THE SPHERICAL FORM OF THE MAMMALIAN ERYTHROCYTE

III. CHANGES IN SURFACE AREA IN DISKS AND SPHERES

By ERIC PONDER

From The Biological Laboratory, Cold Spring Harbor

(*Received* 26 *November* 1936)

In a number of papers, I and my collaborators have considered various methods for measuring the volume of mammalian red cells, either in the discoidal[1] or in the spherical form (Ponder & Saslow, 1930*a*, *b*, 1931; Macleod & Ponder, 1933; Ponder & Robinson, 1934; Ponder, 1935*a*, *b*). Some of these methods, such as the diffraction method, are applicable to spheres only, and others measure only changes in volume such as occur in hypotonic media. So far as this paper is concerned, the two most important conclusions of the investigations are (*a*) that the transformation from disk to sphere, whether effected by the addition of lecithin or one of the photodynamic dyes (Ponder, 1936), or by enclosing the cells between two closely opposed surfaces (Ponder, 1928–9), is accompanied by no change in volume, and (*b*) that at the "critical tonicity" in which haemolysis begins in hypotonic media, the percentage increase in volume is substantially the same irrespective of whether the cells are disks or spheres.

The cell shape thus seems to be of no great consequence so far as volume changes are concerned, but it is a different matter when we come to consider the changes in area which the cell surface undergoes as the volume increases. When placed in a hypotonic solution, a discoidal form can swell considerably by a turning inside out of the biconcavities or by assuming a cup-shaped form, and it can approach, or even reach, the "critical volume" for lysis (some 130–150 per cent of the initial volume) without increasing in area at all; Jacobs (1930), indeed, has always assumed that the area remains substantially constant as the volume increases, and certain facts in connexion with the action of lysins point in the same direction (Ponder, 1937). In the case of the spherical form, on the other hand, increase in volume must be accompanied by increase in area, i.e. the cell membrane must undergo "stretching". The spherical form, however, is derived from a discoidal form in the first instance, and as the disk turns into the sphere of the same volume, the surface is diminished; the "stretching" which has to occur in a hypotonic solution might therefore involve no greater area increase than that which would bring the area up to that of the original disk once more. Virtually nothing is known about these matters, but their bearing on the structure of the red cell membrane is obvious.

[1] The distinction between the discoidal form and the spherical form is made without respect to the fact that in many cases the discoidal forms may be crenated, and therefore far from typical biconcave disks.

I. CHANGES OF AREA ACCOMPANYING VOLUME CHANGES OF SPHERES

Let us follow the changes in area and volume which occur if we start with a disk in an isotonic solution, convert it into a sphere in isotonic solution, and then make the solution hypotonic so that the sphere swells and finally reaches the critical volume for haemolysis. If the disk is in serum or plasma, or in an isotonic solution which maintains the normal discoidal form, the area can be found by photographing the cell on edge and applying Pappus's theorem (Ponder, 1929), and the volume can be found either by the colorimetric method (Ponder & Saslow, 1930*a*), the haematocrite method, or the conductivity method (Ponder, 1935*b*), together with a sufficiently accurate red cell count. Let us take the rabbit erythrocyte as an example, and suppose that the mean volume of the disk is $58\mu^3$ and its mean area $105\mu^2$. If the disk is in serum or plasma, it can be converted into the spherical form by the addition of lecithin (Ponder, 1936), and if it is in isotonic NaCl, Ringer, or glucose, it can be rendered spherical either by placing it between a slide and cover-glass (Ponder, 1928–9), or by adding one of the photodynamic dyes, preferably rose bengal (Ponder, 1936). The radius of the sphere can then be measured photographically or diffractometrically (Ponder, 1933*a*) and the volume and area found by calculation. The transformation involves no volume change, so the volume is still $58\mu^3$, and the corresponding area, as the cell is a sphere, is $72\mu^2$.

The change from disk to sphere thus involves a $33\mu^2$ reduction in the area of the cell surface, and we may ask how the cell membrane adapts itself to this reduction.[1] There are three possibilities: (1) The first is that the membrane may become thicker as the area decreases. If we think of it as being a layer two molecules thick (Fricke, 1925–6; Gorter & Grendel, 1928), this is certainly unlikely, but to test the hypothesis we can appeal to the same kind of evidence (capacity measurements) as that which points to the normal membrane being bimolecular. Such results as have been obtained from capacity measurements of disks and spheres do not simplify the situation, for Curtis (1936) has shown that the capacities, interpreted as Fricke interprets them for the discoidal form, indicate that the transformation from disk to sphere is accompanied by a *thinning* of the membrane. This conclusion, together with the results obtained by Schmitt *et al.* (1936) from birefringence measurements, makes me very doubtful of the validity of the calculations by means of which the bimolecular structure of the red-cell membrane is arrived at. (2) As the area of the membrane is reduced, molecules forming part of the membrane may leave it and pass into adjacent regions of the cell envelope. I have recently suggested (Ponder, 1935*a*) that the reverse of this process, i.e. a movement into the membrane of molecules situated in neighbouring parts of the envelope, may occur when the membrane is stretched. This hypothesis was based on two lines

[1] The distinction between the cell *envelope* and the cell *membrane* is a convenient one, although it may turn out to be unnecessary. The envelope is the cell wall as a whole, a structure which is visible when stained, and which has a complex ultra-structure (Schmitt, *et al.* 1936). The membrane is the part of the envelope in which the semi-permeability properties reside, and capacity measurements indicate that it is a layer only a few molecules thick, situated in, or at the surface of, the envelope.

of evidence: (*a*) that derived from capacity measurements, pointing to the stretching of the membrane being unaccompanied by thinning, and (*b*) that arising from a relation between extension of the membrane in hypotonic solutions and the initial volume of the cell. In view of Curtis's recent results, I am inclined to discount this interpretation of the earlier capacity measurements, and the apparent constancy of the ratio (increase in area)/(initial volume) is susceptible of a different explanation (see below). (3) When the area of the cell surface is reduced, the membrane may adapt itself to the reduction by folding, and thus following, more or less, the visible changes which take place in the surface as crenation and formation of the sphere occurs (Ponder, 1928–9). It may be difficult to think of a bimolecular membrane as "crenating", but a folding analogous to crenation is quite possible in a complex structure such as Schmitt *et al.* (1936) have shown to be present at the surface of the erythrocyte. Of the three possibilities, this last one is the simplest and the most satisfactory.

Next consider the stretching of the membrane of the spherical form in hypotonic solutions. In isotonic solution, the spherical form of the rabbit red cell has a volume $V_0 = 58\mu^3$, and an area $A_0 = 72\mu^2$; calling the tonicity of the isotonic solution 1·0, the volume V in a solution of tonicity T, less than 1·0, is[1]

$$V = V_0 [RW(1/T - 1) + 100]/100. \qquad \ldots\ldots(1)$$

In this expression W is the amount of water contained in the cell, expressed as a percentage by volume, and R is a constant which expresses the extent to which the cell behaves as a "perfect osmometer" (see Ponder & Saslow, 1931); the expression also assumes that the volume of the hypotonic medium is very great compared with the volume of the cell water, as it usually is in fragility experiments. As the tonicity is reduced, the volume of each cell increases until it reaches a "critical volume", V_c, at which the cell haemolyses, and measurements of the distribution of the diameters of spherical cells in partially haemolysed systems lead to the conclusion that the critical volume V_c is substantially equal to CV_0, where C is a constant. Thus in the tonicity T_c for commencing haemolysis, or in any tonicity corresponding to any given degree of lysis, both large and small cells haemolyse in the proportion in which they are present in the original distribution of red cell sizes (Ponder, 1935*c*).

The determination of the maximum critical volume which a spherical cell of mean dimensions can attain is not so simple a matter as appears at first sight, for, according to its water content and its perfection as an osmometer, it may reach critical volume and haemolyse in any tonicity from that at which lysis begins to a tonicity much lower. One accordingly proceeds in the following way. Serum is obtained from defibrinated rabbit blood, and is diluted with water so as to give a series of tonicities, from $T = 0{\cdot}56$ to $T = 0{\cdot}30$, differing by 0·02 tonicity unit (the tonicity of the undiluted serum being considered as 1·0). To 1 c.c. of each of these hypotonic sera, and also to 1 c.c. of the undiluted serum, is added 1 drop of the defibrinated blood (weight about 20 mg.); the systems are then allowed to stand

[1] This expression is wrongly printed in Ponder (1935*c*).

for about an hour, at the end of which time the cells are gently thrown down. All stages of haemolysis, from just commencing lysis to complete lysis, are usually found within the range $T=0{\cdot}56$ to $T=0{\cdot}30$.

The cells in each tube are then made spherical by adding lecithin. About 0·2 c.c. of the supernatant fluid is transferred to a watch-glass, and a few milligrams of lecithin is emulsified in it by rubbing. A drop of this lecithinated serum is placed on a slide. Nearly all of the supernatant fluid remaining in the tube is discarded, and the cells are resuspended in the rest; a small drop of this suspension is then added to the lecithinated serum on the slide. The mixture is covered with a cover-glass vaselined at the edges, and a sufficient number of cells (100–200) is photographed as described by Ponder & Millar (1924). The mean diameter of the cells of each system is found by measurement from the plates.

A typical result of this procedure is shown in Table I. The first column gives the tonicity T, the second the mean red-cell volume in μ^3, and the third the mean red-cell volume expressed as a percentage of the initial volume. The fourth column gives the value of R, calculated from expression (1), with a value for W of 60, and the last column shows the mean cell area corresponding to the mean volume.

Table I

T	V, μ^3	V, %	R	A, μ^2
1·00	60	100	—	74
0·50	86	143	0·72	93
0·46	88	147	0·66	95
0·40	93	155	0·61	99
0·36	99	165	0·61	103

In this experiment, just perceptible haemolysis occurred in a tonicity of 0·50, the mean volume of the cells being $86\mu^3$, and their mean area $93\mu^2$. These cells, however, consist of a relatively small number which, because of their large water content and their perfection as osmometers, have almost reached their critical volume in this tonicity, and are about to haemolyse, and a very much larger number which contain less water or which are less perfect osmometers, and which have not approached their critical volume in the tonicity 0·50. (A third group could be added: those cells which have reached their critical volume, and which have disappeared.) The mean volume, $86\mu^3$, is accordingly less than the maximum critical volume, and the mean area, $93\mu^2$, less than the maximum "critical area". As the tonicity is decreased, however, the mean volume and area become greater, and it will be clear that if we could measure the mean volume and area of the cells in a system the tonicity of which was only infinitesimally greater than that required for complete lysis, we would obtain values for the critical volume and the critical area of the cells which were initially of mean size. In practice, one can do this approximately by plotting mean area against tonicity and extrapolating to $T=0{\cdot}32$, the tonicity for complete lysis in this experiment. This gives a critical volume of $105\mu^3$, and a critical area of $107\mu^2$. Thus we arrive at the interesting conclusion

that *the surface of the spherical form can be stretched until it is about the same as that of the disk from which the spherical form was derived originally*, and that if it is stretched more, the cell haemolyses.

II. CHANGES IN AREA ACCOMPANYING VOLUME CHANGES OF DISKS

When the discoidal rabbit erythrocyte, with its initial volume of $60\mu^3$ and its initial area of $105\mu^2$, is placed in a hypotonic medium, its swelling is accompanied by shape changes which are so various that they are difficult to describe exactly. Many of the cells are cup-shaped, while others increase in volume and yet retain their biconcave form, and there is, on the average, a decrease in diameter of about 8 per cent as the volume increases about 45 per cent (Ponder, 1933*b*, 1935*b*). The important point is that each cell becomes perfectly spherical as its critical volume is approached, and shortly before it haemolyses. This makes it very easy to determine the critical volume for any cell, for cells which have reached their critical volume are spherical while others are not; in the case of cells treated with lecithin, on the other hand, all the cells are spherical, and the mean critical volume can be found by extrapolation only (see above).[1]

The mean critical volume and the mean critical area reached by discoidal cells in hypotonic systems is found by suspending the cells in media sufficiently hypotonic to produce various degrees of partial lysis, photographing the cells, and measuring only those which are spherical. The results show (*a*) that when the cells are in hypotonic serum, *the mean critical area is substantially the same as the mean area of the discoidal form in isotonic serum*, i.e. about $105\mu^2$, and (*b*) that when the cells are in hypotonic NaCl, the critical volume and the critical area are less than they are in hypotonic serum. The first conclusion is the same as that reached in the case of spherical lecithinated cells in hypotonic systems, and means that the discoidal form is able to swell from about $60\mu^3$ to about $102\mu^3$, or about 170 per cent, without its surface being stretched; this it does, of course, by undergoing an alteration in shape, so that the surface/volume ratio changes from that for the disk to that for the sphere.

The second conclusion is substantially the same as that reached by Ponder & Robinson (1934), who observed that the mean volume of cells in a tonicity showing just commencing haemolysis is greater if the medium is hypotonic plasma than when the medium is hypotonic NaCl. This point is of importance for two reasons: (1) In studies on the rate at which lysin occurs in hypotonic solutions under different conditions, it is usually assumed, following Jacobs and Parpart, that the critical volume remains unaffected by such factors as temperature, *p*H, etc.; if there is one clear case in which the critical volume can be shown to vary with the properties of the system, however, the possibility of such a variation can never be overlooked. (2) There is a considerable amount of evidence that the properties

[1] The shape changes shown in Fig. 3 of Haden (1934) are purely diagrammatic, and do not correspond to what is seen under the microscope. A symmetrical swelling of the red cell in hypotonic solutions is the exception rather than the rule.

of the red-cell membrane depend on whether it is bathed with serum or plasma, or with saline media. The disk-sphere transformation between slide and cover-glass does not occur if serum or plasma are present, irregular crenation is less commonly observed in serum and plasma, and the R values for red cells in hypotonic serum or plasma are generally greater than they are in hypotonic NaCl (Ponder & Robinson, 1934); all these observations thus point to the presence of some plasma constituent, probably protein, as being necessary for the maintenance of the normal properties of the membrane. The existence of a difference in critical volume in hypotonic serum and hypotonic NaCl respectively is therefore a matter of considerable interest.

The most satisfactory determinations which I have made were done in the following way. Serum is obtained from defibrinated rabbit blood, and its depression of freezing-point determined; a NaCl solution of the same depression of freezing-point is then prepared. The serum and the NaCl are diluted with water to give a series of tonicities 1·0, 0·6, 0·58, 0·56 ... 0·40, the tonicity of the undiluted serum or NaCl being taken as unity, and 5 c.c. of each of these hypotonic media are placed in a series of vials. To each vial is added 1 drop of the blood, the drop being delivered from a burette cut off at the end, like a stalagmometer. The systems are allowed to stand for about 1 hour at room temperature, and the vials showing just commencing haemolysis are picked out. Two sets of determinations of volume are then made, the first by haematocrite, and the second by diffraction. For the former determinations, 2 c.c. from (*a*) the vial containing undiluted serum, (*b*) the vial containing undiluted NaCl, (*c*) the vial showing just commencing lysis in the hypotonic serum, and (*d*) the vial showing just commencing lysis in the hypotonic NaCl, are placed in calibrated Hamburger haematocrite tubes, and spun at 8000 r.p.m. for 30 min., by the end of which time constant volume is reached. Measurement of the columns of cells gives the percentage increase in volume in the hypotonic serum and NaCl respectively. For the diffractometric measurements, 1 c.c. from each of vials (*a*), (*b*), (*c*) and (*d*) is transferred to small tubes; the cells are thrown down gently, the supernatant fluid lecithinated, and a small quantity of the lecithinated fluid added again to the cells, which are resuspended in it. Preparations are made from the suspensions, and the mean volume of the cells found with the diffractometer.

The two methods agree very closely, and Table II shows a set of typical results.

Table II

	Serum	Saline
Initial volume, μ^3, diffraction	57	57
Initial area, μ^2	72	72
Critical tonicity	0·48	0·50
Critical volume, μ^3, diffraction	85	78
Critical volume, %, haematocrite	149	136
Critical area, μ^2	93	88
R value	0·75	0·6

These critical volumes and areas are those for commencing lysis, and so are considerably smaller than the mean critical volume and the mean critical area, but

there is no doubt that, in either case, the critical volumes and areas are smaller in hypotonic NaCl than they are in hypotonic serum, and that the critical area in hypotonic NaCl is less than the initial area of the disk in an isotonic medium. Without stressing the point unduly, this observation may be related to another observation which it is difficult to be quite sure about, since it rests on an impression one gets when examining the wet preparations from hypotonic systems. One gets the impression that the spherical form which the cell assumes just before lysis is not merely the result of a continuous swelling and deformation up to a point limited by the greatest volume compatible with an unstretched surface, but that it is assumed suddenly, the cell changing from a swollen spheroidal or cup-shaped body into a perfect sphere in much the same way as it changes into a sphere under the influence of a "chemical lysin" such as saponin. The "chemical lysins" first affect a "form factor" in the red cell surface, with the result that the forces maintaining the special shape of the cell give way, the cell becoming spherical; this is followed, after a variable interval, by the membrane becoming permeable to pigment. The indications are that something of the same sort occurs in hypotonic haemolysis, in which case we have to look upon the effects of hypotonic haemolysis as something more complex than mere mechanical effects. It must be borne in mind that we are dealing with the deformation of a surface which has a complex ultra-structure, and whose initial shape depends, almost undoubtedly, on a special molecular arrangement in its surface layers. When the cell increases in volume without changing its surface area, this surface-molecular structure is subjected to stress, and the molecular orientation responsible for shape may break down before that responsible for semi-permeability, while the latter may break down when the surface actually begins to be stretched, or before it begins to be stretched, according to the physico-chemical conditions prevailing at the surface. The principal factor determining the critical volume and area seems to be that the red-cell membrane cannot be appreciably stretched beyond its initial area without breaking down, but this is a limiting factor, and the nature of the hypotonic medium (e.g. NaCl) may exert subsidiary effects, so that the breakdown of the membrane occurs before the point of stretching is reached.

III. THE RELATION OF FRAGILITY TO CELL SHAPE

The principal result of this investigation has been to show that the swelling of the red cell in a hypotonic medium involves a shape change, and is limited by the fact that the surface area cannot be stretched beyond that of the original disk; the extent to which the cell volume can increase without the cell haemolysing must therefore be related to the initial cell shape. This is an idea which has been put forward recently by Haden (1934, 1935), who has pointed out that the fragility of the cells of different animals, and of human cells in different pathological conditions, depends on the length/thickness ratio for the cell, or on the extent to which it can swell before becoming a sphere. Let us consider this suggestion with relation to the two well-known difficulties which arise in connexion with osmotic haemolysis: (*a*) that the cells of different animals show different resistances to hypotonic

solutions, and (*b*) that different red cells of the *same* animal show different resistances, so that a resistance distribution of roughly symmetrical form arises with respect to tonicity.

(*a*) From measurements of the initial and the critical volumes of red cells of the sheep, the ox, the rabbit, and man, and influenced by the idea of a stretching of the cell membrane as the volume increases, I suggested the existence of the relation (Ponder, 1935*a*)

$$dA = KV_0, \qquad \text{.....(2)}$$

V_0 being the mean cell volume, and dA the mean increase in area which can occur before haemolysis. These measurements of initial and critical volume were made on cells rendered spherical by the addition of lecithin, and the increase in area dA was the difference between the area of the spherical form of initial volume V_0 and that of the spherical form of critical volume V_c (for commencing lysis).[1] If, as has been shown above, the maximum area for a spherical form of volume V_c is the same as the area of the discoidal form of volume V_0, dA is equal to the decrease in area which occurs when the disk of volume V_0 becomes the sphere of volume V_0, i.e. the reduction in area which occurs when the disk-sphere transformation takes place in an isotonic medium. Thus:

$$dA = 2\pi a^2 + 2\pi ab.\sinh^{-1} e/e - 4\pi a^2, \qquad \text{.....(3)}$$

where a is the semidiameter of the cell and b 0·67 times the thickness, and where $e = \sqrt{a^2 - b^2}/b$ (Ponder, 1934). The magnitude of dA is therefore a function of the length/thickness ratio for the cell. Since the increase in volume which can occur without the cell haemolysing is a function of dA, the critical volume must be a function of the length/thickness ratio, and, the values of such constants as R and W in expression (1) being substantially the same from one animal to another (see Ponder, 1935*a*), the tonicity T_c in which the critical volume is reached must be a function of the length/thickness ratio also.

This idea is borne out by such measurements of the length/thickness ratios as exist, although few of these are reliable. Haden uses Emmons's figures for the mean thickness of the cells of various animals, and in his own determinations finds the mean thickness by dividing the mean red-cell volume (haematocrite and red-cell count) by $\pi D^2/4$, where D is the cell diameter. Emmons's values, however, were obtained by measuring cells in rouleaux, and this does not give the true thickness (Ponder, 1929), and Haden uses the diameter of the *dried* red cell in finding mean thickness. The only satisfactory figures we have are those for man, the rabbit, and the sheep; the mean length/least thickness ratios are 8·38, 7·30 and 5·20 respectively, and these are in the order of the decreasing resistances observed (Ponder, 1929).[2]

[1] The figures for "extended area" in Table II of Ponder (1935*a*) are less than the figures for the mean area of the discoidal red cells of the various animals, because the measurements were made on cells in systems showing only just beginning lysis.

[2] The values for the ratio length/greatest thickness, however, are 3·56, 4·29 and 2·74 for man, the rabbit, and the sheep respectively, and these are not in the order of the observed resistances. The cell shape, in fact, cannot be defined in terms of one ratio, and what is required is a relation between resistance and some such measure of shape as the "form factor X" which appears in Fricke's equation for volume concentration as a function of conductance (see Ponder, 1935*b*). I propose to make the necessary determinations shortly.

This interpretation of the experimental results is different from, and more satisfactory than, the one put forward in 1935 (Ponder, 1935*a*), in which it was suggested that the relation $dA = KV_0$ arises from the cell membrane being augmented by "reserve" material when it is stretched, the amount of the reserve material being proportional to the initial volume V_0, or, more likely, to the thickness of the cell envelope, itself proportional to V_0. At the time, the idea was supported by results which showed that the capacity of the cell surface, per unit area, remains unaltered when the surface is stretched, indicating that the thickness of the cell membrane remains unchanged. The explanation now is that the disk-sphere transformation in an isotonic medium results in a decrease dA in the cell surface, but that, as the envelope crenates, the cell membrane crenates with it, becoming wrinkled and folded so as to adapt itself to the smaller area. If the cell is measured as a spherical form in isotonic solution and as a spherical form in a solution in which it has reached its critical volume, the increase in area will appear to be dA, but this does not correspond to a stretching of the membrane; it corresponds to the difference between the extended area of the membrane and the area covered by it when it is in a folded state. In fact, instead of dA being a measure of stretching, it is a measure of compression, or more properly of folding or crenation, and it exists only because the measurements are made exclusively on cells in the spherical form. Strictly speaking, the area of the membrane remains unchanged.[1]

(*b*) In the case of the red cells of the *same* animal, the volume V attained in a tonicity T is

$$V = V_0 RW(1/T - 1) + 100/100,$$

if V and V_0 are measured in μ^3, and if V is measured as a percentage volume,

$$V_{pc} = RW(1/T - 1) + 100.$$

If lysis occurs when the cell volume has increased to such an extent that the surface area of the cell (now spherical and of critical volume V_c) is equal to that of the disk of initial volume V_0,

$$V_c/V_0 = \text{const.}, \quad V_{pc} = \text{const.}$$

for the cells of any one shape, and so, in a given tonicity in the range for partial haemolysis, there will be lysis of all the cells of the same shape. Expressed as a percentage of the initial volume, each cell will have increased in volume by a constant amount; if the volumes are measured in μ^3, and if we compare the distribution of sizes in a medium of tonicity T with the initial distribution of sizes, we should find that the two distributions become the same if the volume of each

[1] It must be borne in mind that in all experiments which have been done hitherto, the cells are swelling in hypotonic media as disks, even though the measurements of volume and area are made on the spherical form. Apparent increases in area are thus the result of the method of measurement used, and have no actual existence, for the evidence points to the fact that swelling in hypotonic systems is accompanied by shape changes only, and not by area changes. If we were to lecithinate the cells first, and then put them in hypotonic media, there would be area changes corresponding to the volume changes, but such systems have been used on only one occasion (Ponder, 1936). Cells suspended in lecithin-treated plasma are more resistant to hypotonic media than are cells in untreated plasma, but the increase in resistance is due to a decrease in the R value rather than to an increase in the critical volume.

cell in the initial distribution is increased by a constant factor. This is the result obtained experimentally. For cells of another shape, V_c/V_0 will have a different value. Thus the cells which show the least resistance will be those for which the length/thickness ratio is least, the cells which show the greatest resistance will be those for which the length/thickness ratio is greatest, and the relation of cell number to tonicity, or the frequency distribution of resistances, will be related to the distribution of the population with respect to shape. That the length/thickness ratio for the red cells of any one animal is very variable has already been shown by direct measurement (Ponder, 1929).

The distribution of cell numbers with respect to tonicity must, however, be very complex, for the preceding has taken no account of variations in the constants R and W of expression (1). If the product RW varies, the cells which reach critical volume in a given tonicity T will not be all of the same shape, for cells with a greater length/thickness ratio, but with a value of RW less than the mean value, and also cells with a smaller length/thickness ratio and a value of RW greater than the mean, will haemolyse in the same tonicity. A discussion of the possible effects of variations in R and W is beyond the scope of this paper, but there is one further point which may be commented on. The value of R may not only vary from cell to cell of the same shape, but may itself be a function of the tonicity of the system. Ponder & Robinson (1934) have shown that the R value tends to fall as the tonicity is decreased, and the values in Table I show the same sort of thing. A decrease in the value of R, with decreasing tonicity, would lead to the frequency distribution of resistances becoming negatively skew, some of the cells remaining intact in much lower tonicities than usual. In the case of rabbit red cells in NaCl buffer, these asymmetrical distributions are quite often observed, although the distribution is typically symmetrical.

SUMMARY

Red cells placed in hypotonic media swell until a certain "critical volume" is reached, after which the cell haemolyses. Shortly before reaching this critical volume, the cell becomes a perfect sphere, and in hypotonic serum the area of the sphere is substantially the same as the area of the disk in an isotonic medium. Thus the increase in cell volume is not accompanied by an increase in cell surface, but rather by a change in cell shape, and lysis occurs when the volume has increased to such an extent that the cell membrane is subject to stretching forces. In hypotonic NaCl the critical volume is smaller than it is in hypotonic plasma, and the cell haemolyses before its membrane is subject to extension.

The volume increase which a cell can undergo by becoming a sphere with the same surface area as that of the disk obviously depends on the shape of the disk, and approximately on its length/breadth ratio, so cells of different shapes haemolyse at different critical volumes, attained in different tonicities. This idea, originally due to Haden, can be made to explain quantitatively the differences in resistance which are observed in the case of the red cells of different mammals, and also to account for the frequency distribution of resistances found in the case of the different red cells of the same animal.

REFERENCES

CURTIS, H. J. (1936). *J. gen. Physiol.* **19**, 929.
FRICKE, H. (1925–6). *J. gen. Physiol.* **9**, 137.
GORTER, E. & GRENDEL, F. (1928). *J. exp. Med.* **41**, 439.
HADEN, R. L. (1934). *Amer. J. med. Sci.* **188**, 441.
—— (1935). *International Clinics*, **1**, series 45, p. 69.
JACOBS, M. H. (1930). *Biol. Bull. Wood's Hole*, **58**, 104.
MACLEOD, J. & PONDER, E. (1933). *J. Physiol.* **77**, 181.
PONDER, E. (1928–9). *J. exp. Biol.* **6**, 387.
—— (1929). *Quart. J. exp. Physiol.* **19**, 29.
—— (1933*a*). *Quart. J. exp. Physiol.* **23**, 287.
—— (1933*b*). *Quart. J. exp. Physiol.* **23**, 305.
—— (1934). *The Mammalian Erythrocyte and the Properties of Haemolytic Systems*. Protoplasma Monographien, **6**, 42. Berlin: Borntraeger.
—— (1935*a*). *J. Physiol.* **83**, 352.
—— (1935*b*). *J. Physiol.* **85**, 439.
—— (1935*c*). *Proc. Soc. exp. Biol.*, N.Y., **35**, 156.
—— (1936). *J. exp. Biol.* **13**, 298.
—— (1937). Protoplasma (in the press).
PONDER, E. & MILLAR, W. G. (1924). *Quart. J. exp. Physiol.* **14**, 67.
PONDER, E. & ROBINSON, E. J. (1934). *J. Physiol.* **83**, 34.
PONDER, E. & SASLOW, G. (1930*a*). *J. Physiol.* **70**, 18.
—— —— (1930*b*). *J. Physiol.* **70**, 169.
—— —— (1931). *J. Physiol.* **73**, 267.
SCHMITT, F., BEAR, R. S. & PONDER, E. (1936). *J. cell. comp. Biol.* **9**, 89.