XXX. THE NATURE OF THE RED BLOOD CORPUSCLE.

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THIS investigation began as an attempt to elucidate the process of haemolysis, but it soon became clear that it was necessary first to understand the organisation of the corpuscle. The subject concerns biologists, not only for its own sake, but also for the light which it throws on the nature of animal cells in general; physicists, too, should be interested by the hypothesis that the red corpuscle is a non-spherical liquid droplet.

HISTORICAL.

Since the early days of microscopy, the question of the solid or liquid nature of the red blood corpuscle has excited interest, and opinion has fluctuated between the two hypotheses.

Leeuwenhoek [1798] states: "The blood is composed of exceeding small particles, named globules, swimming in a liquor called the serum," and "Particles...larger in fishes, they being of a flat and oval shape; whereas on the contrary, so far as I could judge from my eye, they in this animal (the bat) were spherical."

For a long time mammalian corpuscles were held to be spherical globules. Hewson [1777] was the first to recognise that human red corpuscles are in reality "flat bodies." This proves, he concluded, that they are not fluid, "because every fluid swimming in another which is in larger quantity, if it be not soluble in that other fluid, becomes globular." Yet he decided to call them "flat vesicles."

The conception of fluid vesicles was maintained by De Blainville [1829]: "The Globules are formed of a little bladder filled with fluid...in which is found the colouring matter; and each of these globules encloses others smaller, though of similar structure." He appears to have had the erroneous idea that they contain nuclei.

Schwann [1839] made the same mistake in believing that mammalian red blood corpuscles contain a nucleus. He says, quaintly: "If they were but a very little smaller they would escape observation altogether, and the blood corpuscles would then appear to be cells without nuclei." His anxiety to reduce all animal tissue to the type of nucleated cells caused him to stretch the evidence to support his theory. His idea that animal cells are typically

vesicles (the idea arising from comparison with plant cells) led him to believe that red blood corpuscles are vesicles. This belief was strengthened by the observation, first made by Hewson, that the nucleated corpuscles of the toad swell in water and become round, and the nucleus can be seen to roll about "like a pea in a bladder." In another matter he anticipated a very recent discovery. Pointing out that the cell contents differ from the surrounding medium, he says: "It might perhaps be conjectured from this peculiarity of the metabolic phenomena in cells, that a particular position of the axes of the atoms (? meaning molecules) composing the cell-membrane is essential for the production of these appearances." This exactly accords with the most modern views of molecular orientation at liquid surfaces [Clayton, 1923].

In recent years, Sharpey-Schäfer [1912] has strongly maintained the hypothesis of the fluid nature of the corpuscles, for the following reasons. (1) In hypotonic solutions they assume a globular form; whereas a solid body imbibing water would, in swelling, retain its former shape. (2) As the corpuscles circulate in the capillaries, slight variations of pressure cause distortion, which would be impossible if they were solid. (3) In the corpuscles of ovipara the nucleus readily becomes displaced.

Koeppe [1903] supports the same hypothesis, but not unreservedly: "The representation of a red blood corpuscle as a bladder filled with fluid contents is quite reasonable; it agrees very well with the observed appearances, but may not correspond to reality."

Most recent writers, following the lead of Rollett [1900] and Hamburger [1898] favour the hypothesis of a stroma containing endosoma in its pores. The great stumbling-block which has prevented acceptance of the fluid hypothesis is that which Hewson recognised, and which is re-stated by Rollett as follows: "How can one imagine that a bladder with a flexible membrane and floating in a liquid, should assume a discoid shape?" An answer to this question was given by Norris, and the point will be elaborated later. Rollett describes appearances in corpuscles treated with water or with solution of ammonium chloride, which he interprets as indicating the presence of a stroma; in numerous microscopical observations of normal blood, I have never seen anything which conveyed the same idea to my mind.

The work of Norris [1882] is referred to last, because he appears to have approached most nearly to the truth. His conclusions have not received the attention they deserve, probably because they were associated with some erroneous views on haematogenesis. The following quotations will make his position clear:

"The extensibility and retractility of blood corpuscles is that of liquids, and the elasticity of such a substance as caoutchouc is by comparison rigidity itself. If we could separate this substance into minute masses of the size of red corpuscles, they would, when submerged in liquid, appear as absolutely rigid bodies. The small motor influences, such as currents, impacts, etc., which are in operation in a specimen of blood, would not affect their form in the least. Even oil globules of the same size as the blood corpuscles are inextensible

under such delicate influences, and if the so-called stroma was no more limpid than oil the corpuscles could not possibly display any elasticity."

"The remarkable properties displayed by myelin (lipoids) at once relieve us from the necessity of considering that one liquid or solution submerged in another must inevitably take on the globular or spheroidal state. The fact is, this substance appears to represent the extending or spreading-out tendency, as opposed to the gathering-up or sphere-forming property. The biconcave and the annular forms seem to be related to a kind of balancing of these two properties."

The Corpuscle a Liquid Droplet.

Our knowledge of vital processes leads us to expect the liquid state, which would allow freer diffusion in the interior of the corpuscles. Pascucci's observation [1905] that haemoglobin crystals may separate freely inside a corpuscle, indicates the absence of internal septa. Reference has already been made to the reasons adduced by Sharpey-Schäfer. Very strong evidence is furnished by the following experiment. Although the observation is so simple, it has apparently never before been made; at least it has not to my knowledge been placed on record. When we consider the number of investigations, such as Wassermann tests, in which washed blood corpuscles are used, it is wonderful that the alteration in shape, when a change is made in the medium of suspension, should have escaped notice[1].

Exp. 1. A small quantity of blood is obtained from a puncture in the ear or finger, and it is allowed to stand until the clot has separated from the serum. The clot is then picked out, placed in a centrifuge tube and teased until most of the corpuscles have escaped from the fibrinous mesh, which is then removed. If some corpuscles and serum are mixed on a slide in the proportion of 1 in 50, covered, and examined with a 1/12th inch oil-immersion objective, a clear view is obtained of the red corpuscles in the form of biconcave discs with rounded edges. The corpuscles are washed in three changes of 0·85 % solution of sodium chloride, in order to free them from serum. The tube containing the serum is also spun in order to throw down any corpuscles which may remain in it. We have now some serum free from corpuscles, and some corpuscles suspended in normal saline solution. A microscopic preparation of the latter (1 in 50) is made and examined as before. It is seen that the corpuscles are now all in the form of spherical droplets, with a diameter about a quarter less than that of the corpuscles in their usual form. There is no "double contour." The stage of the microscope may be tilted and the cover glass touched with the point of a needle, so as to cause some commotion in the film; but however much the corpuscles move about, no phase is seen other than the single phase presented by a sphere. Any possible doubt will be dispelled by seeing the spherical and discoid forms side by side, as will be described later.

[1] Norris [1882] was familiar with the spherical shape in saline solutions, and gives photomicrographs of the same, but he did not know the reverse change.

Next, some of the washed corpuscles are mixed with serum in the proportion of 1 in 50 and a microscopic preparation made. It is seen that the corpuscles have nearly all resumed their former discoid shape. The familiar double contour and all the phases of the normal corpuscles are seen. A certain number fail to regain their normal shape, but remain as spherical droplets; in a preparation carried through without delay, not more than 1 or 2 % fail, but the proportion is greater when they have remained for some hours in the saline solution. The change of shape takes place almost instantaneously, and occurs equally well at 15° and at 37°.

It seems impossible to account for these appearances except on the hypothesis that the red corpuscle is a liquid droplet. If only the first part of the observations were considered, it might be supposed that the corpuscles change from solid to liquid by reason of some damage sustained while being washed. But the reverse change would remain unexplained: if the liquid droplet were to re-solidify on re-suspension in serum there would be no reason why it should change its shape.

A considerable number of variations of this experiment have been tried. If the corpuscles are left for a few hours in isotonic saline solution, most of them become crenated; after standing 24 hours, some of them are again discoid, the probable explanation being that a rearrangement of constituents causes a diminution of surface tension. Stronger saline solutions favour the discoid form, and the most effective salts were found to be ammonium oxalate and ammonium chloride. 1 % of either of these, added to a 0·85 % solution of sodium chloride, gives a medium in which corpuscles are nearly all discoid. It may be pointed out that these salts of ammonium readily pass through the envelope of the corpuscle, and are therefore available for reducing surface tension both from without and from within.

The serum of other animals has the same effect as homologous serum, but egg-albumin and a solution of haemoglobin proved ineffective. Dilutions of serum were tried; it was found that serum, diluted with normal saline solution in the proportion of 1 in 8, causes many corpuscles to return to their normal shape, but a dilution of 1 in 16 has little effect. In the first case spheres, discs and crenated forms can all be seen in the same preparation. There are scarcely any forms intermediate between spheres and the normal biconcave discs, just a very few oval biconvex discs: it seems as if the balance nearly always inclines one way or the other, and is very rarely found poised in a neutral position.

The Contents of the Corpuscle.

The corpuscle contains about 30 % of solids, nearly all haemoglobin: the lipoids are no more than 1 % of the total solids. From general principles, we should expect a hydrophile colloidal system, for most constituents of animal tissues are included in this class: the dispersed phase would consist of haemoglobin with water and probably salts; the continuous phase would consist of

water and salts. The following experiment, in which Hedin's [1895] haematocrit method was employed, gave results which favour this view.

Exp. 2. Some sheep's blood was taken and clotting prevented by the addition of half its volume of a 2 % solution of sodium citrate. The corpuscles were freed from serum by being washed in three changes of normal saline solution. Nearly all the supernatant fluid was removed, only sufficient being left to allow the suspension to be manipulated with a Wright's capillary pipette. A series of ten solutions of sodium chloride of various strengths from 0·6 % to 4·0 % was prepared, and equal volumes of each solution were measured into a series of small glass test-tubes. The same volume of corpuscular suspension was added to each tube, and thoroughly mixed with the solution of sodium chloride. By means of a very fine capillary pipette, these mixtures were transferred to a series of haematocrit tubes, each being filled to a height of 10 cm. and labelled. As haematocrit tubes, pieces of quill-tubing, 11 cm. long with 1 mm. bore, sealed at one end, were used. The whole were then spun for 3 hours in a water-centrifuge: at the end of that time, the height of the deposit in each tube was measured.

It is necessary to calculate the percentage of sodium chloride in the solutions at the end of the experiment. In each tube were placed at the beginning 5·0 cm. of sodium chloride solution of various strengths and 5·0 cm. of suspension of corpuscles. From the deposit in tube 4, it is seen that the amount of corpuscles was 3·0 cm., and therefore 2·0 cm. of 0·85 % solution of sodium chloride was introduced along with them. The percentage of sodium chloride in any tube is therefore given by the formula

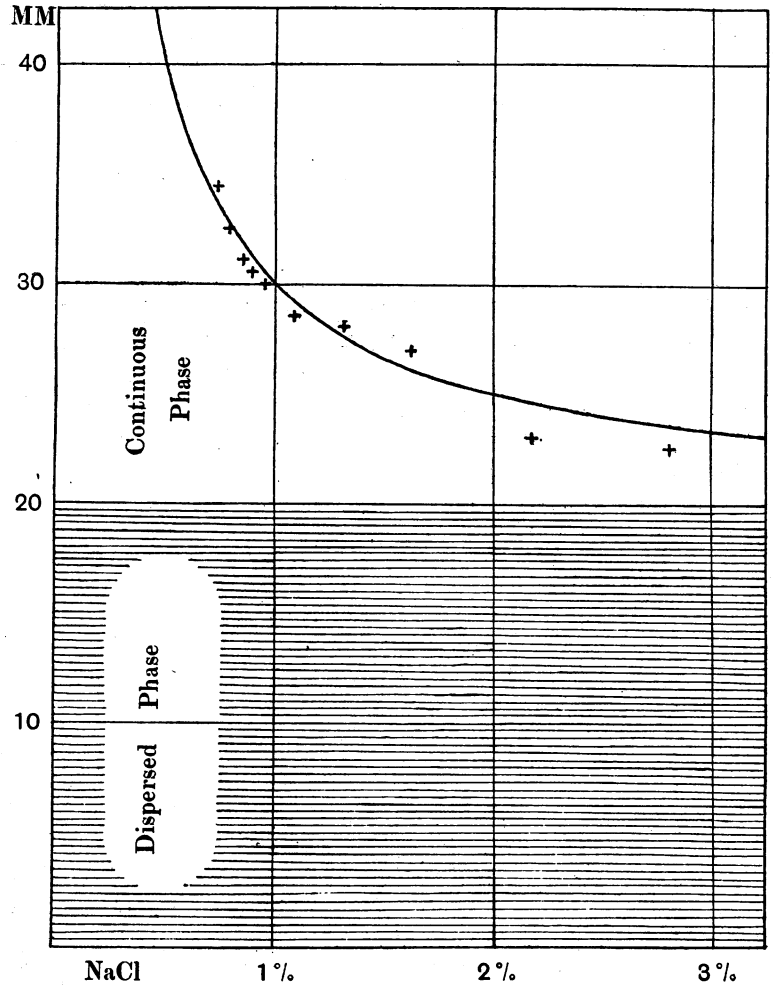
$$\frac{5 \times P + 2 \times 0{\cdot}85}{10 - D},$$

where P is the percentage of sodium chloride in the solution at first, and D is the height of the deposit in centimetres. This is not absolutely correct, since the solution in the interstices between the corpuscles is not taken into consideration; but the error is negligible. The results are given in the following table:

Tube	Sodium chloride solution used %	Calculated percentage at end	Height of sediment of corpuscles cm.
1	0·6	0·72	3·45
2	0·7	0·77	3·25
3	0·8	0·83	3·1
4	0·9	0·89	3·05
5	1·0	0·96	3·0
6	1·2	1·08	2·85
7	1·5	1·28	2·8
8	2·0	1·60	2·7
9	3·0	2·17	2·3
10	4·0	2·80	2·25

The results are represented graphically in the diagram, the ordinates representing volume of corpuscles and the abscissae concentration of solution: the observed results are marked by crosses. There is much evidence to show

that the envelope of the corpuscle is approximately "semipermeable" to most neutral salts such as sodium chloride. This being so, the volume of the contents concerned in osmotic equilibrium should vary inversely as the concentration of the surrounding fluid. It will be seen that the values do fulfil the requirements of the osmotic theory if we exclude a volume of 2·0 cm. (two-thirds of the normal bulk of the corpuscles) as taking no part in osmotic



equilibrium. The values calculated on this supposition are represented by the continuous curve and the observed values lie fairly close to it. Perfect agreement cannot be expected, for there are at least three sources of discrepancy: (1) as the corpuscles shrink, the increased concentration of saline contents may cause an alteration in the amount of water imbibed by the haemoglobin; (2) a gradual permeation of sodium chloride through the envelope, which is not

absolutely semipermeable; (3) alteration in surface tension and therefore in intracorpuscular pressure. We may conclude that the corpuscle contains a hydrophile colloidal system, the dispersed phase being haemoglobin (30 %) associated with 35 to 40 % of bound or imbibed water and probably with salts, the continuous phase being the remaining 30 to 35 % of water with dissolved salts.

The dispersed phase constitutes 65 to 70 % of the whole volume. Now, a large number of small equal spheres, packed as closely as possible without deformation, occupy 74 % of the total volume. Hence, if the dispersed phase is composed of equal spheres, they are not packed quite so closely as possible, and there is no serious impediment to free diffusion within the corpuscle. But the margin is small and it is easy to imagine that increased concentration of the body-fluids might impair the oxygen-carrying capacity of the corpuscles.

The Surface Film.

Pascucci [1905] showed that this is composed of lipoids and proteins. From physico-chemical principles, it is clear that substances which reduce surface energy will become concentrated at the surface, and this applies to substances from the interior of the corpuscle as well as those from the surrounding medium. It sometimes happens that such a concentration is so great as to lead to the formation of a solid surface film: we may now consider whether this is the case with the corpuscle.

When blood is laked by the addition of water, the envelopes of the corpuscles remain as "ghosts" and the appearance with high magnification suggests that these are collapsed bladders with extremely thin solid walls. The "ghosts" are seen both when blood is laked with cold water and when it is dropped into water at 37°: this indicates that the surface film is solid at the temperature of the body. "Ghosts" are also seen when blood-corpuscles, previously washed in normal saline solution at 37°, are laked in water at the same temperature or at room temperature. This shows that the surface film constituting a "ghost" is not removed by washing away the serum: if it is formed by solidification of constituents of the serum, re-solution does not readily occur.

The action of polyvalent ions throws light on the nature of the surface film. It is known that suspensoid colloids are agglutinated by polyvalent ions in extremely dilute solution, whereas emulsoid colloids are not nearly so sensitive; also, the ions causing agglutination carry an electric charge opposite in sign to that of the colloid particles.

Exp. 3. Sheep's blood was taken, and clotting prevented by the addition of half its volume of 2 % solution of sodium citrate. The corpuscles were freed from serum by being washed in six changes of 0·85 % solution of sodium chloride. A series of dilutions of the test substance was prepared, 0·85 % of sodium chloride being present in every one. About 0·2 cc. of each dilution was placed in a small tube and to each was added one platinum-loopful of the

washed corpuscles. The tubes were gently agitated until their contents were thoroughly mixed, they were then allowed to stand for 12 hours at room temperature. In the absence of agglutination the corpuscles settled as a sediment with a perfectly level surface, and none adhered to the sides of the tube above; agglutination was recognised by flocculi adhering to the sides of the tube.

By this method it was found that sheep's red corpuscles are agglutinated by

$CeCl_3$	...	...	1 in 1,000,000
$La(NO_3)_3$	...	...	1 in 1,250,000
$Th(NO_3)_4$	...	...	1 in 80,000
$FeCl_3$	...	...	1 in 300,000
$CaCl_2$	...	...	1 in 400
$K_4Fe(CN)_6$	...	...	1 in 100

These may be compared with the following results:

Human serum gave a precipitate with $CeCl_3$ 1 in 2000, but gave no precipitate with $K_4Fe(CN)_6$ 1 in 200.

Sheep's corpuscles laked by the addition of distilled water gave a slight precipitate with $CeCl_3$ 1 in 100,000. On microscopical examination, this precipitate was found to consist of leucocytes, with an amorphous substance in which "ghosts" could not be identified with certainty. On the surmise that lipoids might be concerned in the agglutination, a test was made with "Wassermann antigen" composed of the lipoids of heart muscle. It gave a precipitate with $CeCl_3$ 1 in 12,500, but none with $K_4Fe(CN)_6$ as strong as 1 in 125.

From these results it may be concluded that the red corpuscle has a solid surface film, which carries a negative electric charge when the medium of suspension is a solution of sodium chloride. The effective constituents of the film are probably the lipoids: it is true that these do not show agglutination or precipitation in such a high dilution as the corpuscles, but the phenomenon is more easily detected in the last, since a minute quantity of lipoid film has a relatively huge coloured content which renders any agglutination easily visible.

A rough calculation shows that if all the cholesterol and lecithin in the corpuscles were present as a continuous film on the surface, the film would be 2 to 3 $\mu\mu$ thick. As an atom of hydrogen is estimated to have dimensions of 0·25 $\mu\mu$ and a molecule of starch 5 $\mu\mu$, it is an interesting speculation that the lipoids may be present as a single layer of molecules.

Although the evidence points to the existence of a solid surface film, yet this may undergo changes, as will be suggested later in discussing crenation.

The *permeability* of the surface film has been the subject of a large amount of research, the results of which can only be summarised here [see Hedin, 1897; Gryns, 1896; Stewart, 1899, 1900; Rywosch, 1907]. Substances fall into four classes, according as they pass through the membrane freely, gradually, very slowly, or not at all. Freely permeating substances include water, urea, alcohols, ethers, esters and some ammonium salts: glycerol permeates gradually:

most neutral salts pass through very slowly indeed: the sugars are completely excluded. These differences depend not only on the size of the molecules, but also on their chemical nature.

The permeability is readily altered in many ways. The resisting power of the film is increased by hardening agents, such as formaldehyde and salts of silver, mercury and zinc. On the hypothesis of the corpuscle as a liquid vesicle, haemolysis is explained simply as the passage of the fluid contents through the damaged envelope, and it will be well to review the different kinds of haemolysis so as to find whether that explanation is adequate.

1. *Haemolysis in water* or in too dilute saline solutions is brought about by endosmosis of water until the distension is so great that the surface film becomes porous and allows the contents to leak out.

2. *Freezing and thawing.* Koeppe [1903] suggested that at some stage of the cycle, ice-crystals melt and a too dilute solution is formed in certain places: haemolysis then occurs as in (1). But cold may also alter the state of aggregation of particles in the surface film, and its permeability may be thereby increased.

3. *Heat.* An obvious explanation is that haemolysis is due to melting of the fatty film [Koeppe, 1903].

4. *Electric shocks* appear to act by mechanical injury to the envelope [Rollett, 1900]. Continuous currents cause haemolysis by the liberation of acid and alkali at the poles. Alternating currents cause a rise of temperature, which lakes the corpuscles.

5. *Fat solvents* remove some essential constituent of the film [Koeppe, 1903].

6. *Substances lowering surface tension*, such as saponin and bile-salt, probably displace the lipoids and so disintegrate the surface film.

7. *Inert substances* in the form of fine powders, such as barium sulphate and kaolin, adsorb some constituent of the film.

8. *Acids.*
9. *Alkalis.* } These change the lipoids substances to fatty acids and soaps respectively.

10. *Strong saline solutions*, such as 10 % solution of sodium chloride, may damage the surface film by taking away water which is necessary for its integrity.

11. *Venoms and toxins.*
12. *Natural haemolysins.*
13. *Specific haemolysins.* } In spite of the tremendous amount of research during the past quarter of a century, it cannot be said that a completely satisfactory explanation is available, but the view that haemolysis is brought about by the disintegration of a surface film composed of a mosaic of lipoids and proteins is an attractive one. It may be that the specificity of these reactions is related to the special pattern of the mosaic as well as to the special nature of the proteins and lipoids. According to Taniguchi [1921], lipoids are an essential constituent of the "receptors" of sheep's corpuscles concerned in heterophile antiserum haemolysis, while those acted upon by isophile immune body are of protein nature.

14. *Spontaneous haemolysis.* One element appears to be a gradual permeation of salts into the interior of the corpuscles, until the surrounding solution becomes hypotonic.

It will be seen that explanations of all the varieties of haemolysis can be founded on the vesicular hypothesis.

The Characteristic Shape.

Calculation shows that a sphere having the same volume as a human red blood corpuscle would have a diameter of about 5μ. Its surface would be about 20 % less than that of the corpuscle: the discoid shape is therefore advantageous in presenting a larger surface for interchange between the plasma and the contents of the corpuscle. A more important consideration is that no point in the interior of the corpuscle is distant more than $0{\cdot}85\mu$ from the surface, whereas the centre of the spherical body would be $2{\cdot}5\mu$, or about three times as far, from the surface: the discoid form therefore favours rapid diffusion from the surface of the corpuscle to every part of the interior. There is a slight disadvantage in that the larger diameter needs a large calibre of capillaries.

The question arises how the corpuscle assumes its peculiar shape. It might be supposed that the vesicle has a solid envelope of a definite shape: it is difficult to reconcile this view with the observations described under Exp. 1 and with those to be described in connection with crenation: if this view were accepted, we should have no conception how the envelope took its definite shape in the first instance, and that would be as great a mystery as the one supposed to be elucidated.

I believe with Norris [1882] that "the Biconcave Shape is due to the operation of physical conditions and not to structural restraint." The simple geometrical figure suggests its production by a few simple factors. The envelope of the corpuscle is practically semipermeable; from which it follows, in accordance with the laws of osmosis and imbibition, that the volume of the corpuscle must have a definite value, which depends on the surface tension and on the tonicity of the surrounding medium. At the surface of the corpuscle, there is a solid film enclosed between two liquid films, those of the corpuscular contents and of the surrounding fluid. The solid film is elastic but its extensibility is limited. The tension of the two liquid films would reduce the corpuscle to a sphere, were there no opposing force. What is the nature of this opposing force? I suggest that it is a repulsion between the dispersed particles of the colloidal system in the interior of the corpuscle: it may be an electrostatic repulsion caused by the similar electric charges of the particles, or some chemical force may be responsible. There may be a similar state of affairs in the solid film itself (the "spreading-out tendency" of Norris). If the surface tension were very great compared with the repelling force, the shape would be spherical. When repulsion predominates, the result is the familiar biconcave disc with rounded edges.

It may be asked why the same phenomenon is not commonly seen in emulsions. In ordinary emulsions the droplets are homogeneous and forces of repulsion do not arise in their interior. As Norris pointed out, droplets composed of myelin may assume a biconcave or annular form. In the case of spheroidal cells, it is probable that their contents are composite, the different constituents neutralising the affinities of one another in such a way that forces of repulsion do not develop.

It is now possible to understand the change of shape seen in the first experiment. In serum there are many constituents which reduce surface energy and the surface tension is therefore very small: repulsion predominates, and the discoid shape results. With corpuscles washed free from serum and suspended in isotonic salt solution, the surface tension is much greater, the forces of repulsion are overcome, and the spherical form is produced. When the corpuscles are re-suspended in serum, the original conditions are present and the original form is resumed.

In the case of nucleated non-mammalian corpuscles, if we imagine an elongated nucleus floating in a corpuscle otherwise resembling that of a mammal, it is easy to see that the dispersive forces will cause the haemoglobin-system to occupy the periphery, and the nucleus will be forced to the centre. The oval form of camel's corpuscles is not yet explained.

Crenation.

This may affect the entire surface of the corpuscle or it may be limited to the margin of the disc. The processes may be very fine, resembling when seen under an oil-immersion lens the hairs on a gooseberry: from this there are all degrees of coarseness up to the stage where three or four processes take up the whole surface of the corpuscle. As a rule, the projections are conical, but sometimes they are rounded.

The conditions determining the state of crenation are elusive. Two preparations may be made as nearly alike as possible; one will show crenated corpuscles and the other will show none; in fact, in the same microscopic field, the corpuscles on one side may all be smooth and those on the other side all crenated. Corpuscles become crenated after standing for some time in normal saline solution or in serum: the phenomenon is often very striking when the medium of suspension is serum diluted with about eight times its volume of normal saline.

Crenated corpuscles can be brought back to the state of smooth spheres (in normal saline) by warmth, or by the action of dilute acid or alkali. The optimum temperature for producing the change is 45°: 40° is ineffective and 50° causes destructive changes. The strengths of hydrochloric acid and sodium hydroxide found best were 0·002 % and 0·01 % respectively, each being associated with 0·85 % of sodium chloride.

Crenation is usually explained as being a shrinking in hypertonic solutions.

This is evidently not the case, since it is best seen in isotonic solutions, and the wrinkling of corpuscles in hypertonic solutions is not the same thing.

The following explanation is suggested. The surface film of the corpuscle is under normal conditions uniform, at least so far as any microscopical examination can detect. With lapse of time and fall in temperature, there is liable to be a change in the state of aggregation of the micellae of the film: in some parts there may be denser masses and in other parts there may be rarefaction of the envelope; the change being analogous to agglutination or precipitation. The thin parts yield to the intracorpuscular pressure, and, according as the pattern of dense and thin portions is fine or coarse, so fine or coarse will be the crenations. When the temperature is raised, or in the presence of a trace of acid or alkali, the micellae spread out evenly and the original shape is resumed.

Summary.

1. The red corpuscle is a fluid droplet. The following evidence leads to this conclusion:

(*a*) Haemoglobin crystals separate freely in the interior of a corpuscle.

(*b*) The corpuscles assume a spherical form in hypotonic solutions.

(*c*) The nucleus of oviparous corpuscles is readily displaced.

(*d*) Slight forces produce deformation.

(*e*) In isotonic saline solutions a spherical form is assumed, and the reverse change to the discoid form takes place when the corpuscles are again placed in serum.

2. The contents form a hydrophile colloidal system. The change of volume in solutions of different concentration shows that the continuous phase (salts and water) constitutes one-third of the whole. The dispersed phase (haemoglobin, salts and water) forms the remaining two-thirds.

3. The surface film contains lipoids in a solid state; this is shown by the microscopic appearance of "ghosts," and by the agglutinating action of polyvalent kations in extremely dilute solutions. Its permeability by different substances is mentioned. The permeability is diminished by fixing agents: increased permeability results in haemolysis. All varieties of haemolysis can be explained as due to disintegration of the envelope of the corpuscle.

4. The advantages of the discoid shape are pointed out. The characteristic shape is the result of two sets of forces:

(*a*) tension of liquid surfaces tending to cause a spherical shape;

(*b*) repulsive forces between the dispersed particles of the corpuscular contents.

5. Crenation is briefly described. It is explained as due to protrusion through weak areas in the envelope of the corpuscle.

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