ANALYTICAL REVIEW

The Pathogenesis of Spherocytes and Leptocytes (Target Cells)

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The size, shape and survival time of normal human erythrocytes are marvellously constant. Their uniform size, for example, is such a reliable characteristic that abnormalities have long been the basis for a standard classification of anemias. It is only recently, with increasing knowledge of causative factors, that the terms microcytic and macrocytic anemia have lost their old nosologic precision. The survival time of the red cell has been carefully scrutinized in recent years. This, too, has proved to be remarkably constant and has been rather definitely established at about 120 days. It is now generally appreciated that shortening of the red cell survival time is synonymous with hemolytic disease.

The shape of the red cell is also significant. Bizarre shapes (poikilocytosis) are characteristic of red cells in the process of fragmentation and in some diseases of the bone marrow there are red cells shaped like tear drops, tennis rackets, dumb bells or belaying pins. Of immediate interest in this study are variations from the normal shape which involve the red cell's thickness.

The normal human red cell is a biconcave disc. It is composed of a fixed framework of protein (stromatin) in which is contained a heavily concentrated, almost crystalline solution of hemoglobin. The outer surface of the cell is covered with a thin layer of lipoid most of which is in lipoprotein combination with the underlying stroma. Mitchison has recently suggested that the stromal envelope of the red cell is much thicker than had been previously supposed. Instead of a layer several molecules deep, it appears from these new observations that a framework of fibrillar stromatin extends into the cell about 0.5 μ. The inner sides of this structure probably touch at the center of the cell's biconcavity. The surface structure of the red cell actually comprises over 50 per cent of the volume of the cell. Much of the hemoglobin must lie within the fibrillar framework of this thick surface structure. It is apparently held there by an electrostatic bond between the molecules of the pigment and those of the protein. When the electrostatic charge of the stromatin is neutralized by reducing the pH to its isoelectric point the hemoglobin escapes from the red cell. Even after hemolysis by weak acid or distilled water, the depigmented stroma retains the shape of a biconcave disc. The stromal surface of the red cell is, in effect, a "stressed skin." The shape is moulded in the structure and the resilience of the stroma causes the cell to resume the normal shape after distorting forces have been removed. The red cell is extremely flexible.

Although we speak of a "fixed" framework it is fixed in the sense that it is...
not fluid, and the shape of the cell may be modified despite the mould of the stroma. A definite shape implies a definite volume. When the volume of an object changes, the shape or size or both must alter accordingly. Red cells that are thicker or thinner than normal may owe their abnormalities to certain disproportions between the volume and the surface area of the cell.

It is proposed to examine the variations in cellular thickness and to show that they may be correlated with the volume-surface area ratio. Several interesting concepts are revealed thereby. The small, thick spherocyte possesses a disproportionately small amount of surface for its volume. The thin target cell is, in a sense, the antithesis of the spherocyte and has a greater than normal surface-to-volume ratio. It is proposed also to examine the pathogenesis of these cells. There are different sorts of spherocytes and target cells and they are not all produced in the same manner. The historical background of these abnormal red cells is also of some interest, for our concepts of hemolytic anemia have developed step by step with investigations of abnormal red cells, especially the spherocyte.

Vanlair and Masius were evidently the first to propose that a disease might be due to an abnormally rapid destruction of red blood cells. These Belgian physicians in 1871 recorded their studies of a patient with anemia and “microcythemia” and described the red cells as being small in diameter (5 μ) and spherical in shape. Their report was illustrated with lithographs which demonstrated the spherical character of the red cells which they called dwarf cells or microcytes and defined as “red cells on the way to destruction.” Vanlair and Masius proposed that the patient’s jaundice was a reflection of the excess bile pigment formed when hemoglobin was released from the “senile” red cells. In 1877 a second case of anemia and jaundice with spherical microcytes was reported by Litten who agreed with the concepts of Vanlair and Masius. Litten also described a case of hemoglobinuria (associated with puerperal sepsis) and observed that the red cells were normal in appearance. He suggested that not all diseases of blood destruction are associated with spherocytosis. These works apparently attracted little attention at the time of their publication, perhaps because a more facile concept of anemia was in vogue. Biermer had studied a large group of cases, many of them occurring in young adult silk workers with a fulminating disease characterized by profound anemia and purpura. He called these cases primary pernicious anemia. For many years thereafter almost every case of anemia which was not obviously due to hemorrhage was dropped into Biermer’s catch basket, and hundreds of cases of “primary pernicious anemia” were recorded in the latter decades of the nineteenth century. Probably only a small proportion of these patients would be diagnosed today as pernicious anemia. Eichhorst collected a large series of these cases, probably representing various types of anemia including hemolytic disease. In many of them he noted the presence of small red cells, which he proposed were pathognomonic of primary pernicious anemia. William Hunter later suggested the use of the term “Eichhorst’s corpuscles” for these small red cells. Hunter did much to define the disease which is now recognized as pernicious anemia, separating it from the many other types of anemia by such hallmarks as glossitis, gastro-enteritis and the presence of abnormal hemolysis. Hunter, in fact, coined the word hemolytic to describe the anemia which occurred in this disease and in the experimental anemia of dogs produced by hemolytic chemicals. Hunter observed the small round “Eichhorst corpuscles” in the experimental anemias and became convinced of their importance in the pathogenesis of hemolytic disease.

* This is the manner in which Hunter first used the word “hemolytic” (italics are his): “In haemopoeitic anemias we do not usually find in the blood the small yellow microcytes, perfectly spherical in form and deep in colour, which point to an anemia of haemolytic origin.”
The observations of William Hunter and those who preceded him were made on preparations of fresh, unstained blood. Meanwhile, Ehrlich had introduced the aniline dyes, and the study of fresh red cell preparations was largely abandoned. Three dimensional cells became two dimensional on the stained smear. Thus when the small red cell was rediscovered in cases of familial hemolytic jaundice by Chauffard in 1907, its spherical quality was not appreciated. Chauffard did, however, point out that these cells were abnormally fragile in hypotonic salt solutions. The spherical shape of the cells was established by Christophers and Bentley in a remarkable monograph on blackwater fever published in 1908.

These British Army medical officers who were assigned to India to study that problem made some very careful descriptions of the characteristics of this cell and proposed the diagnosis of spherocyte, a term usually ascribed to Naegeli. The latter, however, stated that the spherocyte was pathognomonic of familial acholic jaundice, a dictum which tended to canalize thinking about hemolytic disease for the next fifteen or twenty years. Von Boros extended Naegeli’s concepts, showing that the diameter of the cell could be correlated with its thickness: as the diameter became smaller the thickness increased, the volume of the cell remaining constant. Haden correlated the abnormality of shape with osmotic fragility. He demonstrated that normal disc shaped red cells in hypotonic solutions became increasingly thick as they took up water. The spherocytes of congenital hemolytic jaundice were thick to begin with and could therefore take up less water before reaching capacity, when they became hemolyzed. Increased osmotic fragility was thus indicative of spherocytosis. Guest later showed that the normal red cell and the red cell in hereditary spherocytosis are in fact nearly perfect osmometers.

Naegeli’s concept of the spherocyte as a pathognomonic sign of familial acholic jaundice was finally invalidated by the work of Dameshek and Schwartz who, in 1938, demonstrated that acquired hemolytic anemia was frequently associated with spherocytosis, and that experimental hemolytic anemias produced by hetero-antibodies were also associated with spherocytosis and increased hypotonic fragility. Thus they demonstrated that the spherocytic abnormality could be acquired, and postulated that the spherocyte was an injured red cell on its way to destruction.

During the period reviewed above, considerable discussion took place concerning the pathogenesis of the spherocyte and the mechanism of hemolysis in hereditary spherocytosis. There have been three schools of thought regarding these matters. (1) The bone marrow is abnormal and produces an abnormal red cell. (2) The red cells are normal as they issue from the marrow but are injured in the circulation by an autohemolysin or other noxious agent. (3) The disease is due primarily to an abnormal spleen which injures and destroys the red cells. This last concept was suggested by Vanlair and Masius whose attention was directed to the spleen by its size in their patient and her mother. Vanlair and Masius believed the spherocyte to be an injured cell and the spleen to be the site of injury. The spleen, they said, did not destroy the cells for if that were the case the spherocytes would not be found in the circulating blood. Hunter subscribed to the theory of injurious agents in the circulation. He saw the red cells in his experimental animals become small and round after he injected lytic agents. He
extended his beliefs to pernicious anemia and suggested that the abnormal hemolysis of that disease (and in fact the disease itself) was due to absorbed intestinal toxins.

After the demonstration by Chauffard\(^1\) of the increased osmotic fragility in hereditary spherocytosis the hemolytic process was often ascribed to the "increased fragility"\(^5\) of the red cells although it was conceded that the hypotonic conditions which might exploit this fragility do not exist in the circulation. Attention was directed to the spleen when splenectomy was found to cure hereditary spherocytosis. As late as 1949 it was proposed that the disease was primarily splenic\(^13\) although this is difficult to correlate with the observation that, while splenectomy cures the hemolytic anemia, it does not correct the spherocytosis.* Patients examined many years after the operation continue to show this abnormality.\(^12\) There can be no doubt that passage through the spleen increases the osmotic fragility of some of the abnormal red cells,\(^15\)\(^-\)\(^21\) but the means whereby the cells are injured and destroyed remains obscure.

Dameshek and Schwartz\(^11\) favored the theory of a circulating hemolysin to explain the spherocytosis of the hereditary disease. They proposed an analogy between the appearance of spherocytes after injection of anti-erythrocyte sera and the appearance of spherocytes in the hereditary disease. The reticulocytes in hereditary spherocytosis are disc shaped as they issue from the bone marrow, and become spherical as they mature. Might there not be, asked Dameshek, a circulating hemolysin in hereditary spherocytosis which injures the patient's mature red cells? This concept gave rise to some lively discussions, but studies of the survival of transfused red cells have tended to disprove it.

Dacie and Mollison\(^7\) showed that normal cells transfused into patients with hereditary spherocytosis survive normally (120 days). On the other hand, spherocytic blood transfused into a normal circulation survives a very short time (10 to 20 days). Recently Schrumpf\(^39\) has demonstrated that spherocytic red cells transfused into the circulation of a person whose spleen has been removed survive an almost normal length of time. Paolino's\(^44\) meticulous measurements of the reticulocytes in the bone marrow and blood of patients with hereditary spherocytosis have shown that, although these reticulocytes are disc shaped, they are in fact smaller in diameter and thicker than the reticulocytes of corresponding age found in normal blood and bone marrow. All of these studies indicate that there is almost certainly no "extrinsic" mechanism acting upon the red cells in this hereditary disease, but that the inherited defect involves the red cell structure. The spleen is at fault only insofar as its peculiar structure results in trapping of spherocytes and thus in their increased destruction.

The consensus, then, at the present time is this: in the hemolytic anemia of hereditary spherocytosis the structure of the mature red cell is faulty, its fault being a variant toward the spherical shape which invites destruction by the

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* In hereditary spherocytosis the red cells remain spheroidal after splenectomy although they do show some modification of their shape in the direction of normal. Osmotic and mechanical fragilities are reduced, but both fragility tests remain decidedly abnormal.\(^5\) The slight change in shape of the spherocyte is probably related to an alteration of the cellular surface of the sort found in the red cells formed by a normal bone marrow after splenectomy. (See below the paragraph on the post-splenectomy target cell.)
spleen. Otherwise the hereditary spherocyte seems normal. As far as the carriage of oxygen is concerned the cell is functionally adequate and when the spleen is out of the circulation it can live a nearly normal span of days. The increased mechanical fragility (in vitro) of the hereditary spherocyte persist after splenectomy although it is less marked. This abnormal fragility, like the abnormal osmotic fragility of the sphereocyte seems to be little or no impediment to its survival once the spleen has been removed.

On the other hand, the sphereocyte of acquired hemolytic anemia is released from the marrow as a normal red cell and is then injured by an immune-body hemolysin or some other agent. The spherical red cell that results is just as susceptible to destruction by the spleen as the hereditary sphereocyte. But there is an important difference. Because the acquired sphereocyte has been injured by an extrinsic agent, such as an hemolysin, removal of the spleen may not be enough to spare it from premature destruction. Frequently the injurious agent persists after splenectomy, and when this happens the operation does not cure the hemolytic disease.

**COMMENT**

It has always been emphasized that the spheral red cell represents a primary abnormality of shape. Morphologically this is the most obvious defect and functionally it probably accounts for the destruction of such cells by the spleen. Actually, however, the increased thickness and small diameter of the red cells would appear to depend upon a relationship which exists between the red cell volume and its surface area. Haden2° mentioned this concept, ascribing it to von Boros, but Barrett described it as the "essential" of Haden's theory of hypotonic fragility.

A normal, disc shaped human red cell, with a diameter of 8.4 μ has a surface area of approximately 155 sq. μ and a volume of about 90 cu. μ. A change in either the volume or the surface area would result in a change in shape (table 1). If the surface area were made smaller than 155 sq. μ the cell would have to be thicker and more spherical to accommodate a volume of 90 cu. μ. If the volume were increased beyond 90 cu. μ and the surface remained 155 sq. μ again the cell would have to thicken. A true sphere represents a shape which is able to contain a given volume within the least surface. Any alteration in a disc shaped red cell which increases the volume or decreases the surface results in a change of shape in the direction of a sphere. Such alterations of the volume-surface proportion may arise in a variety of ways.

1. *The "Osmotic" Spherocyte*

When red cells are placed in hypotonic solutions they behave as osmometers and take up water in amounts which are proportional to the tonicity of the solution. The cell volume increases but the surface area remains unaltered. The red cell becomes spheroidal, and the change in shape is a result of the change in volume (table 1). It may be calculated mathematically that a normal red cell, with a surface of 155 sq. μ and an original volume of 90 cu. μ would become a complete sphere when its volume is increased to 180 cu. μ, its surface meanwhile remaining unchanged.*

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*Actually osmotic hemolysis occurs before the red cell becomes a perfect sphere. An
2. The "Acquired" Spherocyte

This term is used here to designate a cell that has become a spherocyte after beginning its career as a red cell of normal size, shape and life expectancy. It is the fragile short-lived red cell of acquired hemolytic anemia, a cell that has become spheroidal because of injury initiated in many cases by antibodies. The patient's own red cells undergo this alteration of shape as do normal cells transfused into such a patient. Anti-red-cell serum injected into healthy animals induces a similar change in the animal's normal red cells. The volume of the acquired spherocyte is not affected during the change in shape. The disproportion between volume and surface that is characteristic of spherocytes is due to a diminution of surface area. Injury causes the surface to contract, and the cell becomes spheroidal, thereby containing its unaltered volume within the shrunked surface area.

<table>
<thead>
<tr>
<th>Table 1.—Dimensions of Red Cells of Several Shapes</th>
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<tr>
<td>Diameter (μ)</td>
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<td>---------------</td>
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<tr>
<td>Normal Red Cell</td>
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<tr>
<td>Hereditary Spherocyte</td>
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<tr>
<td>Postsplenectomy Target Cell</td>
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<tr>
<td>Normal Reticulocyte</td>
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<tr>
<td>&quot;Acquired&quot; Spherocyte</td>
</tr>
<tr>
<td>&quot;Osmotic&quot; Spherocyte</td>
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To permit ready comparison of surface areas, the figures in this table are based on a red cell with a corpuscular volume of 90 cu. μ. The "acquired" spherocyte represents a red cell whose surface has shrunked tightly around the unaltered cellular contents (90 cu. μ). The "osmotic" spherocyte results from the exposure of a normal red cell to a hypotonic environment until its volume is increased to fill completely the available surface area of 155 cu. μ.

The cell diameters are those found in wet preparations and are larger than those found in dry smears. The thickness shown for the normal red cell is less than the actual measurement at its thickest point to allow for the thinness at the biconcavity. The diameter of the reticulocyte was deliberately selected as representing a rather large and therefore a young cell.

All of the figures represent values of single red cells. Surface and volume of disc shaped red cells are computed by the following formulas which treat the cells as thin cylinders.

\[ \text{Surface area} = 2 \pi r (h + r) \]
\[ \text{Volume} = \pi r^2 h \]

Surface and volume of the true spheres were calculated by the formulas:

\[ \text{Surface area} = 4 \pi r^2 \]
\[ \text{Volume} = \frac{4}{3} \pi r^3 \]

where \( r \) is radius and \( h \) is thickness.

Increase from 90 to 180 cu. μ doubles the volume of the cell (table 1). But Guest has shown that the cell becomes hemolized when its volume is about 1.7 times the original. This suggests that the cell in hypotonic solution is not destroyed by "bursting." Perhaps dilution of the ionic or molecular concentration within the cell weakens the electrostatic bond that exists between the hemoglobin and stromatin molecules. Osmotic hemolysis is not the all-or-none reaction of a pricked balloon. After blood has been treated with distilled water the sediment contains some intact red cells and some quite free of hemoglobin, but most show intermediate degrees of hemoglobin loss.
A similar contraction of the surface membrane occurs when normal red cells are treated in vitro by a variety of hemolytic agents. The cells become spheres without a change in volume after passing through stages of crenation. The uneven contours of the crenated red cell indicate an irregular shrinking of the surface. As the shrinking continues it involves the crenations themselves. The cell becomes smooth again but it is now a sphere, and its surface is tightly contracted about the unaltered cellular contents. If, during this change, the red cell volume has remained at 90 cu. μ, the surface area of the final sphere may be calculated to be approximately 100 sq. μ (table 1), a diminution of about one third.

3. The “Spherocyte of Stasis”

When red cells are allowed to incubate in their plasma—either in a test tube at 37 C. or in the sinusoidal spaces of the spleen—they tend to swell and become spheroidal. Bergenhem and Fahraeus proposed that the change was due to injury of the cell by lysolecithin evolved from plasma lipids by the enzyme, lecithinase. This theory has been seriously questioned. The spherocyte formed by lysolecithin in vitro shows no increase in volume or osmotic fragility, whereas the spherocyte that results from incubation shows both. The “spherocyte of stasis” doubtless increases its volume by taking up water. Since the change occurs while the cell lies in its own plasma it is not likely that osmotic swelling could result from hypotonicity of the environment. Yet the acceptance of water by a red cell is an osmotic phenomenon, the water moving in the direction of increased osmotic activity.

The reason for the swelling of the “spherocyte of stasis” has recently been demonstrated by Maizels who has shown that the increased osmotic activity in the red cell is related to the stiffing of the cell’s metabolism by a lack of glucose. The glycolytic metabolism of the red cell provides energy whereby the potassium concentration within the cell is kept high and the sodium concentration low as compared with the concentration in the plasma of these two ions. When blood is allowed to stagnate in spleen or test tube the available glucose is expended and cannot be replaced. The mechanism for maintaining a high potassium concentration fails for lack of energy and the potassium leaves the cell. The sodium-excluding mechanism also fails and sodium enters the cell. But the exchange is not equivalent. The red cell gains more ions than it loses and the increase of ionic concentration requires that water also enter to maintain osmotic equilibrium. Hence the “spherocyte of stasis.” This ionic exchange and consequent swelling can be prevented in vitro by adding glucose to the incubating blood. Maizels has also shown that the change is a reversible one. Red cells were stored at 4 C. until the potassium-sodium exchange had taken place. The blood was then transfused into a normal subject, and within a few hours the cells had lost the sodium and regained their normal potassium. Such storage does not affect the survival of transfused red cells in a normal circulation. One might argue by analogy that the swelling caused by stasis in the spleen is also a benign, completely reversible reaction. There is no reason to believe that the normal spleen does anything to hasten the destruction of normal red cells. Their survival is unaffected by splenectomy.
4. The Anti-Sphering Factor

Furchgott and Ponder\textsuperscript{17} have demonstrated the presence of a factor in the albumin fraction of plasma which is essential to maintain the normal disc shape of red cells. When the “anti-sphering factor” is removed from the red cells (by adsorption on glass) they become spheres without a change in volume. Stated otherwise, the surface area diminishes. The change is analogous to that which occurs when the normal red cell is injured by hemolytic substances, but in this instance the change is evidently accomplished without injury and is completely reversible, the sphere becoming a disc when it is again coated with this albumin fraction. Molecules of the anti-sphering factor may be imagined to enter tiny crevices in the red cell’s surface and by widening these crevices to increase the surface area of the cell.\textsuperscript{37}

5. The Hereditary Spherocyte

The abnormal red cell of hereditary hemolytic anemia derives within the bone marrow from an erythroblast of normal proportions. The cell is delivered to the circulating blood as a reticulocyte which is disc shaped\textsuperscript{11, 36} although somewhat smaller in diameter and somewhat thicker than the normal reticulocyte.\textsuperscript{36} The hereditary spherocyte encloses a normal cellular volume and a normal amount of hemoglobin. It appears, however, that during its development, the surface of the red cell becomes abnormally diminished (table 1). In order to contain a normal volume within an abnormally small surface area, the red cell must be spheroidal.

A better understanding of the derivation of the hereditary spherocyte may be had by first examining the development of the normal red cell. A reticulocyte is the most immature form of red blood cell that can be identified in the normal circulation. Aside from the presence of the reticulum substance, the reticulocyte has other characteristics which distinguish it from the mature red cell. It resembles the mature red cell in that both are disc shaped, although the reticulocyte is larger; it is greater in diameter, greater in volume and greater in surface area (table 1). The hemoglobin content is approximately the same as that of a mature red cell since, during maturation of the reticulocyte, no significant amount of hemoglobin is created.\textsuperscript{38} Because of the larger cellular volume the 30 \gamma of hemoglobin in the reticulocyte is less concentrated than that of the mature red cell. Most of the greater volume of the reticulocyte consists of water.\textsuperscript{38} Maturation from a relatively large reticulocyte to a smaller red cell is, in one sense, a process of shrinking. The diameter shrinks, the volume shrinks, the surface shrinks but they all remain proportional to one another, and the shape remains a disc. Hemoglobin content remains practically unchanged, and it is probable that the loss of volume is due to loss of water. Ponder states that the difference in surface area between a reticulocyte and a mature red cell is proportional to the difference between the lipid content of the two cells.\textsuperscript{37} Since lipid is an important constituent of the surface ultrastructure of red cells, Ponder’s correlation of these differences may indicate that the shrinkage of the maturing red cell surface is accompanied by an actual loss of lipid molecules from the surface ultrastructure.

One may speculate that the abnormality of the surface of the hereditary spherocyte is also associated with a loss of lipid. There is some evidence that the mature
hereditary spherocyte contains less lipid than the normal red cell, and that the difference is proportional to the difference in surface area (table 2). Although this work is unconfirmed, it is possible that the observed low lipid content of the hereditary spherocyte is an actual indication of fewer molecules in its surface than are found in the normal red cell. The reduced surface area in the hereditary

<table>
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<tr>
<th></th>
<th>Surface Area</th>
<th>Lipid per RBC mg. × 10⁻¹¹</th>
<th>Ratio 2/1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal RBC</td>
<td>155</td>
<td>400</td>
<td>2.6</td>
</tr>
<tr>
<td>Hereditary Spherocyte (postsplenectomy)</td>
<td>115</td>
<td>330</td>
<td>2.9</td>
</tr>
<tr>
<td>Reticulocyte</td>
<td>250</td>
<td>650</td>
<td>2.6</td>
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It is known that there is enough lipid in a red cell to provide on the surface of the cell a continuous layer about 2 molecules thick. It is also known that most if not all of this lipid is actually at the surface where its fatty acids dominate the electrostatic and electrophoretic characteristics of the cell. In this table is shown that the values for lipid in these three cells vary directly with differences in surface area. The value for the reticulocyte is based on a statement by Ponder that the higher than normal amount of lipid in this cell is proportional to its greater surface area. The small amount of lipid in the hereditary spherocyte suggests that the surface membrane of this abnormal cell is not merely a normal membrane that has shrunken but actually contains less substance than its normal counterpart. As the reticulocyte matures it loses a certain amount of lipid and perhaps other materials as well. In the maturation of the hereditary spherocyte it appears that an abnormally great amount is lost. An abnormally small surface results.

Values for lipid in the normal red cells and the hereditary spherocytes are those reported by Erickson and her co-workers. The normal value is an average of determinations made in 16 healthy children. The hereditary spherocytes were obtained from 3 children after splenectomy when they were no longer anemic. Before splenectomy the value was 390 × 10⁻¹¹ mg. This higher value may have been due to the reticulocytes that are present before splenectomy.

The ratio of lipid to surface area, while of the same order in all three of these examples, appears to be greater in the spherocyte, suggesting a qualitative difference in the surface of these cells. This is not necessarily the case. The values for surface area shown in this table are those from table 1 which were purposefully selected to fit mature red cells with a volume of 90 cu. μ. In table 2 the ratio of lipid to surface area of the spherocyte would equal that of the normal red cell had we selected instead a spherocyte with a surface area of 125 sq. μ. Unfortunately Erickson did not push her calculations of the surface area of the red cells that she analyzed for lipid content.

Erickson and her co-workers studied the lipid values in many kinds of anemia. They were not especially impressed by the differences they found. They concluded, "An examination of the data has failed to disclose any relationship of the lipid ... content or distribution in either plasma or erythrocytes to the characteristic abnormal shape, size or resistance of the cells in the anemias under consideration. There are slight indications that there may be some relationship between the cell area and the concentration of lipids."

This is an important point and it merits further investigation.

spherocyte is not due to a reversible “wrinkling” of the surface, which is evidently the case with the acquired spherocyte. One may say that the process of normal maturation from normoblast to mature red cell involves a proportionate loss of volume, surface area, diameter and lipid. In the maturation of the hereditary spherocyte there is a disproportionately great loss of surface area and presumably
of the materials that comprise the cellular surface. Thus the fundamental defect in hereditary spherocytosis may reside in a quantitatively abnormal development of the red cell surface.

**THE LEPTOCYTE OR TARGET CELL**

Compared with the spherocyte and the attention shown it, its opposite number, the target cell has been neglected. In 1877 Litten described a “pessary cell” which he observed in drops of fresh blood. This may have been the cell now under discussion, but his description is open to several interpretations. In the illustrations of an excellent paper by Muir and McNee in 1912 both target cells and spherocytes are very evident. In 1933 Haden and Evans described a “Mexican-hat cell.” Observed in three dimensions it resembled a sombrero. The edge of the brim and top of the crown contained hemoglobin. Viewed from above or in stained smears the perimeter of the cell was seen as a rim of hemoglobin surrounding a central island of hemoglobin with a clear zone between. This suggested the appearance of the bull’s eye in a target, hence the name “target corpuscle” proposed by Barrett. Target cells are by no means pathognomonic of any condition although they are believed by Dameshek and others to be of fundamental significance in Mediterranean anemia and sickle cell anemia. They also occur in iron deficiency anemia, posthemorrhagic erythropoiesis, liver disease, the postsplenectomy state and severe dehydration. Although the target cell is associated with several types of hemolytic anemia, the hemolytic mechanisms apparently do not depend upon its peculiar conformation. In sickle cell anemia for instance, the sickling phenomenon is evidently a critical factor. In Mediterranean anemia, fragmentation of the red cells is perhaps of primary significance.

In the target cell as in the spherocyte there exists a disproportion between red cell surface and red cell volume, but the disproportion is in the opposite direction. The target cell is thin. It possesses more surface area per unit of volume than does the normal red cell and much more than the spherocyte (table 1). The hemoglobin may be of normal quantity but it is inadequate to fill the oversized target cell in a normal manner. For some unexplained reason all of the hemoglobin disposes itself at the periphery of the red cell except for a small island in the center. It has, in fact, been suggested that the target-like conformity may be an artefact which appears during the drying of the smear. This may be true, but the relative hypochromia and thinness which predispose to the “artefact” are certainly real. Because of its relatively small volume or perhaps because of its high proportion of stroma the target cell in hypotonic solutions is able to accommodate a relatively large amount of “extra” water as compared with a normal red cell. Increased osmotic resistance has been shown to be a characteristic of target cells.

The disproportion between surface area and volume which exists in the target cell may come about in several ways.

1. **The “Osmotic” Target Cell**

   Hypertonic solutions in vitro and severe dehydration in vivo produce reversible target cells by osmotic withdrawal of water from the cell. Because the surface area remains fixed and the volume decreases, a thin cell results.
2. The "Hypochromic" Target Cell

In Mediterranean anemia there is inadequate formation of hemoglobin so that
the red cells are poorly filled. The surface area of such a cell may be normal or
even large, but the cell is thin because of a disproportion between its contents
and its surface area. The same disproportion exists in iron deficiency anemia and
one encounters target cells but not to the same extent as in Mediterranean ane-
mia. One reason for this may be the smaller diameter of the red cells in iron
deficiency anemia. In the smaller cell there is less stroma and the small mass of
hemoglobin would comprise a greater proportion of the cell's volume. This is,
however, only a relative correction. Even with a small diameter, the red cell of
iron deficiency is hypochromic, low in its volume-surface area ratio and thin.
It demonstrates increased osmotic resistance.

3. The "Postsplenectomy" Target Cell

In normal persons after splenectomy, target cells appear in the cir-
culation. These cells have a normal volume and normal hemoglobin content,
but the diameter and computed surface area are above normal (table 1). It may
be said that the disproportion between volume and surface makes their thinness
inevitable. The reason for the abnormally large surface area is unknown, but is
evidently tied up with the removal of the spleen. Barrett has studied the ap-
pearance of target cells in patients whose spleens were removed for thrombocy-
openia. He found that the target cells gradually increase in numbers for several
weeks after the operation. Similar findings occur in dogs. This suggests that
the postsplenectomy target cell is produced by the bone marrow after the operation.
It does not, however, preclude the possibility that red cells already in the cir-
culation may be modified to become target cells. Because the target cells have a
normal mean corpuscular volume the difference in shape must be the result of
a large surface area, and when the area is computed this is found to be the case
(table 1). The postsplenectomy target cell may be due to relaxing or stretching
of its surface or perhaps there is a maturation defect of the cell surface asso-
ciated with the absence of a spleen. Stephens has demonstrated that the
postsplenectomy target cell in the dog has a different electrophoretic mobility
than that of the normal red cell. Although this would indicate that the surfaces
of the two are qualitatively different, there is a possibility that the slowly moving
cells which Stephens observed were reticulocytes.

4. The Target Cell of Liver Disease

In many sorts of liver disease, especially those associated with regurgitative
or obstructive jaundice, the red cells are found to have an increased diameter*
and decreased thickness, while the cellular volume remains normal. The com-
puted surface area is found to be enlarged. It is apparently an "acquired" alter-
ation. The target cells of hepatitis, like others, show an increased resistance in
hypotonic solutions. When a patient with virus hepatitis or biliary obstruc-
tion is transfused, the normal donor red cells become more resistant than normal as
though they too had become target cells. That they do actually become target

* This increase in diameter is not to be confused with the macrocytosis which occurs
with advanced cirrhosis. Apparently the large red cells in cirrhosis are partly the result of
a deficiency state and partly of reticulocytosis.
cells has been noted by Dacie in a splenectomized patient whose red cells all contained iron staining inclusion bodies. When this patient was given transfusions during an attack of virus hepatitis the donor red cells, which contained no inclusion bodies, were found upon examination of stained smears to have become target cells. Before and after the attack of hepatitis the donor cells in the circulation were not target cells. The abnormally large corpuscular surface in acute liver disease may thus be an acquired characteristic. It is also reversible. For instance, it has been shown that when biliary obstruction is relieved the patient's red cells revert to a state of normal osmotic resistance in eighteen days.

5. The Target Cell of Sickle Cell Anemia

The erythrocytes of sickle cell anemia are normochromic and of normal volume, yet they appear hypochromic in stained smears and many of them are target cells. This is because the cells are thin with a relatively large diameter, and the surface area is disproportionately great. One would therefore expect the lipid content of the erythrocytes of sickle cell anemia to be abnormally high. And so it is, as Erickson has shown. Complete atrophy of the spleen is uncommon in sickle cell anemia, but it is doubtful if the target cells of this disease are due to "spontaneous splenectomy." Target cells are found in children with sickle cell anemia who have large spleens.

Summary

1. The history of knowledge regarding the spherocyte and the target cell together with the theories of their pathogenic significance are reviewed.

2. Although these abnormal erythrocytes are generally considered to represent primary abnormalities of shape, it is pointed out that the abnormalities are secondary to disproportions between the cellular volume and the surface area.

3. The spherocyte is a thick red cell because—as compared with the normal erythrocyte—its surface area encloses a relatively larger volume. The disproportion may come about by increasing the volume, which occurs in hypotonic solutions and during periods of stagnation, either in the spleen or in the test tube. The disproportion may also result from shrinkage of the cellular surface, a change which occurs when a normal red cell is injured by various "sphering agents" including antibodies or when the natural "anti-sphering factor" is removed from its surface. In hereditary spherocytosis the cellular surface area is computed to be abnormally small. This apparently occurs as the result of a developmental defect.

4. The target cell, which is a thin erythrocyte, is the antithesis of the spherocyte. Compared with the normal red cell its volume is found to be small for the surface area. The disproportion occurs if the volume is reduced as when cells are exposed to a hypertonic environment. It occurs in hypochromia, as a consequence of too little hemoglobin. Target cells also result when a normal cellular volume is enclosed in an overlarge surface. This occurs in normal persons after splenectomy where it may be due to an absence of some splenic effect on the maturation of the red cell surface. In hepatic disease the large surface area is an acquired change affecting even transfused red cells. It is reversible. In sickle cell anemia the large surface is apparently an hereditary characteristic.
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Analytical Review: The Pathogenesis of Spherocytes and Leptocytes (Target Cells)

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