ON THE SPHERICAL FORM OF THE MAMMALIAN ERYTHROCYTE

PART II. SPHERICAL FORMS PRODUCED BY LECITHIN AND THE PHOTODYNAMIC DYES

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SOME time ago I published an account of the change in shape, from disc to sphere, which occurs when mammalian red cells, suspended in saline, are placed between a slide and a closely applied cover-glass (Ponder, 1929). Later I briefly described a similar transformation which occurs in plasma or serum to which small quantities of lecithin have been added, the normally discoidal cells again becoming perfect spheres without change in volume (Ponder, 1933), and recently I have observed a disc-sphere transformation in systems containing red cells and dyes of the fluorescein series. To avoid difficulties associated with the peculiar shape of the mammalian red cell, several observers have worked with the spherical form in lecithin-treated plasma (Ponder, 1933, for finding volumes, 1935, in conductivity studies; Race, 1934, in sedimentation experiments; Fricke and Curtis, 1935, in conductivity and capacity experiments); no detailed description of the phenomenon itself, however, has yet appeared, and the disc-sphere transformation which occurs with the dyes of the fluorescein series is altogether new.

What is known about these spherical forms is best described by enumerating a series of experiments which illustrate various points.

I. THE SPHERICAL FORM IN LECITHIN-TREATED PLASMA

(i) To obtain the spherical form, one proceeds as follows. Blood is withdrawn from the animal, and plasma or serum separated. Any anticoagulant may be used, or the blood may be defibrinated. About 5 mg. of lecithin are taken for each c.c. of plasma, and the lipoid is emulsified in the plasma by rubbing in a mortar. The plasma may then be filtered either through a hard paper or through a Barkefelt filter. Before the filtration a considerable proportion of the lecithin separates out as oily droplets, and this makes it difficult to say how much is really emulsified.

If this lecithin-treated plasma is mixed in about equal proportions with whole blood or with whole blood diluted with untreated plasma, the cells undergo a change of form. First they crenate and assume the familiar "thorn apple" form; the crenations then gradually become finer and fewer, and ultimately disappear

altogether, leaving the cells as perfect spheres. Dark ground examination sometimes shows the surface of the spheres to be mottled, the appearance being similar to that described by Millar (1925) in the case of the spherical forms which occur between slide and coverslip. The spheres remain unchanged for a period which depends on the amount of lecithin added, and finally undergo haemolysis.

(ii) The disc-sphere transformation can easily be reversed by washing the cells in untreated plasma or in isotonic NaCl. When the reversal occurs, all the changes of the disc-sphere transformation are seen in reverse order; first fine crenations appear, these then become coarser, and finally discs appear, these often being a little crenated. The disc-sphere transformation and its reversal can be repeated several times (Race, 1934).

(iii) Unlike the disc-sphere transformation which occurs between slide and cover-glass, the change of form in lecithin-treated plasma does not depend on the cells lying between two closely approximated surfaces, for it occurs in an uncovered or hanging drop, nor does it depend on the cells being immersed in NaCl, for it takes place irrespective of whether NaCl, KCl, Ringer's solution, citrate, serum, or plasma is used as the suspension medium. As will be seen below, different quantities of lecithin are required to bring about the change of form in the case of different kinds of red cell, but the phenomenon seems to be essentially the same for all the kinds of mammalian red cell examined.

(iv) The effect is produced either by "lecithin ab ovo, Merck", a dark and impure substance, or by lecithin prepared in the laboratory and pure white in colour. Cephalin, on the other hand, is without effect, as are all oils and waxes which I have tried.

(v) In view of the claims which have been made regarding a lecithin-cholesterol antagonism (Brinkman and van Dam, 1920), I have tried to modify the phenomenon by adding cholesterol to the lecithin-treated plasma. Such attempts have been unsuccessful.

(vi) The volume of the spherical forms is a matter of the greatest interest, for if the change of form is unaccompanied by a change in volume, the volume of the red cell can be measured by suspending the cells in lecithin-treated plasma and then finding their volume, as spheres, either photographically or diffractometrically. This method of measuring volume has already been used (Ponder, 1933; Ponder and Robinson, 1934), but the evidence that no volume change occurs during the transformation has not yet been published. Two cases arise: (*a*) when the cells are in normal plasma, and (*b*) when they are in hypotonic plasma.

(*a*) When the cells are in normal plasma, the identity of volume between discoidal cells and spherical cells in lecithin-treated plasma can be demonstrated in the following way. The volume of the discoidal cells is found by measuring the percentage volume with a high-speed haematocrite (12,000 r.p.m. for 30 min.), and dividing the figure obtained by the red cell count $\times 10^{-7}$ (see Ponder, Dubin and Gordon, 1934). The volume of the spherical cells in lecithin-treated plasma can be found either by photographing the cells (Ponder and Millar, 1924), and calculating the mean volume from the mean radius of the spheres, or by measuring the mean

radius diffractometrically, and calculating the mean volume from the figure found. Table I shows the agreement between the results of the three methods.

Table I

Animal	Discs, μ^3 Haematocrite	Spheres, μ^3	
		Photography	Diffraction
Man	77·5	76·3	78·1
Man	85·5	85·0	86·0
Rabbit	62·0	60·5	60·0
Rabbit	58·0	56·5	58·0
Rabbit	56·5	58·5	57·0
Sheep	31·0	31·5	30·5
Sheep	28·5	28·0	27·5

Such discrepancies as exist are within the limits of error of the methods, and so the change of form from disc to sphere does not seem to be accompanied by a change in volume.

(*b*) When the cells are suspended in hypotonic plasma the determinations are more difficult to make, for the only method which can be used for finding the volume of the discs is the haematocrite method (as under (*a*), above), the results of which depend largely on the rate and time of spinning and on the degree of hypotonicity. One can make serious errors if one uses the same rate and time of spinning for cells in hypotonic plasma as gives complete packing in the case of cells in undiluted plasma; the error can be greatly reduced, however, by using a high-speed haematocrite (at least 10,000 r.p.m.), and spinning until constant volume is reached. This takes at least an hour in the case of cells in hypotonic plasma. Two Hamburger haematocrite tubes are taken, and in the first is placed 2 c.c. of undiluted plasma, while in the second is placed 2 c.c. of a hypotonic plasma just too concentrated to bring about lysis. One drop of whole blood is added to each tube, and the tubes are spun until the heights of the columns of cells show no further decrease. From the heights of the columns the volume of the cells in the hypotonic plasma is obtained relative to that of the cells in the undiluted plasma. Lecithin is then emulsified in undiluted plasma, cells added, and their volume determined diffractometrically. To 1 c.c. of the hypotonic plasma is added a drop of blood, and after a few minutes the cells are gently thrown down. Lecithin is emulsified in the supernatant fluid, which is replaced in the tube and mixed up with the cells; the volume of the spherical cells is then measured diffractometrically. Their volume is divided by the volume found in lecithin-treated undiluted plasma, and the figure so obtained should be the same as that found by the haematocrite method, provided the addition of the lecithin has the effect of changing shape only, and not volume.

It is sufficient to say that the agreement between the results of the two methods is sufficiently good to warrant the conclusion that the volume of discs in hypotonic plasma is the same as that of spheres in hypotonic plasma treated with lecithin after the cells have reached their equilibrium volume. Occasionally the ratio obtained by the haematocrite method is a little greater than that obtained by diffraction, but

the small discrepancies can be fairly attributed to the failings of the haematocrite method.

(vii) The quantity of lecithin required to bring about the disc-sphere transformation depends on the kind of red cell under consideration. Quantitative determinations of this amount are best carried out by emulsifying the lipoid in saline. In the case of the erythrocytes of man, concentrations of lecithin in excess of 30 mg./litre, when completely emulsified, produce the spherical form; in the case of rabbit red cells, 25 mg./litre is sufficient, while the cells of the rat require about 28 mg./litre, and the cells of the ox about 50 mg./litre. As might be expected from the above, the cells of various animals show differences in the ease with which they can be converted into spheres, and even when adequate quantities of lecithin are used the velocity of the disc-sphere transformation varies considerably. The cells of man and of the rabbit are very easily transformed, those of the sheep less easily and rapidly, and those of the ox the least easily of all. Further, the interval which elapses between the assumption of the spherical form and the subsequent lysis of the cell varies from animal to animal; in the case of rabbit cells the interval is relatively long (15–30 min.), while in the case of ox cells it is much shorter (about 5 min. under comparable conditions).

(viii) Red cells suspended in lecithin-treated plasma are more resistant to hypotonic haemolysis than are cells in untreated plasma. This increase in resistance is related to the quantity of lecithin present in rather a curious way. For human red cells in lecithin-treated saline, the form and the resistance are unchanged when the quantity of added lecithin is less than about 20 mg./litre, but as the lecithin concentration increases, the resistance increases to a maximum when the concentration is about 30 mg./litre, which is the same lecithin concentration as that which produces the spherical form. The increase in resistance observed at this concentration may correspond to as much as 0·1 tonicity unit, *i.e.* cells which begin to haemolyse in untreated hypotonic plasma at a tonicity of 0·47 may not begin to haemolyse in lecithin-treated hypotonic plasma until a tonicity of 0·37 is reached. As the concentration of lecithin is further increased the resistance falls off again, and a decrease in resistance of as much as 0·1 tonicity unit may be found when the lecithin concentration is about 45 mg./litre. This decrease is probably related to the fact that lecithin is a haemolysin. The increase in resistance is less easy to explain, but it ought to be pointed out that it is not an isolated phenomenon, for many lysins and sensitising agents, *e.g.* the urethanes, the bile salts, colloidal silicic acid, and brilliant green, produce a similar increase in resistance when in sublytic concentrations. Since the critical volume in which the treated and untreated cells haemolyse is substantially the same, this increase in resistance means that the R value for the treated cells is less than that for the untreated, which is altogether in keeping with the idea that "injury" to the red cell usually results in a lowering of the R value (see Ponder and Robinson, 1934).

(ix) Just as the addition of lecithin and the transformation of the disc into the sphere alters the resistance to hypotonic solutions, so does the immersion of the cell in hypotonic solutions alter the ease with which added lecithin converts discs into

spheres. In isotonic plasma the transformation is always relatively easy to effect, but when the cells are in hypotonic plasma even rabbit cells are difficult to transform, and ox cells often cannot be transformed at all. The stretched red cell membrane is apparently much less easily affected by lecithin than is the membrane of the normal cell.

II. THE SPHERICAL FORMS PRODUCED BY DYES OF THE FLUORESCEIN SERIES

(i) Mammalian red cells suspended in isotonic NaCl, NaCl-phosphate (pH = 7·0), or Ringer's solution, rapidly become spherical if small quantities of fluorescein, eosin, erythrosine, or rose bengal are added. To produce the disc-sphere transformation with rose bengal, 0·2 c.c. of a "standard" suspension made by suspending the thrice washed red cells from 1 c.c. of rabbit blood in 20 c.c. of saline (0·96 per cent. NaCl) are mixed with 1 c.c. of $M/10^5$ rose bengal in isotonic NaCl or Ringer. Within a few seconds all the cells appear spherical in a hanging or uncovered drop. If the same number of cells are added to smaller quantities of the dye, *e.g.* 1 c.c. of $2M/10^6$, "thorn apple" forms appear, and if still smaller quantities are used, the discoidal form is maintained.

(ii) The amount of dye required to effect the transformation varies in the case of the four dyes of the fluorescein series. Using 0·2 c.c. of "standard" cell suspension and 1 c.c. of dye, one requires a dye concentration of about $M/10^5$ in the case of rose bengal, $M/10^4$ in the case of erythrosine, $M/10^3$ in the case of eosin, and $M/10^2$ in the case of fluorescein to turn rabbit red cells from discs to spheres. This dependence on molar concentration shows that the dye molecule exerts a specific effect, different in the case of each dye. It is interesting to observe that the minimal dye concentration in which the disc-sphere transformation is obtained is of the same order as that required to give haemolysis when the dye-cell systems are exposed to light, but at least ten times less than the concentration which gives haemolysis in the dark (Blum, 1935).

(iii) Taking the surface area of the rabbit red cell, when spherical, as $80\mu^2$, the total surface area of the cells in the above systems is at least $4.(10^9)\mu^2$. In 1 c.c. of a concentration of rose bengal of $5M/10^6$, which produces spheres and "thorn apple" forms, there are $3.(10^{15})$ molecules, and so there are $8.(10^5)$ dye molecules available for each μ^2 of cell surface. If the rose bengal molecule were to cover the same area as does the molecule of sodium oleate, we would require about 10^6 molecules per μ^2 of surface in order to form a monomolecular layer; the molecules of rose bengal present in a system in which there is just enough dye to produce the disc-sphere transformation are accordingly just sufficiently numerous to cover the cell surfaces, provided that all the dye in the system is concentrated at the surfaces. As a matter of fact, not all the dye is so concentrated, and so we are led to the conclusion that the transformation from disc to sphere can be brought about when there are fewer dye molecules than can cover the surfaces of the cells.[1]

[1] Calculations of this sort, however, are always open to the objection that few of the more complex dyes are pure substances of known molecular weight.

(iv) The spherical cells in rose bengal systems can be immediately reconverted into discs by the addition of serum or plasma. Using rose bengal in a concentration of $M/10^5$, the spherical cells produced by mixing 1 c.c. of the dye with 0·2 c.c. of a "standard" rabbit red cell suspension can be reconverted into discs by adding 0·002 c.c. of rabbit serum. The component of the serum which produces the reversal seems to be a protein, and, assigning to it a molecular weight of the order of 10,000, the quantity necessary for the reversal is of the order of $M/10^5$, *i.e.* of the same order as that of the quantity of the dye used to produce the spheres from discs. In the case of all the dyes of the fluorescein series, however, the discs which appear when plasma or serum is added are not always of the typical form; often they are cup-shaped, and they show, further, a tendency to stick to the slide (cf. the stickiness of discs produced by adding serum to the spherical forms between slide and cover-glass, Ponder, 1929).

(v) The spheres produced by minimal concentrations of the dyes of the fluorescein series can also be reconverted into discs by diluting the system with saline, or by washing the cells (cf. the reversal of the spherical form produced by the addition of lecithin).

(vi) The spheres produced by the addition of the photodynamic dyes have the same volume as the discs from which they are formed. This can be shown in the same way as that by which the identity of volume is demonstrated in the case of discs and spheres in lecithin-treated plasma (see above).[1]

(vii) Haematoporphyrin in a concentration of $M/10^5$ in NaCl or Ringer will produce the change from discs to spheres in the same way as does rose bengal, and in this case too the spheres are reconverted into discs by the addition of serum or plasma. The shape transformation, however, does not appear to be produced by all the photodynamic dyes, for it does not occur with methylene blue.

III. THE SPHERICAL FORM OBSERVED BETWEEN SLIDE AND COVER-GLASS

Since the first paper of this series was written (1929) little light has been thrown on the cause of the spherical forms which appear between a slide and a closely applied coverglass, but the investigations of Tietel-Bernard (1932) and of Waller (1930, 1935) have a bearing on the matter, and can be conveniently considered here. Both these investigators are concerned with crenation, which is a stage in the formation of the spherical form.

Teitel-Bernard's observations and views can be summarised as follows. He regards crenation as due to a change in the molecular configuration of the haemoglobin in the cells, the pigment becoming less dispersed, and the ionic activity in the cell interior accordingly becoming lessened; this results in a fall in the intracellular tonicity, and water leaves the cell, giving crenation and a decreased cell volume. By making measurements of the thickness of cells in rouleaux, he shows that the

[1] The volume of cells made spherical by the addition of the photodynamic dyes cannot, however, be found by the photographic method, for the photosensitised cells haemolyse when they are brought into the brilliantly lit microscopic field.

thickness is increased in hypotonic saline, and diminished in hypertonic saline; this he accounts for on the ground that the cell loses water in the latter case, although the discoidal form may be retained provided the degree of hypertonicity is not too great. He further shows that in hypertonic solutions the haemoglobin of the cell may alter its molecular state even to the extent of crystallising, particularly at a *p*H of 6·5 (the isoelectric point of haemoglobin), when the cells may show birefringence. His observations are varied and interesting, but I think it can fairly be said that the most important conclusion which he draws from them is that crenation is due to changes in the red cell interior. He also discusses a curious form of crenation which appears when a micro-dissection needle is approached to a red cell, but this phenomenon has little bearing on the points with which this paper is concerned. He also restates, and further develops, the idea of Gough (1924), that the normal discoidal shape of the erythrocyte is due to the haemoglobin molecules in the cell being arranged in a liquid-crystal form, so that the repulsive force along the equator of the cell is greater than that in the direction of its polar axis. This idea again assumes that the forces which are responsible for the red cell shape are situated in the interior.

Looked at in the light of all our existing information regarding changes in red cell shape, Teitel-Bernard's interpretation of his results can be criticised on at least two grounds. (*a*) The substances which produce crenation most readily, and in its most extreme form (the spherical form), are not, in general, substances which would be expected to penetrate into the cell interior and bring about a change in the molecular state of haemoglobin. Because of their high molecular weight, neither lecithin nor the dyes of the fluorescein series are likely to pass across the cell membrane, and in the case of rose bengal and haematoporphyrin the changes of form are brought about when there are barely enough molecules to cover the cell surface, let alone to penetrate and exert an effect on the haemoglobin. Further, the change from sphere to disc occurs immediately on the addition of serum to rose bengal cell systems, and the serum proteins, which seem to be responsible for the reversal, can scarcely be regarded as substances likely to enter the interior of the cell. All the evidence, in fact, points to the changes of shape being due to the action of substances at the red cell surface, and in this connection we have also to bear in mind that a change of shape from disc to sphere always precedes haemolysis, which is the result of an effect on the surface membrane. (*b*) His elaboration of Gough's idea that the discoidal form of the cell is brought about by the contained haemoglobin being in a mesomorphic state can be set aside, as can Gough's original statement of the hypothesis, by the fact that red cells are discoidal, although crenated, after haemolysis by freezing and thawing or by treatment with hypotonic saline followed by a "reversal" of the haemolysis with NaCl. If the discoidal form is due to the presence of some substance in a mesomorphic state, that substance is certainly not the haemoglobin; it is more likely that it is a component of the cell envelope (see Ponder, 1934).

Waller's published observations are contained in a short note in the *Proceedings of the Physiological Society* (1930), but he has recently placed at my disposal a much more extensive description of his observations, with the idea that I should use them

in connection with the material with which this paper is concerned. Waller is emphatically of the same opinion as Saslow and myself (Ponder and Saslow, 1930), that crenation is not a phenomenon necessarily associated with diminution in red cell volume, and points to an observation made by Hewson, as early as 1773, to the effect that the shape of the red cell remains unchanged even if equal volumes of 3 per cent. NaCl are added to the serum surrounding the cells, and to a statement in Kirkes' text-book (1888) to the effect that crenation occurs in hypotonic saline. Waller, Brinkman and van Dam (1920), Gough (1924), and Ponder and Saslow (1930), in fact, all disagree with the statement that crenation is an effect caused by immersion of the cells in hypertonic saline, and Ponder and Saslow have shown that it is not always associated with a decrease in cell volume. As Waller observes, crenation and final assumption of the spherical form occur when the cells are contained in thin layers between a slide and a cover-glass, but does not occur in a parallel manner in the case of red cells suspended in saline in a test-tube; moreover, the rapidity of the change in form depends on the ratio of the surface of the glass to the volume of the enclosed fluid. Waller's view, however, is that the crenation, and the ultimate assumption of the spherical form, is brought about by diffusion of alkali from the glass; in fact, he regards crenation as a stage in the haemolysis of red cells by alkali. His evidence for this conclusion is: (*a*) that the change is quicker if the film between the surfaces is thin, (*b*) that the addition of serum causes the discoidal form to be retained, (*c*) that the addition of suitable buffers also causes the discoidal form to be retained, and (*d*) that if the glass surfaces are thoroughly cleaned with ether, the discoidal form persists between them. Like Teitel-Bernard, Waller is inclined to attribute the change of shape which results in crenation and the assumption of the spherical form to a change in the properties of the haemoglobin.

There are several objections to Waller's idea that crenation and the final assumption of the spherical form between slide and cover-glass are due to diffusion of alkali from the glass. (1) The first is a point to which Waller himself calls attention, viz. that crenation and the spherical form are observed between quartz surfaces. (2) The spherical form is never observed except between *two* glass surfaces, although there may be irregular crenation when the cells are in an uncovered or a hanging drop. (3) Although it can readily be shown that alkalis produce a spherical form of the cell, and that this occurs as a stage in alkali haemolysis, the spherical form does not appear in an uncovered drop until the *p*H is about 11·0, and haemolysis soon follows; in the case of the spherical form between slide and coverglass, on the other hand, the *p*H of the medium can be shown by the addition of indicators to be not greater than 7·5, and haemolysis does not take place for a very long time. Waller seems to have taken two superficially similar phenomena for the same phenomenon, and although the retention of a crenated discoidal form between slide and coverglass in phosphate buffers of *p*H less than 7·0 is an observation which can be confirmed, the essential factor does not appear to be the *p*H of the medium. The effect of the acid phosphate buffers in preventing the appearance of the spherical form is similar to that of ammonium salts (Gough, 1924), and is something which has yet to be explained.

IV. DISCUSSION

Setting aside the occurrence of the spherical form between slide and cover-glass as a phenomenon yet to be explained, one is struck by the fact that all other cases in which the discoidal form of the red cell is replaced by the spherical form are cases in which the transformation is followed by haemolysis. Thus the assumption of the spherical form is followed by lysis when the cells are treated with saponin, digitonin, the bile salts, the soaps, brilliant green, complement and amboceptor, lecithin, haematoporphyrin, and the dyes of the fluorescein series, and the only difference to be observed is in the concentrations of the lysins which produce the spherical form and the ensuing lysis, or, alternatively, in the length of time which elapses between the shape change and the haemolysis. For example, the shape change is almost immediately followed by lysis in the case of saponin and the bile salts, whereas in the case of lecithin and the photodynamic dyes and lecithin the shape change and the haemolysis occur in concentrations of different orders. The simplest way of looking at the matter is to suppose that there are two cell components involved, the first concerned with the maintenance of the special shape, and the second with the semi-permeability to pigment, that both are attacked by all lysins, but that the first is always affected before the second, and more readily by some lysins than by others.

The nature of these two components is still a matter for speculation. As regards the "shape component", the weight of evidence at the present time is that the special shape is due to a peculiar molecular arrangement in the cell envelope (Ponder, 1934), and the fact that the disc-sphere transformation is so readily reversible necessitates our thinking of the cell surface as a liquid film, the molecules of which are free to re-orient themselves when the disc turns into a sphere, or *vice versa*. The "permeability component", on the other hand, is at present thought of as a layer a few molecules thick (Fricke, 1925–6), although it may be considerably thicker (see Danielli, 1935; Curtis, 1935), and it seems apparent that its properties are not substantially altered when the "shape component" undergoes a change, for the permeability of spherical red cells is certainly not greatly different from that of cells in the discoidal form. All one can conclude at the present time is that the "shape component" and the "permeability component" are essentially different, and such electrical evidence as there is supports this view. Curtis (1935), for example, has shown that there is no appreciable change in the cell resistance, the capacity per unit area of the cell surface, or the frequency dependence of the capacity, when the disc-sphere transformation takes place, and Abramson (1934) has shown that the disc-sphere transformation which occurs in saponin solutions is not associated with any measurable change in ζ potential. It seems that the electrical properties of the red cell surface are principally associated with the "permeability component", as one might expect.

Finally, it is interesting that many of the properties of the red cell membrane depend on whether the cells are bathed with serum or plasma or whether they are bathed with saline media. Thus the disc-sphere transformation which occurs be-

tween slide and cover-glass and the transformation which follows the addition of photodynamic dyes do not take place in the presence of serum or plasma, which reconvert into discs any spheres which may be present. Again, the irregular crenation which is often seen in the cells of saline suspensions does not occur either as frequently or as markedly when serum or plasma is the surrounding medium, and the extent to which red cells can swell, and their membranes stretch, without losing pigment into hypotonic solutions, is greater in hypotonic serum or plasma than in hypotonic saline. There is some evidence, too, that the permeability of the cell membrane is not the same when it is stretched in hypotonic plasma and in hypotonic saline respectively (Ponder and Robinson, 1934). All these observations point to the presence of some plasma constituent, probably protein, as being necessary for the maintenance of the normal properties of the membrane.[1]

SUMMARY

1. The change in form of the mammalian red cell from that of a biconcave disc to that of a sphere in saline, serum, or plasma to which lecithin has been added is studied in detail. The transformation is reversible, and is unaccompanied by a change in cell volume. Different quantities of lecithin are necessary to bring about the shape change in the cells of different animals.

2. A similar change from disc to sphere is produced by the addition of the dyes of the fluorescein series to red cells in saline. The order of increasing activity is: fluorescein, eosin, erythrosine, and rose bengal. Haematoporphyrin also produces a disc-sphere transformation, and in all these cases the spheres can be turned back into discs by the addition of serum or plasma. The shape change is not accompanied by a volume change, and, in the case of rose bengal, it is apparently brought about when there are scarcely sufficient dye molecules to cover the cell surface.

3. The disc-sphere transformation which occurs between a slide and a closely applied cover-glass is discussed in the light of recent experimental results. The cause of this transformation is still obscure.

4. It is pointed out that in all cases in which the disc-sphere transformation occurs (with the exception of the case in which it occurs between slide and cover-glass), the change in form is ultimately followed by lysis. The component of the cell membrane responsible for the special shape seems to break down earlier, and sometimes very much earlier, than does the component responsible for the semi-permeability, but the breakdown of the former is invariably followed by the breakdown of the latter. The breakdown in the shape component, however, appears to modify the permeability and electrical properties of the cell surface remarkably little.

[1] It is a curious fact, however, that the electrical properties of discoidal red cells in serum or plasma are substantially the same as those of discoidal cells (Curtis, private communication). The extent to which an adsorbed film (*e.g.* of protein) would affect the electrical properties of the cell surface would, however, depend largely on the conductance of the adsorbed substance, and if this were great the effect on the electrical properties might be very small.

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