CCLII. THE ESCAPE OF POTASSIUM FROM RABBIT RED CELLS INTO HYPOTONIC SOLUTIONS.

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RECENTLY it has been shown, by a number of methods, that the volumes attained at equilibrium by mammalian red cells in hypotonic solutions are less than those which would be expected if the cell were a "perfect" osmometer, containing that amount of osmotically active water which it is known to contain and possessing a membrane impermeable to cations [Ponder and Saslow, 1930, 1, 2; 1931; Macleod and Ponder, 1933; Ponder and Robinson, 1934]. This result can be accounted for in several ways, but the best explanation is that a loss of salts occurs from the cell into the hypotonic medium. The extent of this loss, which varies with the way in which the suspension of cells is treated, can be calculated from volume measurements [Ponder and Saslow, 1931], but although both Kerr [1929] and Davson [1934] have shown leakage of Na and K actually to occur, their data are not sufficient to enable us to tell whether the leakage observed is of the right magnitude to account for the volume changes. Further, both Kerr and Davson used ox erythrocytes, whereas the most reliable volume determinations have been made with rabbit cells, and Kerr's determinations of leakage were made after the cells had been standing in hypotonic solutions for several hours. Our idea is, not only that the cells may lose osmotically active substances on standing, but that they do so in the very short space of time during which they are coming to equilibrium with the hypotonic solution. This Jacobs and Parpart [1933] seem to doubt, although they admit the possibility of loss of salts on standing, at least in the case of some kinds of red cell. They believe that they have shown, by an indirect method, that there is very little loss of salts from the ox cell, if, indeed, there is any at all, a point of view which is almost untenable in the face of Kerr's and Davson's analytical data. What is required to make the idea of cation impermeability in hypotonic solutions wholly untenable is a demonstration (*a*) that the quantity of salts lost by the cell, and detected by analysis, is substantially the same as that predicted from the volume measurements, and (*b*) that the volumes from which the calculations are made are attained, not after hours of standing, but within a few seconds. It would then be difficult to escape from the conclusion that the loss of salts occurs during that short time when the cell is swelling to equilibrium. This paper is concerned with such demonstrations.

1. *The loss of potassium.*

In these experiments we have availed ourselves of the fact that the rabbit erythrocyte contains no sodium, and that it can be suspended in NaCl which is K-free. The cations lost from the cell into a hypotonic solution can then be determined as K.

The blood is either oxalated or defibrinated in such a way that no haemolysis occurs (with a glass rod and without touching the sides of the beaker). The

freezing-point of the plasma is obtained by Johlin's [1931] method, and a solution of NaCl of equal freezing-point prepared. With this the cells are washed twice, and a suspension is prepared of about the same volume concentration as that of the original blood. The percentage volume occupied by the cells is found by means of a high speed haematocrit (12,000 r.p.m.). About 10 ml. of this suspension are delivered into a centrifuge-tube and the cells spun down, and the supernatant fluid is drawn up into a small separating funnel, the stem of which has been drawn out into a capillary and turned back upon itself for a short distance at the tip. To this is added enough water to bring the tonicity to about 0·6 % NaCl: the contents of the funnel are then run back into the centrifuge-tube, which is thoroughly shaken. This is the only method which we have found by means of which the tonicity of the suspension medium can be sufficiently reduced without haemolysis occurring. The tonicity of the suspension medium is, of course, a little higher than 0·6 after mixing is complete. The suspension is allowed to stand for 10 minutes.

A second haematocrit determination is now made on this diluted system, and from the two haematocrit determinations the amount of swelling, the exact tonicity, and the apparent quantity of osmotically active water in the cell are calculated [see Ponder and Saslow, 1931; Ponder and Robinson, 1934]. The quantity of water actually present in the cell is determined by weighing packed cells before and after drying, with suitable corrections for the incompleteness of packing [see Macleod and Ponder, 1933]. The cells are thrown down from the rest of the suspension, and the supernatant liquid is drawn off for K determinations. At the same time a quantity of the original suspension (isotonic) is similarly treated: in this way we determine the amount of potassium present in an isotonic NaCl solution surrounding the cells and that present in a hypotonic solution from which the cells have gained water. The method used for determining K was that of Kramer and Tisdall [1921], as described by Peters and Van Slyke [1932].

The results can be expressed in tabular form. The quantities of K lost are expressed as percentages of the total quantity of K present in the cells used in the experiment, assuming each ml. of cells to contain 5·2 mg. of K. In this way the experimental results are most easily compared with the values calculated from Ponder and Saslow's equations. The last column of Table I gives the quantity of K, again expressed as a percentage, lost from the cells into the isotonic NaCl in which the suspension was originally prepared.

Table I.

Tonicity	R	K lost determined	K lost calculated	K lost isotonic NaCl
0·68	0·88	2·1	4·0	0·5
0·68	0·89	4·3	3·5	0·5
0·68	0·77	5·1	7·5	0·3
0·68	0·83	6·8	5·5	—
0·68	0·80	3·0	6·5	—

It is clear that the quantity of K lost by the cells is of the order required by Ponder and Saslow's equations, but there are several points to be specially remarked upon. (1) The R values are rather high, particularly as oxalated blood was used in all the experiments except the first. After a considerable experience with the various kinds of methods used, we think that the R values always tend to be higher when the cell suspension is concentrated than when it is dilute, and that there must be some factor, at present unrecognised, which accounts for this. (2) While the experimental and the calculated values for the K loss are of

the same order, they do not agree very well. The discrepancy is certainly not due to error in the analyses, for all duplicates agreed to within 8 %. There are at least two possible explanations of the discrepancy. The first is that the equations from which the K loss was calculated are based on premises too simple in nature. The second is that the loss of K which occurs at equilibrium may be affected by the leakage of K which occurs even into the isotonic NaCl, this being presumably associated with a Na-K exchange. Davson also has observed that a loss of K occurs (from ox cells) into isotonic NaCl, and even into undiluted serum; if a Na-K exchange actually occurs, the result might well be that the osmotically active substance lost by the cell into a hypotonic solution is not K alone, but also Na, which was not tested for. The initial "isotonic" conditions are, in fact, so much in doubt that we cannot expect a better agreement than has been obtained, particularly as all the methods are being used at the extreme limits of their accuracy.

2. *The rate at which the equilibrium volume is reached.*

In the foregoing experiments, the volumes found were those occupied by the cells after 10 minutes' standing in a hypotonic solution, and it is possible, so far as these experiments alone are concerned, that the salt loss which corresponds to the abnormally small volumes took place gradually over the same length of time. The equations of Ponder and Saslow, however, require that the loss of osmotically active substances shall take place within the time during which the cell is swelling to its equilibrium volume, *i.e.*, that the loss should be very rapid indeed, taking probably only a few seconds. It is obviously impossible to determine K analytically with sufficient rapidity to show that this is so, but we can show that the equilibrium volume from which the extent of the loss of K can be approximately calculated (see above) is attained within 15 seconds, and from this we can infer that the loss of K occurs within this time.

The method is as follows. A series of NaCl solutions is prepared, of such tonicities that tonicities of 0·5, 0·6, 0·7, *etc.*, result when 1 ml. of a suspension of washed cells in isotonic NaCl, of volume concentration about 40 %, is mixed with 6 ml. of the solutions. One such solution is taken and 1 ml. of the suspension added: the mixture is then allowed to stand for 15 minutes. At the end of this time the volume of the cells is measured diffractometrically [Ponder and Saslow, 1931]. After the measurement has been made, the setting of the diffractometer is left unchanged. The rapid measurement requires two persons. One blows from a pipette 1 ml. of the red cell suspension into 6 ml. of the same NaCl solution and then shakes the tube violently. The other takes a small sample from the tube with a capillary pipette and runs a small drop between a slide and a cover-slip, previously placed on the stage of the diffractometer. As quickly as possible, he looks through the telescope at what is at first a field in which no diffraction patterns are visible. As the cells turn into spheres between the slide and cover-glass, the diffraction patterns begin to appear: at first they are indistinct and shifting from smaller angles to larger ones, but they soon assume a brilliant appearance and come to rest. When they do so, they occupy the same position (which the observer can judge by their relation to the cross hair, left in the same position as for the former reading) as those obtained when the cells had stood for 15 minutes in the solution of the same tonicity. As it usually takes about 15 seconds, from the moment of mixing, for sharp patterns to be observed, this means that the volume of the cells is the same after 15 seconds as it is after 15 minutes.

The limiting factors in experiments of this kind are the time which it takes to mix the cells with the hypotonic solution, to transfer the drop to the slide and

cover-glass, and for the cells to assume the spherical form. We have, however, been able to make measurements of the equilibrium volume in as short a time as 13 seconds, and Table II shows the kind of result obtained. The volumes are expressed as percentages of the volume of the cell in isotonic NaCl.

Table II.

Tonicity	Volume 15 mins.	Volume in time t	Time t after mixing, secs.
1·0	100	—	—
0·7	113	113	16
0·6	124	124	13
0·5	137	137	15

The swelling curve obtained by plotting volume against tonicity is one for an "imperfect" osmometer, with an R-value of between 0·5 and 0·6, and the curve is the same whether we measure volume after 15 seconds or after 15 minutes. If, as Jacobs and Parpart are inclined to think, the cells swell as "perfect" osmometers, and the loss of K which can be detected analytically after some 10 minutes occurs gradually as a result of the cells being in a hypotonic solution, we would have the following extraordinary state of affairs. In some time less than 13 seconds, the cells would require to swell to about twice the volumes given in Table II (*e.g.*, in a tonicity of 0·5, to about 165 % of their initial volume), and then they would require to shrink again in order to take up the volumes shown in the table at the end of about 15 seconds. Either during this hypothetical period of shrinking, or thereafter, they would also require to lose the K which is detected analytically in the suspension medium, and yet their volume would have to remain unchanged from 15 seconds after mixing to at least 15 minutes after mixing. It is far more probable that the K is lost in that short time during which the cell is taking in water and assuming its equilibrium volume, and that it is the K lost during this short time which is later detected in the suspension medium.

SUMMARY.

1. When rabbit red cells are suspended in hypotonic NaCl, K is lost from the cells, and the quantity which escapes is of the right magnitude to account for the observed volume changes.

2. The cells reach their equilibrium volume in a hypotonic solution within 15 seconds, and it is presumably within the time during which they are swelling to equilibrium that the loss of K occurs.

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