LETTERS TO THE EDITOR

[Brief letters to the Editor that make specific scientific reference to papers published previously in THE JOURNAL OF GENERAL PHYSIOLOGY are invited. Receipt of such letters will be acknowledged, and those containing pertinent scientific comments and scientific criticisms will be published.]

Osmotic Behavior of Hemoglobin In Vivo and In Vitro

Dear Sir:

In 1964 Savitz, Sidel, and Solomon (1964) produced evidence that the relation of red cell volume to the reciprocal of the external osmotic pressure was linear in a range from 480 to 192 milliosmols. From Savitz, Sidel, and Solomon's results, it may be calculated that the mean fraction of cell water which is apparently osmotically active was 0.81 over the hypertonic ranges of their experiments, and 0.80 over the hypotonic ranges. The hypertonic value is in agreement with some previous results, notably those of Ørskov (1946) and Lefevre (1964), and may probably be accepted (although Sidel and Solomon (1957) and Villegas, Barton, and Solomon (1958) obtained various values, 0.64 in man, 0.97 in the dog, and 0.55 in beef, which are not quoted by Savitz et al. [1964]). However, the hypotonic result is in conflict with many previous studies, which give values in the range 0.90 to 0.99 (Guest and Wing, 1942; Ponder, 1944; Ørskov, 1946; Guest, 1948; Ponder, 1950; Hendry, 1954; Dick and Lowenstein, 1958). Savitz et al. suggested that the discrepancy in the case of Dick and Lowenstein's (1958) results might be due to the immersion refractometry method employed by the latter authors; it was supposed that this might measure only the osmotically active water instead of the total cellular water content. However, the specific refraction increment of hemoglobin, which formed the basis of the refractometry method, was measured by Stoddard and Adair (1923) and Roche et al. (1932) in terms of the concentration of dry hemoglobin per unit volume of solution. The solid and water concentrations measured by the refractive index method are therefore the same as those obtained by drying the cells and lead to values of relative cell volume (and of apparent volume of osmotically active water derived from them) identical with those estimated by the hematocrit or other techniques.

In a recent paper Gary-Bobo and Solomon (1968) reported that the fraction of apparently nonosmotic water (column 6 of Table II) varied with tonicity even when referred to a single reference osmolality, π_i, being less over hypotonic ranges than over hypertonic ranges. They nevertheless claimed (footnote to Table II) that the results were consistent with the linearity of V plotted against $1/\pi$, which was described by Savitz et al. (1964). This is evidently impossible since from their equation

$$V = W_{\text{eff}}(\pi_i/\pi) + b^1,$$

it follows that $W_{eff} = 1/\pi_i \cdot dV/d(1/\pi)$. Thus if the fraction of nonosmotic water ($1 - W_{eff}$) and hence the fraction of osmotically active water, W_{eff}, vary when π_i, the reference osmolality, remains the same, $dV/d(1/\pi)$ must vary and thus the plot of V against $1/\pi$ cannot be linear as described by Savitz et al. In fact the results of Gary-Bobo and Solomon are inconsistent with those of Savitz et al. They confirm the difference between the values of apparent nonosmotic water in hypertonic and hypotonic solutions which was first shown by Davson (1936) and Ørskov (1946), and further documented and accounted for theoretically by Dick (1966).

The results of Gary-Bobo and Solomon (1968) may be compared in detail with those of Dick and Lowenstein (1958). Since Dick and Lowenstein for technical reasons used acid solutions of bovine plasma albumin (pH 5.6 or 6.9) for their refractive index measurements, although they used an isotonic reference point for π_i, this would actually correspond with a somewhat hypotonic reference point at pH 7.4 owing to the erythrocyte swelling caused by acid pH. Thus, to obtain a comparable fraction of osmotically active water from Gary-Bobo and Solomon's data at pH 7.4, their value of the apparent osmotically active water content of the erythrocyte in hypotonic solutions must be divided by the total cell water *at the same osmotic pressure as the reference osmolality*. Such a calculation gives an osmotically active water fraction of 0.90 at 192 milliosmols and 0.91 at 194 milliosmols. Gary-Bobo and Solomon's other values for the apparent osmotically active water at low pH (their Fig. 5) are larger still and even exceed the total water below pH 6.7; it seems that low pH has the same effect as hypotonicity owing to the swelling produced. These results agree better with the value of 0.95 obtained by Dick and Lowenstein than they do with the value of 0.80 calculated from Savitz, Sidel, and Solomon's results in the hypotonic range. As already shown (Dick and Lowenstein, 1958; Dick, 1966; Dick, 1967), the value 0.95 is consistent with Adair's (1929) data on the osmotic behavior of hemoglobin in vitro at concentrations and ionic strength comparable to those for erythrocytes in isotonic or hypotonic solutions.

Gary-Bobo and Solomon suggest that the apparent decrease of the osmotically active water content in the hypertonic range might be related to a change in the charge on the hemoglobin molecule with change of concentration. An alternative empirical explanation may, however, be of interest (Dick, 1967). Some previously unpublished data of Adair (1967) show that the osmotic coefficient of hemoglobin rises more steeply with concentration when the ionic strength is raised. This implies that osmotic data on hemoglobin at low ionic strength, such as those of Adair (1929) which were used by Gary-Bobo and Solomon, are not appropriate for in vitro comparison with the behavior of hemoglobin in vivo in the shrunken erythrocyte in which the ionic strength is high. The higher osmotic coefficient of hemoglobin, and the greater rate of its increase at high ionic strength, may provide an explanation for the large fraction of apparent nonosmotic water in the shrunken erythrocyte.

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