NaCl	0.56	0.45
KCl	0.60	0.49
RbCl	0.61	0.49
CsCl	0.59	0.47

Order, Li > K ≧ Rb > Cs > Na.

LiCl, for example, is a little greater than that observed with Dr. Shedlovsky's preparation, and so the latter was used in the experiments of section 2. In all cases the tonicity of the 0.172 M solution was taken as $T = 1.0$, the hypotonic solutions being made by the addition of water. The pH of the solutions varied from pH 6.6 to pH 6.8 (freshly prepared solutions, glass electrode); after the addition of the cell suspension, the pH of the systems was 7.1 ± 0.05.

The completed hemolytic systems are allowed to stand at 22°C. for 5 hours, with occasional mixing by inversion. At the end of that time the cells are thrown dowm, and the amount of lysis is determined from the concentration of Hb in the supernatant fluids; this is found colorimetrically, the whole procedure being very like that already described (Ponder, 1948 *a*).

Table I shows that the tonicities T_0 and T_{50} are not identical in their effects in the case of all the 0.172 M chlorides of the monovalent cations, and that the fragility of the cells is least in 0.172 M LiCl and greatest in 0.172 M NaCl. The order of the salts, with respect to the fragility of human red cells in them, is Li > K ≧ Rb > Cs > Na, which is not the order of either the hydrated or the crystal radius of the ions.[1]

[1] The introduction of corrections for differences in the activity coefficients makes matters worse instead of better, for the order of the activity coefficients is Li > Na > K > Rb > Cs, and LiCl, the strongest electrolyte, is the one which is isoplethechontic

2. *The Tonicity-Volume Relations in Hypotonic NaCl and in Hypotonic LiCl Systems*

The tonicity in which a red cell hemolyzes in a hypotonic system is that in which it reaches its critical volume V_h, a volume determined principally, although not exclusively, by red cell shape. When the quantity of the hypotonic medium is very great, the expression which gives the volume as a function of the tonicity T is

$$V = RW\left(\frac{1}{T} - 1\right) + 1 \tag{1}$$

in which the initial volume V_0 of the cell is represented by unity, in which W is the water in the cell expressed as a fraction of unity, and in which R is a constant which varies from system to system. There are accordingly several ways in which variations in the fragility of a red cell, as measured by the tonicity in which it hemolyzes, can occur. The critical volume V_h itself may vary, the value of R may vary, as when there are changes from one metastable form of the red cell to another (Ponder, 1945), changes in the amount of "bound water," or when there is an escape of osmotically active substances from the cell into the hypotonic medium,[2] or the value of W may vary. The point which is apt to be confusing is that tonicity is classically defined in terms of the volume of the cells of the system under consideration, a solution being isotonic with plasma when it maintains the same cell volume as plasma does, *i.e.*, when it is isoplethechontic with plasma;[3] we are not entitled to expect, however, that the freezing point or the activity of water will be equally depressed in all isoplethechontic solutions unless the red cell can be represented by a model of a special kind.

From a technical point of view, the analysis of the difference between the volume-tonicity relations in hypotonic NaCl and in hypotonic LiCl involves the simultaneous measurement of V_h, R, and W.

Measurement of R and W.—The red cells of human heparinized blood are washed 3 times with 0.172 M NaCl, and are then suspended (*a*) in 0.172 M NaCl and (*b*) in 0.172 M LiCl, in such proportions that the volume concentration of the cells is 0.4.

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with plasma when present in the highest concentration (equimolar with 1.1 gm./100 ml. NaCl). NaCl, a weaker electrolyte, is isoplethechontic with plasma in a concentration of only 0.93 gm./100 ml.

[2] An escape of K from the red cell can be held responsible for a change in the value of R only if the escape is rapid, and only if it is not compensated for by an entry of an equivalent amount of Na. This point has been fully discussed elsewhere (Ponder, 1948 *b*).

[3] This word ("volume maintaining") was introduced by Ponder and Saslow (1930) to avoid the ambiguity associated with the word "isotonic," which is often used without the realization that isosmotic solutions are not always isoplethechontic.

A series of solutions of NaCl and LiCl, varying in tonicity from $T = 1.0$ to $T = 0.4$, are prepared, a 0.172 M solution of each salt being considered, for the time being, as having a tonicity of 1.0; the hypotonic solutions are made by the addition of water. To 2 ml. of each is added 0.5 ml. of the appropriate red cell suspension (red cells suspended in isotonic NaCl being added to the series of NaCl solutions, cells suspended in isotonic LiCl being added to the series of LiCl solutions). After standing for 5 hours at 25°C., with occasional mixing by inversion, small volumes of each system are transferred to Hamburger hematocrit tubes, in which the relative cell volumes, together with the amount of hemolysis, if any, are determined (*cf.* Ponder, 1948 *c*).[4]

The expression for the volume of the red cell, regarded as an osmometer of initial volume $V_0 = 1.0$ and immersed in a limited volume of a medium of tonicity T,[5]

$$V = \frac{RW(a - aT)}{(aT + 1)} + 1 \tag{2}$$

can be rewritten as

$$V = RW\left(\frac{1}{T + 1/a} - \frac{T}{T + 1/a}\right) + 1 = RW \cdot f(T, a) + 1 \tag{3}$$

where a is the ratio of the volume of the surrounding medium to the volume of the cell water. This expression becomes identical with expression (1) when a is infinitely great; when a has a comparatively small value, a series of values of $f(T, a)$ is calculated and used to replace values of $1/T$ (see abscissa of Fig. 1). If the red cell can be treated as an osmometer, a straight line will result when values of V are plotted against values of $f(T,a)$; this line will pass through the origin $T = 1.0, f(T,a) = 0, V = 1.0$, and its slope will be RW. The value of R can be calculated from the slope RW of the straight line when the value of W is known. It can be found by drying a small mass of red cells to constant weight at 60–80°C.

As the tonicity is reduced, a value $T_{h(0)}$ is finally reached at which the least resistant cell in the system hemolyzes. The volume which corresponds to this critical tonicity

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[4] These determinations are made by spinning with a force of 2.7×10^3 G, maintained for 30 minutes. Differences in the centrifugal force applied (and, to a minor extent, differences in the duration of spinning) result in differences in the value of R as well as in differences in the absolute lengths of the columns of packed cells. The most likely explanation for this is that the packing forces change when the cell undergoes swelling and decreases in density. A reduction of the centrifugal force to 0.7×10^3 G results in an increase of from 0.1 to 0.15 in R. I have not been able to obtain the high values of R (average value of 0.98) found by Guest (1948) for human red cells in hypotonic NaCl, except at relatively low rates of spinning. Recently I had the experience of obtaining, in a succession of determinations, R values between 0.85 and 0.95 instead of the usual 0.7 to 0.8 (systems of human red cells in hypotonic NaCl). This was traced to the hematocrit motor needing oil.

[5] The addition of the 0.5 ml. of suspension, which contains 0.3 ml. of NaCl or of LiCl of a tonicity of 1.0 (0.172 M), raises the tonicity of the hypotonic medium to which the cells are added, so that the final tonicities in the series are 1.0, 0.913, 0.826 . . . (common difference 0.087) instead of 1.0, 0.9, 0.8 . . . (common difference 0.1). It is the tonicity of the mixture which is denoted by T.

is $V_{h(0)}$, the critical volume for the least resistant cell in the system. If the tonicity is reduced further, there is hemolysis in the system and we are concerned with the volume of the cells which remain intact; this is $V/(1-p)$, where p is the amount of lysis in the system ($p = 1.0$ for complete hemolysis). The tonicity in which $p = 0.5$ is the tonicity $T_{h(50)}$ with its corresponding critical volume $V_{h(50)}$ for the cell of average resistance. The true critical volumes are $V_{h(0)}/V_p$ and $V_{h(50)}/V_p$, for V_p, the volume of the cells in plasma, is not generally equal to the volume in the solution which has the tonicity denoted by 1.0 (see below).

Adjustments near the Origin.—In some types of experiment, it may be desirable to compare V_0 with a special value of V, the volume V_p of the same number of red cells in plasma. If $V_0 = V_p$, the salt solution of tonicity $T = 1.0$ is isotonic (isoplethechontic) with plasma. If the salt solution of $T = 1.0$ is not isoplethechontic with plasma, V_0 will equal $V_p + \Delta W$, and ΔW, which can be either positive or negative, will be the amount of water which enters (or leaves) the cell when it is transferred from plasma to the solution for which T has been put equal to 1.0. There will be a point on the linear tonicity-volume relation which has coordinates V_p and a special value of $f(T, a)$; from this special value can be calculated a value of T which can be used as a number by which the concentration of the salt solution under consideration must be multiplied in order to give a salt solution isotonic with plasma.

When the salt solution of $T = 1.0$ is not isoplethechontic with plasma, two consequences follow upon the taking of $T = 1.0$ as the origin of the coordinates of the tonicity-volume relation. The first is that red cells immersed in the solution of $T = 1.0$ (supposedly isoplethechontic with plasma, but not really so) will gain (or lose) water; this will change the slope of the line relating V and $f(T,a)$ from RW to RW_1.[6] If V_p is less than V_0, the cell will appear to behave as a better osmometer than it really is; alternatively, if V_p is greater than V_0, the cell will appear to behave as a poorer osmometer than it really is. The second is that if the cells begin to hemolyze at a critical volume V_h, and if the ratio V_h/V_0 is calculated from the value of V_0 found in the solution of tonicity 1.0 (not isoplethechontic with plasma) instead of from V_h/V_p, the critical volume will appear to be spuriously small in relation to the initial cell volume if V_p is less than V_0 and spuriously great if V_p is larger than V_0. Serious discrepancies can be introduced by an error of this kind.[7]

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[6] $W_1 = (W \pm \Delta V_0)/(V_0 \pm \Delta V_0)$.

[7] It is not the purpose of this paper to discuss the concentrations of NaCl and of other salts which have been found to be isoplethechontic with normal human plasma collected under oil. This aspect of the problem has been dealt with by Christensen and Warburg (1928) and by Kirk, Sorensen, Trier, and Warburg (1941); the concentration of NaCl which they found to be isoplethechontic with plasma is about 0.150 M. Other values found, usually with fewer precautions as regards preventing the escape of CO_2, vary from 0.145 M to 0.190 M. The pH of NaCl solutions is generally less than that of plasma and particularly of plasma which has been exposed to air, and so it is to be expected that the cation content of an NaCl solution isoplethechontic with plasma would be somewhat greater than that of plasma itself. The point which this paper emphasizes is that the cation content of a LiCl solution isoplethechontic with plasma would be greater still, the difference between the concentrations of NaCl and of LiCl not being attributable to differences in pH.

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