

DIFFRACTOMETRIC MEASUREMENTS OF THE TONICITY-
VOLUME RELATIONS OF HUMAN RED CELLS IN
HYPOTONIC SYSTEMS

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When red cell volume is measured by a high speed hematocrit, the relation between the volume V and a special function of the tonicity T , $f(T, a)$, is not the linear one expected on the basis of the van't Hoff-Mariotte law which would apply if the cells were perfect osmometers. The slope of the line is usually too small, its value being, not the water content W of the cell, but RW , where R is a constant which is usually between 0.75 and 0.9. Further, when a fraction p of the cells of the system are hemolyzed and when the volume $V/(1 - p)$ of the $(1 - p)$ cells which remain are considered, the departure from the linear relation becomes very pronounced; there are both upward and downward departures, the former occurring when p is small and particularly when the volume concentration of the cells in the system is large, and the latter being usually observed when p is large and in systems containing a small volume concentration of cells (Ponder, 1950 *a*).

One of the explanations for the upward departures, which are due to the apparent volume of the intact cells being larger than expected, is that the column of intact cells is really a column of intact cells plus ghosts of varying degrees of rigidity. This hypothesis has been useful in that it has led to a reexamination of some of the properties of red cell ghosts (Ponder, 1950 *b*). It has been shown that, in hypotonic systems in which the initial volume concentration of the cells is relatively high (>0.1), the ghosts are partially rigid, that they have considerable volume (as much as 0.5 that of the red cell), and that they may contain a higher concentration of Hb than that in the medium surrounding them; it is only when the volume concentration in the system is very small that the volume of the ghost becomes negligible and that the concentration of the contained Hb becomes the same as that in the surrounding medium ("all-or-none hemolysis"). This partial rigidity and the ability to retain surplus Hb are also properties of ghosts in systems containing saponin or the bile salts, and are functions of the lysin concentration.

The properties of ghosts in hypertonic systems are therefore those which are required by the hypothesis which attributes the upward departures from the expected linear relation between $V/(1 - p)$ and $f(T, a)$ to the presence of

rigid ghosts. To this extent the hypothesis is supported, but one would like to test it more directly. This may be done by measuring the cell volume both by hematocrit and by diffraction. Since partially rigid ghosts can contribute to the volume as measured by the hematocrit, but are most unlikely to contribute to the volume as measured diffractometrically, measurements made with the hematocrit should show the upward departures from linearity if the latter are due to the presence of partially rigid ghosts, while measurements made diffractometrically should show much smaller departures or none at all.

Methods

The diffractometric measurements of the volume of the intact red cells in systems of decreasing tonicity were made by the method described by Cox and Ponder (1941). In this method, the cells are rendered spherical by being placed between slide and coverglass.

Some modifications have been made in the diffractometer and in the photometer used for finding the positions of the maxima and minima. The camera lens of the diffractometer has been changed for one of shorter focal length (10.2 cm.); this reduces the size of the rather large diffraction rings produced by cells in the spherical form, and brings them nearer to the centre of the plate. The diffractometer is mounted in a dark room, the light source entering from the Hg arc through an aperture in the wall. This aperture carries a filter for the Hg green line and an electrically operated shutter. A G.E. 100 watt Mazda A-H4 type of Hg arc, which operates with an auxiliary autotransformer (51G96) has been substituted for the arc originally used. Both the light source and the diffractometer are bolted to frames on the wall, one outside the dark room and the other inside, to ensure rigidity; the frame which holds the diffractometer has a set of centering screws by means of which alignment can be secured and maintained.

The Lange thermopile photometer used for the finding of the positions of the maxima and minima has been replaced by an electronic photometer (Photovolt Corporation model 512). The search unit of this photometer fits into the drawtube of the microscope in the moving stage of which the photographic plate is held. The source of illumination is a 100 watt microscope lamp with a voltage stabilizer and a variac in the lamp circuit; the variac is very convenient as a means of adjusting the intensity of the illumination, and the voltage stabilizer is essential. The positions of the first minimum and the first maximum are found with this assembly in exactly the same way as with the assembly previously described, but with much greater convenience and with a somewhat increased precision.

The tonicity-volume relation as obtained by high speed hematocrit was plotted as already described (Ponder, 1950 *a*), dense systems (2 ml. of NaCl-buffer at pH 8.5 and of varying tonicities, plus 0.5 ml. of a thrice washed human red cell suspension of volume concentration 0.4) being used throughout. The preparations for diffractometric measurement of volume were made from the same systems as were used for the measurement of volume by the hematocrit. A pH of 8.5 was selected because the diffractometric measurement of volume depends on the cells becoming spherical when placed between slide and coverglass; the disk-sphere transformation

occurs rapidly and completely in NaCl-buffer at pH 8.5, and all preparations were examined before the photographs of the diffraction patterns were taken, in order to be sure that they contained only smooth spherical forms.¹

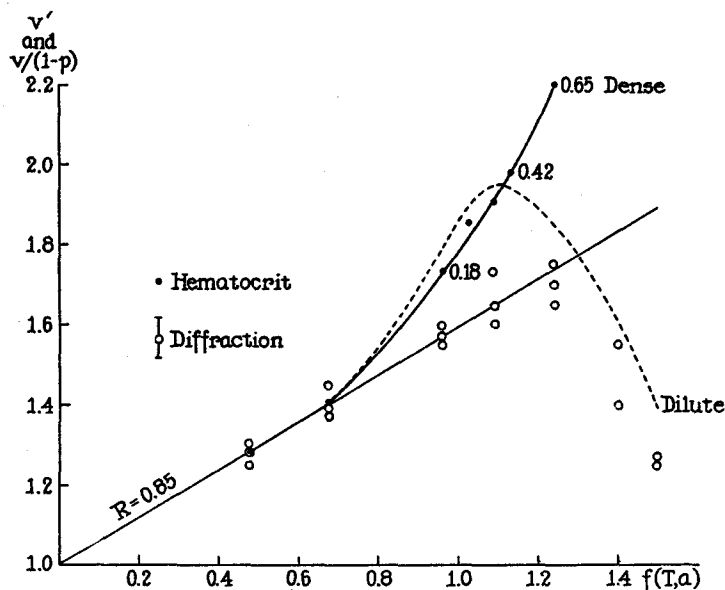


FIG. 1. Ordinate, volume of intact red cells relative to the initial cell volume; abscissa, a function of the tonicity T , (nearly equal to $1/T-1$). The straight line shows the relation expected on the basis of the van't Hoff-Marriott law (except that the value of R is less than 1.0). Solid points, results of determination of volume in dense systems with the hematocrit; circles, results of diffractometric determinations. The dotted curve shows the type of result usually found with the hematocrit in dilute systems. For further explanation see text.

RESULTS AND DISCUSSION

The results of the hematocrit and diffractometric determinations are best presented graphically. Fig. 1 shows the relative red cell volume plotted against $f(T, a)$; in the case of the hematocrit determinations, the volume is $V/(1 - p)$,

¹ This opportunity may be taken to correct 3 typographical errors which have occurred in papers referred to. The areas scanned by the high power objective are about 300μ , not 30μ , in diameter (Cox and Ponder, 1941). In transcribing Equation 3 from Ponder, 1949, to Ponder, 1950 *a*, Equation 1, the term $+1$ has been omitted from the right-hand side. The illumination at any point in the diffraction pattern is given as I , a function of $R^2 \cdot \frac{2}{m} (J_1(m))$; it should be I^2 , the square of this quantity (Ponder, 1929, and several subsequent papers into which the error is copied).

the volume of the $(1 - p)$ cells which remain intact in a system in which a fraction p has hemolyzed, and in the case of the diffractometric determinations it is V' , the mean volume of the intact red cells which give rise to the diffraction patterns. The volumes obtained by diffraction (circles) are those calculated from 3 sets of photographs of 3 sets of preparations, the value of V' being reproducible to about ± 0.05 .

The straight line is a theoretical relation between V' or $V/(1 - p)$ and $f(T, a)$ which would be expected if the value of RW were 0.6 (see Ponder, 1950 *a*); as is usual in experiments of this kind, this value is smaller than that of W , the water content of the cell expressed as a fraction of unity; *i.e.*, R is less than 1.0 and in this case is 0.85. When $f(T, a)$ is greater than about 0.7, *i.e.* when T is less than about 0.6, the volumes found with the hematocrit show an upward departure from the linear relation; the diffractometrically measured values, on the other hand, continue to lie very well along the line until, as T becomes as small as 0.4 and the system shows about 50 per cent hemolysis, the volumes become smaller again, just as they do in systems in which the volume concentration of red cells is small (dotted curve, hematocrit determinations taken for illustrative purposes from Ponder, 1950 *a*, Fig. 2; also *cf.* Guest and Wing, 1939, 1942).

Results similar to these have been obtained in each of seven satisfactory experiments with the red cells of different individuals; some of the experiments being less complete, however, than the one illustrated by Fig. 1, in that only one set of photographs of diffraction patterns was measured instead of 3 sets.² All the experiments agree in showing (1) that the upward departures from linearity met with when the volumes of the intact cells are measured with the hematocrit are not found when the mean volume of the cells is measured diffractometrically; (2) that the diffractometric measurements show the downward departures from linearity when T is small, although the tonicity at which these downward departures occur varies considerably (from 0.40 to 0.46), as does the extent of the downward departure itself; and (3) that the value of R is always less than 1.0, the best value usually being between 0.75 and 0.85.

There is therefore strong evidence that the upward departures from linearity observed when the volume measurements are made with the high speed hematocrit are due to the inclusion of partially rigid ghosts in the column of packed red cells, and that the best explanation for the downward departures found at low tonicities is that in these tonicities the rigidity of the ghost is much re-

² Attempts have been made to eliminate the troublesome photographic step by applying the search unit directly to the plane LL of the diffractometer. The intensity of the patterns is so low that greater amplification than that given by the model 512 photometer would be required for the direct measurement of the positions of the maxima and minima. Instability has resulted from attempts to obtain greater amplification, probably because of small variations in line voltage.

duced, while at the same time the cells which remain unhemolyzed are cells characterized by a small critical volume and also by a small value of RW (Guest and Wing, 1939, 1942; Ponder, 1950 *a*).

SUMMARY

Red cell volumes in media sufficiently hypotonic to produce partial hemolysis have been measured with a high speed hematocrit and by an improved diffraction method. A comparison of the results shows that the upward departures from the linear relation based on the van't Hoff-Mariotte law are not observed when the volumes are measured diffractometrically. Downward departures are observed at low tonicities by both methods. These results provide strong evidence that the upward departures are due to the inclusion of semirigid ghosts in the column of packed red cells which is measured when the volumes are found with the hematocrit.

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