

## THE HUMAN RED BLOOD CELL\*

RUSSELL L. HADEN, M.D.

The erythrocyte is probably the most important single cell in the animal body. It is a container for hemoglobin by which alone sufficient oxygen necessary for the life of all tissue is transported. Biologically, it is one of the most interesting of all cells. The mature erythrocyte functions as a single unit separate from all other cells; it has no nucleus during its active physiologic life; as it utilizes no oxygen, it has no metabolism of its own; it is always in motion. The red cell may be considered as an inanimate body constantly traveling from lungs to tissues and back again. It is different from every other cell.

Besides its great physiologic interest and importance, the red blood cell is remarkable because it was the first cell seen by the human eye. The first cells of any kind to be described and illustrated were those of the cork in Robert Hooke's famous "Micrographia" published in 1665.<sup>1</sup> Before this time, however, Swammerdam observed erythrocytes in the blood of the frog and the louse.<sup>2</sup> He wrote: "The same has been likewise discovered in human blood for several years, it is found to consist of ruddy globules swimming in a clear liquor." The exact date of Swammerdam's discovery is unknown but probably was as early as 1658. Malpighi<sup>3</sup> in 1665 mistook red blood cells for fat cells in the omentum of the hedge-hog. He described them as "fat cells looking like a rosary of red coral." Leeuwenhoek was the third of the great minute anatomists of the classical period of microscopy independently to discover the erythrocyte. On April 5, 1674, he wrote his friend Constantine Huyghens<sup>4</sup> that he had observed blood from his own hand with his microscope and found it to consist of "red globules floating about in a crystalline fluid." He evidently recognized the importance of his discovery as he sent a letter to the Royal Society of London only two days later containing his findings.<sup>5</sup> On June 1st he communicated further observations to the Royal Society. He stated that the diameter of a red globule was 1/25,000th of the grain of sand which he used as his unit of measure,<sup>6</sup> which corresponds to 8.5 microns. This is remarkably close to the correct figure (7.7 microns), considering the crude instrument which he used. He said that he could distinguish the corpuscles with his microscope as clearly as he could see grains of sand on black taffeta without the aid of a magnifying lens.<sup>7</sup> Later he thought he observed that "the sanguinous globules which made the blood red" were firmer when he was indisposed and became softer as his health was regained.<sup>8</sup>

With his characteristic thoroughness, Leeuwenhoek studied the red

---

\*Part I of Porter Lectures, Series X, delivered at the University of Kansas School of Medicine, Lawrence and Kansas City, May 7-8, 1940.

blood cell for many years. He examined the blood of many species of animals; found that the erythrocyte was no larger in the blood of a whale than in the smallest fish, or in the horse than in the harvest mouse; proved that the redness of the blood is due to the globules which he described; noted the variation in shape in different animals; found it to have a green rather than a red color in the grasshopper, and was the first to illustrate it (Fig. 1).<sup>9</sup>

2 *Experimenta & Contemplationes*

adhiberem industriam, ut hanc globulorum coagulationem viderem, nunquam tamen mihi contigit. Eorum tamen ortum & formationem mihi hoc pacto imaginabar; globulos nempe ex quibus farina Tritici, Hordei, Avenæ, Fagotritici, &c. constat, aquæ calore dissolvi, & aquæ coadunari; hac vero aqua, quam cerevisiam vocare licet, refrigescente, multas ex minimis particulis in cerevisia coagulari, & hoc pacto efficere particulam sive globulum, quæ sexta pars est globuli fæcis & iterum sex ex hisce globulis conjungi; ut vero hanc conjunctionem mihi ob oculos ponerem, cepi 6. globulos cereos, cosque ita conjunxi, ut videre est Fig. 1. atque ita composui

fig:1.

1.

1.



& depinxi, ut singuli possent conspici, porro conjunctos hosce globulos ita in manibus convolvi, ut figuram acquirent similem Fig. 2. Mihi enim imaginabar, illud, quod hic convolutione inter manus efficiebam ad comprimendos globulos cereos, ex æquo fieri per agitationem cerevisiæ in comprimendis fæcis globulis, & hoc pacto fæcis globulum perfici. Nemo autem sibi persuadeat, me fæcis globulos suis circumferentiis separatos vidisse, ut hic Fig. 2. depingitur, sæpius enim sex hi globuli mihi ita apparent, acsi uni bullulæ essent inclusi, ubi enim fæcem aquæ puræ imposuisssem, quia cerevisia nimis viscosa erat & crassa, & hos globulos circa fundum convolvi siviisssem, sex illi globuli, qui fæcis globulum constituunt, non a se invicem separabantur: observationes hæ tam nude mihi apparebant, acsi nudo oculo videremus par-

fig:2.



vam

FIGURE 1: The first illustration of the red blood cell of man. From Leeuwenhoek's *Arcana Naturæ Detecta*, Delphis Batavorum, 1695, p. 2.

## THE HUMAN RED BLOOD CELL

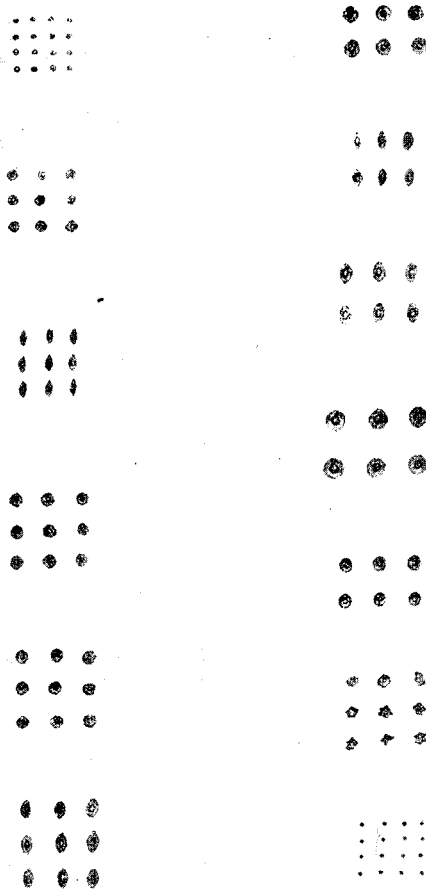


FIGURE 2: William Hewson's illustration (1773) of the red blood cell of different species. The erythrocyte of man is the second group from the top on the left. It is pictured here for the first time as a biconcave disk.

One hundred years after these early observations, William Hewson,<sup>10</sup> the English physiologist, presented a paper before the Royal Society of London on the "Figure and Composition of the Red Particles of the Blood Commonly Called the Red Globules." He pointed out for the first time that the red cell of man is not a globule, but a flat disk, and noted many other characteristics (Fig. 2). He wrote, "What is found so generally among animals must be of great use in their economy," although he had no idea of the function of the red cell. Even the great John

Hunter in his book on the blood<sup>11</sup> considered the red cell, "the least important part of the blood."

Two hundred years after the red cell was discovered, Hoppe-Seyler made his epochal studies<sup>12</sup> on hemoglobin in which he demonstrated that this substance readily takes up and gives off oxygen.<sup>13</sup> Lavoisier had shown, long before, that animal life depends upon oxidation, which is analogous to the combustion of inorganic substances, although he did not know where the oxidation of organic substances took place. It gradually became evident that oxidation occurs in the tissues and not in the lungs or circulating blood as first was supposed. Accordingly, Hoppe-Seyler's discovery provided a mechanism by which oxygen could be conveyed from the lungs to the tissues. Hemoglobin thus assumed great importance as the only means for transportation of oxygen in the body. During this period, it also was shown that hemoglobin is responsible for the red color of the corpuscle. It had been known since the time of Leeuwenhoek that the redness of blood is present only in the erythrocyte. We now have, for the first time, an explanation of the function of the red cell, a question which had puzzled Leeuwenhoek, William Hewson, John Hunter, and all other students of the blood. The tissues of the animal organism cannot live without oxygen, and can be supplied only by hemoglobin carried in the red cell. The red cell is truly a necessary and most important part of the animal economy as suspected by Hewson.

In 1851 Vierordt<sup>14</sup> made the first blood count and Funke discovered hemoglobin. Improvements in cell counting methods followed rapidly and numerous procedures soon were devised for measuring hemoglobin. In 1854 Welcher<sup>15</sup> reported the blood counts and hemoglobin estimations in many different diseases and, in 1863,<sup>16</sup> wrote a most complete article on the "size, the number, the volume, the surface, and the color of the blood corpuscles of man and animals." Welcher also determined the volume of the red cell in cubic microns by an indirect method long before Hedin (1890) described the hematocrit for separating red cells and plasma. Other historic landmarks in the study of the red cell are monographs by Malassez<sup>17</sup> on the counting of red cells (1873), by Preyer<sup>18</sup> on hemoglobin (1871), by Manassein<sup>19</sup> on the dimensions of red blood corpuscles (1872), and by Hayem<sup>20</sup> on the anatomy and physiology of the blood (1878). We are prone to think that finer measurements of the red cells have been made only recently. Malassez<sup>21</sup>, however, recorded the hemoglobin in micromicrograms per cell as early as 1877. In 1881 Mrs. Ernest Hart<sup>22</sup> reported in man and animals the volume of the red corpuscle in cubic microns, and the corpuscular content of hemoglobin in micromicrograms. She also recorded the following measurements in various types of anemia: the hemoglobin per cm. of blood, the hemoglobin per corpuscle in micromicrograms, the number of corpuscles per milligram of hemoglobin, the

## THE HUMAN RED BLOOD CELL

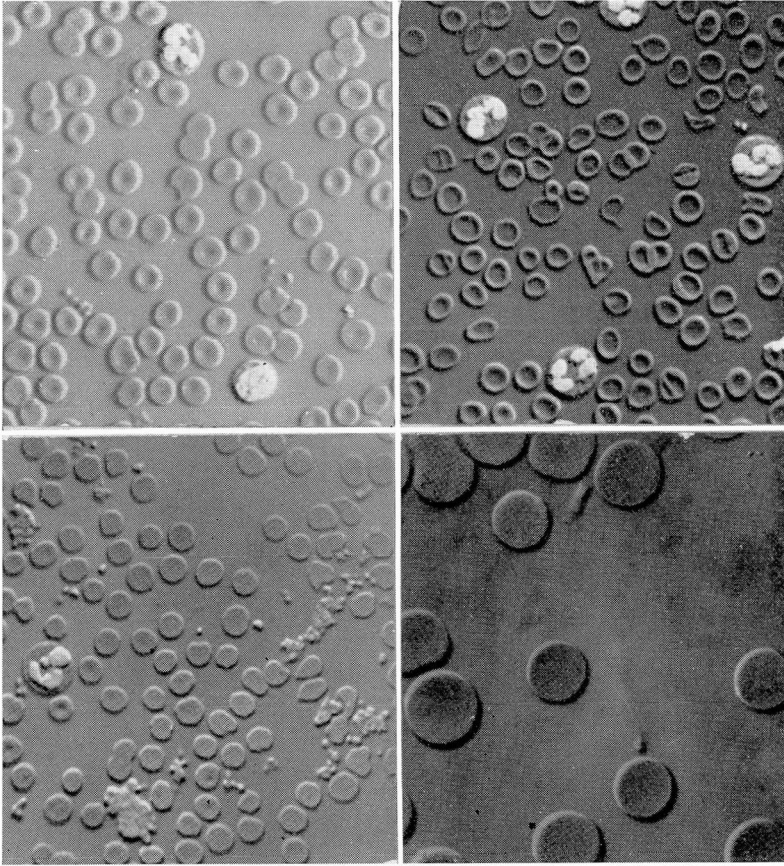


FIGURE 3: Three dimension photomicrograph of red cell. The shape of the red cell may be illustrated by bas-relief photomicrographs made by combining a positive and negative plate. The shallow, thin cells in hypochromic anemia (upper right) and the spherocytes of congenital hemolytic icterus (below) are especially well shown in relation to the normal biconcave disks (upper left).

diameter of the corpuscles, the area in square microns, and the micromicrograms of hemoglobin per unit of corpuscular substance. Certainly, no measurements of the red cell made in modern times have been more complete.

The normal erythrocyte of man is a biconcave disk varying little in volume, diameter, thickness, and hemoglobin content. The shape is best illustrated in a bas-relief photomicrograph (Fig. 3). The constancy of size and shape of the normal cell is remarkable in view of the rapid rate of red cell formation, the short span of life, and the continuous trauma to which the circulating cells are subjected. There seems to be no fixed period of life and activity for an erythrocyte. The length of time any individual cell functions seems to be determined by the chemical and mechanical injury it undergoes in the circulating blood.<sup>23</sup> Prob-

ably the mean span in man is about thirty days. During such a period, a red cell makes about 60,000 round trips to convey oxygen from lungs to tissues.

As the total number of cells in the circulation remains nearly constant in health, almost a trillion cells must be delivered from the bone marrow each day to compensate for those lost. Thus, new cells are produced at the rate of nearly one billion per minute.

The number, shape, size, and hemoglobin content of the red cell in the circulation depends upon numerous variables. The more important factors influencing the red cell are: (1) the condition of the bone marrow in which the cells are formed. If the amount of functioning tissue is decreased by aplasia, or replacement by other tissues as in leukemia, the normal number of new cells cannot be supplied; if the activity of the marrow is impaired by toxemia or by hypometabolism, cells cannot be formed normally. (2) The supply of specific materials necessary for making new cells. If the specific maturing principle (EMF) supplied by liver and liver substitutes is not adequate, the cell stroma cannot be formed normally; if iron is lacking, hemoglobin cannot be formed to fill the stroma. (3) The rate of destruction of red cells. The marrow attempts to compensate for a rapid destruction of cells by forming a larger number. The capacity to do this depends upon the state of the marrow and the extent of the need.

Ponder<sup>24</sup> thoroughly reviewed different ideas concerning the structure of the red cell. Some workers considered it a dense sponge-like body, others a bag with fluid contents. Ponder concluded<sup>25</sup> that the erythrocyte is a "balloon-like structure consisting of a cell membrane or envelope enclosing hemoglobin, salts, and many other substances in solution." Although a cell membrane cannot be demonstrated with the microscope, numerous studies have thrown light on its structure. It has an outer network of protein, probably only a few molecules thick. Within this are not more than four layers of lipid, and within this is another layer of protein, intimately connected with the gel of stromatin which fills the cell interior and holds the hemoglobin (Fig. 4).

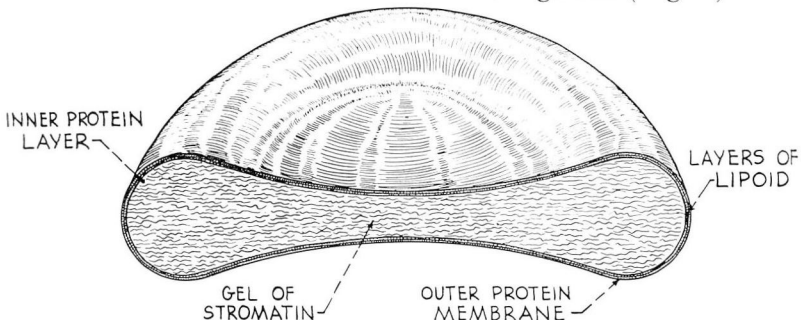


FIGURE 4: Cross-section of a normal erythrocyte of man (After Ponder).

## THE HUMAN RED BLOOD CELL

The reason for the existence of the red cell is often overlooked. Hemoglobin is the essential element in oxygen transportation. Why should this be carried in a cell rather than in solution in the blood plasma? Barcroft<sup>26</sup> answered this question. As the proteins of the blood plasma cannot pass through the wall of a capillary, they exert an osmotic pressure, tending to draw water into and hold it within the lumen of the capillaries. The force of the heartbeat and hydrostatic pressure constantly tend to force fluid out of the vessels. The opposing forces are normally balanced to maintain the proper fluid relation between tissues and vessels. The normal osmotic pressure of the blood proteins is 25 to 30 mm. of water. Fifteen grams of hemoglobin, a protein, free in 100 cc. of plasma, would have an osmotic pressure of 150 mm., and would draw water from the tissues to make an impossible circulatory condition from the physical standpoint (Fig. 5). As the capillary wall is permeable by hemoglobin, smaller meshes in the wall would be necessary to hold back the hemoglobin in solution. This decreased permeability would interfere with fluid interchange between the tissues and circulating blood. Barcroft also pointed out that hemoglobin functions more efficiently in a phosphate medium and in a hydrogen concentration, such as exists in the red cell, than it would in plasma. Therefore, the efficiency of both the plasma and hemoglobin is increased by placing the hemoglobin inside the red cell "in a world all its own," and an excellent physical mechanism is provided for the necessary gaseous interchange.

The reason for the discoidal shape of the red cell is not entirely clear. The purpose of the erythrocyte is to carry the respiratory pigment, hemoglobin, which is constantly taking up and giving off oxygen. Hartridge<sup>27</sup> offered an explanation for the shape of the red cell in relation to this function. About 250 cc. of oxygen per minute is used by a resting man of average weight. With exercise, this may increase to 2,500 cc. per minute, and, in a day, 360 to 3,600 liters of oxygen may be used. This entire amount of oxygen in combination with hemoglobin is carried from the lungs to the tissues as oxyhemoglobin. Jacobs<sup>28</sup> showed that oxygen enters the red cell very rapidly. With the oxygen tension at one-third of an atmosphere, the hemoglobin in the interior of a red blood cell becomes half-saturated in one-hundredth of a second. Hartridge demonstrated that the biconcave disk is most efficient from the standpoint of gaseous diffusion. Since the structure of the red cell is like an elastic envelope containing a homogeneous matrix filled with hemoglobin, the most efficient shape for the corpuscle would allow all the contents to be saturated with oxygen in the same interval of time. In a sphere gases diffuse in at the periphery and reach the center at the same time. Also, with an infinitely thin disk, the gases reach different parts of the stratum at the same time. A sphere and a flat disk thus have the proportions required for uniform diffusion. In a sphere, however,

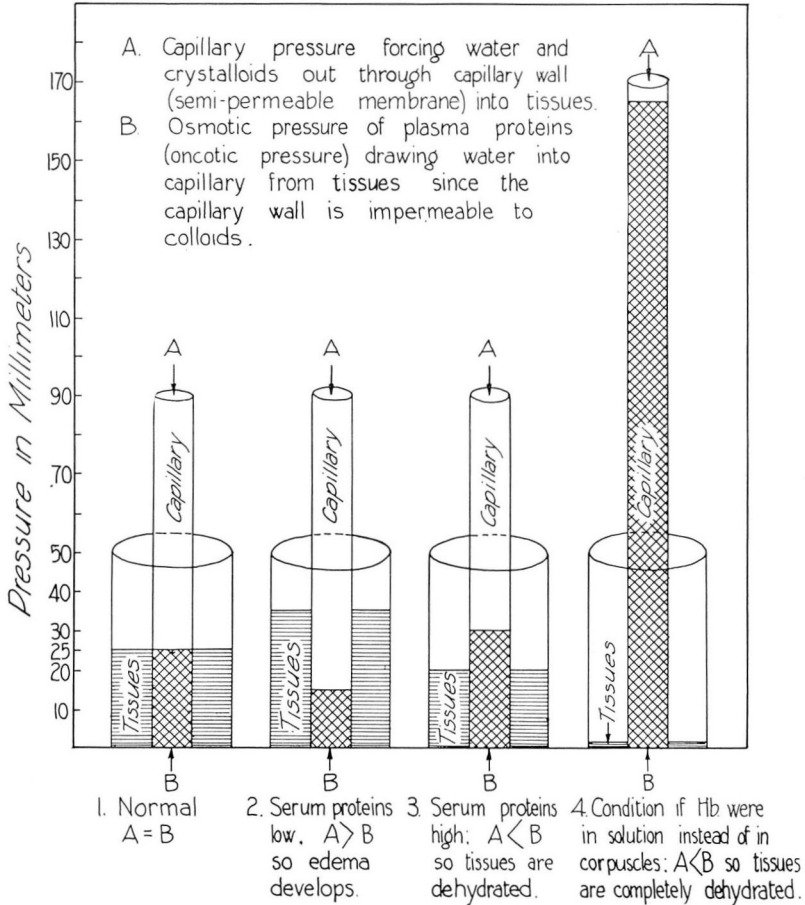


FIGURE 5: Diagram to illustrate Barcroft's explanation of the need for a cell to carry hemoglobin in the circulating blood. The osmotic pressure exerted by the normal amount of hemoglobin (14 to 16 grams) if free in the blood plasma would draw large amounts of fluid into the vessels and so dehydrate the tissues. The hemoglobin in the red cell is outside the plasma and exerts no pressure.

the surface is small for the given bulk, and in a flat disk, the surface is increased at the expense of the contents. The biconcave disk, according to Barcroft, is a compromise between the sphere and the disk. Since the edges are thicker than the center, diffusion is uniform at any given point.

Ponder<sup>29</sup> does not accept this teleological explanation, but considers the discoidal form a lucky coincidence. He pointed out that the biconcave erythrocyte composed of lipoids and proteins is necessarily in a state of strain, maintained by two forces. The first tends to produce contraction and a spherical form to make a minimal surface area for the volume; the second, to produce expansion of surface area and a very flattened form. Balanced against each other, the two forces maintain the



## THE HUMAN RED BLOOD CELL

disk shape. Ponder's concept seems to best fit the facts observed with regard to the change in shape of the red cell in response to various toxic, especially hemolytic, agents.

Since the discoidal form is maintained by a state of stress within the cell, anything which affects the structure of the surface membrane and stromatin may upset the delicate balance of the opposing forces. As the strain is released, a cell tends to become a sphere. Trauma to the cell, whether chemical, mechanical, or thermal, will alter its shape. It seems most likely, however, that the cell membrane is not elastic and cannot expand. As the stromatin and membrane, which maintain the state of strain in the corpuscle, are altered by injury, certain characteristic changes take place. These are best seen in hypotonic hemolysis, first demonstrated by Ponder and his co-workers. Using rabbit's blood,

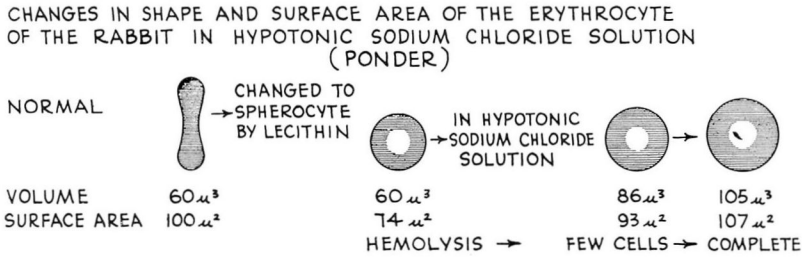


FIGURE 6: Diagram to illustrate Ponder's measurements of the erythrocyte of the rabbit as it undergoes hypotonic hemolysis.

Abramson, Furchgott, and Ponder<sup>30</sup> added lecithin, which affects the stroma of the cell and changes the disk to a sphere. Starting with a normal cell volume of  $60 \mu^3$  and a surface area of  $100 \mu^2$ , the cell is converted into a sphere (Fig. 6). The volume is still  $60 \mu^3$  but the surface area is only  $74 \mu^2$ , as it decreases with the change in shape without altering the volume. If the plasma, in which the spherical cells are suspended, is made more hypotonic the volume of the cell gradually increases. When the mean cell volume is  $86 \mu^3$  and the mean surface area is  $93 \mu^2$ , hemolysis begins; when the mean volume is  $105 \mu^3$  and the surface area is  $107 \mu^2$  hemolysis is complete. From this experiment Ponder concluded that the surface of the spherical form can be stretched until it is about the same as that of the disk from which it was derived originally; if it is stretched more, the membrane breaks. This seems to prove that the membrane covering the cell cannot be stretched without breaking, although the surface membrane can contract.

One of the simplest ways to study the effect of injury on the shape of the cell is to observe a thin layer of red cells between two closely applied surfaces such as a coverglass and slide. Here spherocytes occur and various shapes are produced by changes in the cell membrane as the

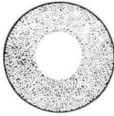

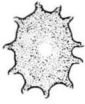


					
VOLUME	$86\mu^3$	$86\mu^3$	$86\mu^3$	$86\mu^3$	$86\mu^3$
SURFACE AREA	$133\mu^2$				$85\mu^2$

FIGURE 7: Diagram to illustrate the changes in a discoidal erythrocyte with injury. The volume is unchanged but the surface membrane first folds, then "puckers" and finally contracts to the area necessary to cover a sphere.

form is altered. First, the surface membrane folds as the surface area decreases, without change in volume; "thornapple" forms then appear. Finally, the biconcave disk becomes a sphere (Figs. 7 and 8). It seems evident that injury to the cell is followed by a loss of internal strain and a consequent alteration of the normal biconcave shape. This injury also may be produced by immune hemolysins, by hemolytic poisons such as acetylphenylhydrazine, by lysins such as bile salts and saponin, by heat, by benzene, or by toxins formed during the course of disease. The toxic agent may act on the envelope, on the lipid fraction, or on the protein of the stromatin.

Bergenheim and Fähræus<sup>31</sup> demonstrated in the spleen a substance, lysocleithin, which they think concerns the normal disposal of cells and has the property of making red cells into spherocytes. After a biconcave

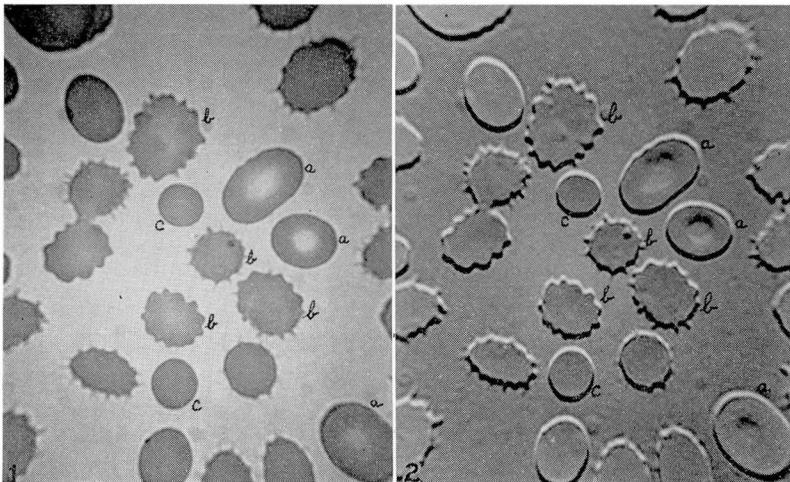


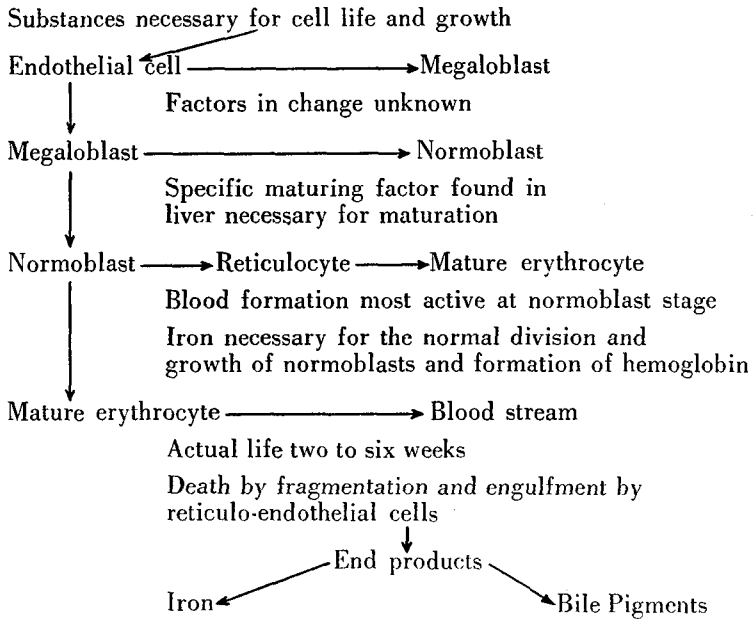
FIGURE 8: Photomicrographs of film from blood cells in the red cell mass at the bottom of an hematocrit tube after centrifuging. Note the biconcave disks (a), the crenated forms (b), and the spheres (c). The abnormal forms are shown especially well in a bas-relief photograph (2) of the same area.

## THE HUMAN RED BLOOD CELL

disk is changed into a spherocyte, it seems to be destroyed easily so that the spherocyte may be considered a stage in the hemolysis of the erythrocyte.

Since the red cell lives only a relatively short time and is replaced by a new cell when disposed of, the life cycle is most important in relation to both normal and abnormal physiology. The normal stages of the life cycle are shown in Table I.

TABLE I  
LIFE HISTORY OF THE ERYTHROCYTE



Certain indicators of red cell activity are necessary to evaluate the formation, circulation, and destruction of red cells (Table 3). The red cell count and the hemoglobin content record only the balance between the formation and destruction of red cells. Young red cells have the property of being stained with certain dyes. The number of reticulocytes or young cells which take the stain is an index of the rate of production of red cells ready to function in the blood stream, or at least the rate of delivery of such cells from the marrow. If the delivery of cells from the marrow is impaired, the marrow may be hyperplastic or hyperactive with a low reticulocyte count in the circulation. If the reticulocyte count in the circulation is high, the marrow is necessarily hyperplastic; if below normal, the marrow may be aplastic, hypoplastic, or hyperplastic.

RUSSELL L. HADEN

When a red cell is destroyed, hemoglobin is freed, iron is split off from the hemoglobin molecule, and bilirubin is formed as the end-product of the pigment metabolism. This bilirubin is adsorbed by protein and is not excreted easily by the kidney. As the capacity of the liver cells to excrete bilirubin so formed is quickly exceeded, an excessive destruction of red cells and hemoglobin is soon reflected in an increased bile pigment content of the plasma. In the absence of biliary obstruction and liver disease, the amount of bilirubin present in the plasma indicates the rate of red cell and hemoglobin destruction. A correlation of the bilirubin content of the plasma and the reticulocyte level is shown in Table 2.

TABLE 2  
RELATION OF BLOOD FORMATION AND DESTRUCTION TO  
BILIRUBIN AND RETICULOCYTE LEVEL

Bilirubin content (icterus index)	INCREASED (over 1.5 per cent)	Reticulocyte count NORMAL (0.5-1.5 per cent)	DECREASED (under 0.5 per cent)
Increased (over 6 units)	Increased blood destruction with active bone marrow.	Increased destruction without good marrow response.	Increased destruction with inactive bone marrow or impaired delivery of red cells.
Normal (4-6 units)	Active bone marrow without excessive destruction.	Decreased marrow without excessive destruction of red cells.	Decreased formation or impaired delivery of red cells without excessive destruction.
Decreased (under 4 units)	Decreased destruction of hemoglobin due to iron deficiency; active cell formation in marrow.	Decreased destruction of hemoglobin. Decreased formation of hemoglobin; normal cell formation in marrow.	Decreased destruction of hemoglobin. Decreased formation of hemoglobin. Decreased cell formation in marrow or impaired delivery of cells.

If all the elements necessary for red cell formation are deficient, the marrow cannot make the normal number of cells at the normal rate. The marrow may function at a low speed, but such cells as are delivered to the circulation usually are normal. Two specific elements, iron and erythrocyte-maturing factor (EMF), are necessary if the marrow is to make a normal cell completely filled with hemoglobin. As the red cells develop in the bone marrow, they multiply actively at the megaloblast stage. They are not ready for delivery from the marrow, however, until completed by a substance formed by the interaction of a secretion of the stomach (the intrinsic factor of Castle) on food elements (the extrinsic factor of Castle) and stored in the liver. This substance has been designated by such names as "liver principle," the "anti-anemic principle of Castle," the "pernicious anemia principle," and "anti-megalocyte principle." As its fundamental action is to mature the red cell, or pre-

## THE HUMAN RED BLOOD CELL

pare it for emergence from the marrow, we have designated it the erythrocyte-maturing factor (EMF). Since it is always necessary to know, in studying an anemia, whether or not there is a sufficient supply of this essential factor, there must be some indicator of its lack. The cell to which this substance (EMF) is supplied becomes smaller, so a decrease in volume of the cell is characteristic of the maturation effected by the erythrocyte-maturing factor. A macrocytosis is indicative of its lack. Although a macrocytosis usually indicates a deficiency of the erythrocyte-maturing factor (EMF), cells of increased size may be due to other causes. A hyperplastic marrow, overactive in response to a great demand for red cells, may deliver red cells larger than normal, because of rapid removal from the marrow before maturation is complete rather than a lack of erythrocyte-maturing substance (EMF). Accordingly, the hyperplastic marrow may deliver macrocytic cells in response to rapid destruction of red cells in phenylhydrazine poisoning or in spherocytic jaundice. However, in response to increased cell loss a chronic hyperplasia of marrow usually leads in time to the formation of cells smaller than normal.

Iron is the second specific element necessary for normal red cell formation. Without iron, hemoglobin cannot be formed. It is possible also that iron stimulates the growth and multiplication of red cells at the normoblast stage when division is most active. With a decrease in the amount of hemoglobin a decrease first occurs in the concentration of hemoglobin in the red cells, or in the color index. Since there is no

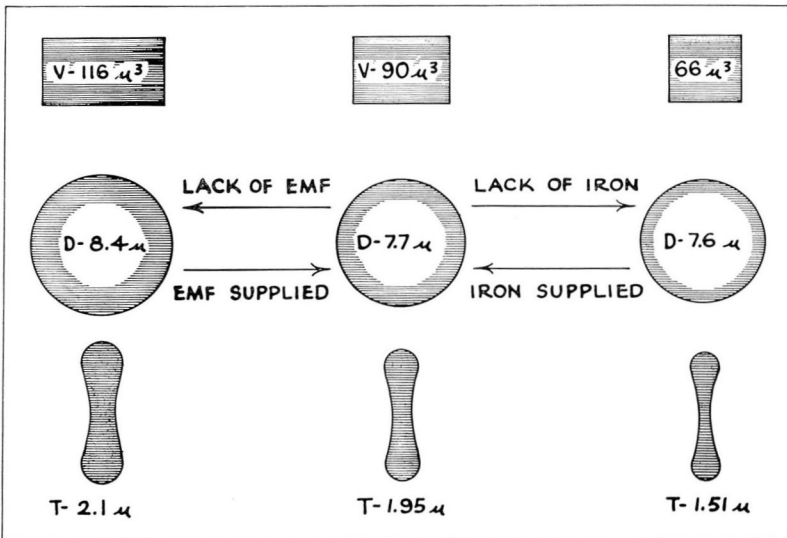


FIGURE 9: The effect of a deficiency in iron or in the erythrocyte-maturing factor (EMF) of liver on the red cell. The lack of iron produces microcytosis; iron relieves microcytosis if due to a deficiency. The lack of EMF produces a macrocytosis; liver or liver substitutes containing EMF relieve macrocytosis if due to a lack of this substance.

RUSSELL L. HADEN

value in having red cell stroma without hemoglobin to fill it, and if the color index continues to be low, the cells become smaller and the volume index decreases. The hypochromia shown by the lessened color index and volume index is a measure of the lack of iron. The effect of the erythrocyte-maturing factor (EMF) and iron on the red cell are shown in figure 9.

Accurate indicators are available to show the balance between red cell formation and destruction (red cell count and hemoglobin content), the rate of destruction of red cells (icterus index), the rate of regeneration or delivery of red cells (reticulocyte count), the lack of the erythrocyte-maturing factor or EMF (macrocytosis), and a deficiency of iron (hypochromia and microcytosis) as shown in Table 3. The relation of the blood findings to red cell formation and destruction are shown in Table 4.

TABLE 3  
MEASURES OF RED CELL ACTIVITY

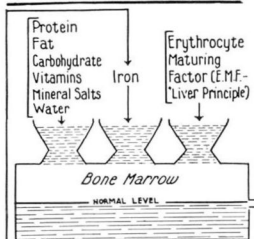
FACTOR	INDICATOR
Balance of red cell and hemoglobin formation and destruction.	Red cell count and hemoglobin content.
Rate of destruction of red cells.	Level of bile pigment in plasma.
Rate of delivery of red cells.	Level of reticulocytes in circulation.
Deficiency of iron.	Hypochromic and microcytosis of red cells.
Deficiency of erythrocyte-maturing factor (EMF).	Macrocytosis of red cells.

TABLE 4  
RELATION OF BLOOD FINDINGS TO RED CELL FORMATION AND DESTRUCTION

Active bone marrow.	Increased number of reticulocytes, basophilia, nucleation. Slight increase in mean erythrocyte volume if reticulocytosis is marked. Often an increase in leukocytes and platelets unless destruction is more active than normal. The number of cells is increased.
Inactive bone marrow.	Decrease or absence of reticulocytes, basophilia, and nucleation. If blood destruction is normal or increased, the cell count decreases.
Increased red cell and hemoglobin destruction.	Increase in bilirubin content of plasma; decrease in number of cells unless compensated for by increased marrow activity.
Decreased hemoglobin destruction.	Decrease in bilirubin content of plasma.
Deficiency in erythrocyte-maturing factor (pernicious anemia).	Anemia with increase in mean erythrocyte volume (increased volume index).
Deficiency in iron (iron deficiency anemia; chronic hemorrhagic anemia).	Anemia with hypochromia of red cells (decreased color index); microcytosis (decreased volume index) if hypochromia continues.
Hemolytic anemia.	Anemia with increased icterus index; reticulocytosis if marrow responds to increased need.
Anemia due to decrease in amount of activity of marrow (aplastic or hypoplastic anemia).	Anemia with cells of normal size and hemoglobin content; decrease in reticulocytes.

# NORMAL RED BLOOD CELL

## FORMATION

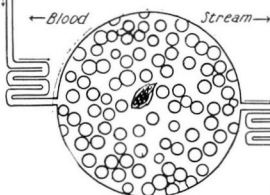


### NORMAL ADULT

Red cells formed largely in ribs, skull and vertebrae  
 Total normal volume of marrow is 1400 c.c. Normal color is greyish red  
 For red cell formation, non-specific substances necessary for all cells are required as well as two specific substances, iron and the erythrocyte maturing factor (E.M.F.)  
 One trillion cells and 25 gms. HB. formed each day  
 Rate of formation measured by reticulocyte percentage in circulation  
 Normal life of red cell about 30 days

## CIRCULATION

Twenty-five trillion red cells in blood stream. Each cell during its life, makes 50000 to 100000 complete circuits from lungs to tissues. Functions as conveyor for hemoglobin which carries oxygen from lungs to tissues  
 Blood count represents balance between red cell and hemoglobin formation and destruction

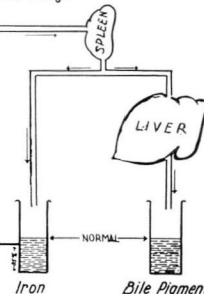


### EXAMPLE

R.B.C. 5.0 Million  
 H.B. 100% (15.4 gms)  
 Volume Index 100 (90 cubic microns)  
 Color Index 1.00 (312 micromicrograms)  
 Saturation Index 1.00 (34.7%)  
 Icterus Index 46 units  
 Reticulocytes 0.5 - 1.0%

## DESTRUCTION

Old red cells taken out by reticulo-endothelial cells, largely those of spleen  
 1 trillion red cells destroyed each day  
 25 gms. HB destroyed each day  
 100 mg. of iron released  
 65 mg. used again  
 15 mg. lost and replaced by food  
 500 c.c. bile formed and excreted by the liver containing 300 to 400 mg. of bile pigment  
 Rate of destruction gauged by iron released and bile pigment formed  
 Only clinical laboratory measure is icterus index or quantitative Van den Bergh



Eighty-five percent of iron released returns to marrow to be used again

FIGURE 10: Normal red cell physiology. The cells are being formed and destroyed at a constant rate. The cells vary little in volume and hemoglobin content. The rate of delivery of new cells is gauged by the reticulocyte count and the rate of destruction by the icterus index. The blood count and hemoglobin content show only the balance between the formation and destruction of red cells.

The mechanism of red cell function and destruction may be illustrated by a diagram in which the bone marrow is considered as a grist mill with three hoppers supplying materials for making red cells (Fig. 10). One hopper supplies the nonspecific elements and the other two, the specific elements. Normally, the hoppers are full. The level in the mill indicates the relative fullness of the bone marrow. To maintain a normal balance between formation and destruction, nearly one trillion cells and 25 gm. of hemoglobin must be formed daily. In the circle showing the normal circulation are one hundred red cells with one

reticulocyte. The cells are of normal size and hemoglobin content. The normal findings are shown below the circle. Old red cells are taken out by the reticulo-endothelial cells, largely those of the spleen. If the blood count remains constant, as it normally does, the same number must be taken out as is delivered to the blood stream by the marrow. As the hemoglobin is destroyed, iron is split off. Some of the iron is excreted but the larger part (85 per cent) is returned to the marrow to be used again. The end-product of hemoglobin destruction is bilirubin which is excreted by the liver. The normal amount of bile pigment and iron formed is indicated by the level of these substances in the containers in which they are received. The mill is considered as functioning at a constant rate of speed to supply the same number of cells with the same hemoglobin content as is destroyed each day. The normal mean elapsed time between the release of the cell and its ultimate disposal is thirty days.

It is now possible to make very complete and very accurate measurements of the red cell. Besides the routine counts and hemoglobin estimation, the total mass of circulating erythrocytes or erythron is determined from the hematocrit reading and the total blood volume, as measured by a dye method. The mean red cell volume is calculated from the count per cu. mm. and the total red cell mass in a given amount of blood as measured with a hematocrit.<sup>32</sup> We have found the mean cell volume to be 90 cubic microns when using isotonic sodium oxalate (1.4 per cent) as an anti-coagulant, and centrifuging until packing is complete. The mean cell diameter may be determined in a number of ways. We have employed the Haden-Hausser erythrocytometer<sup>33</sup> which utilizes the principle of diffraction of light. This method is simple, accurate, and gives a normal mean cell diameter of 7.7 microns. The mean cell thickness is calculated from the volume and diameter, and the surface area from the diameter and thickness by considering the cell as a cylinder. The thickness may also be read directly from a three-dimensional chart.<sup>34</sup> Any unusual and abnormal shape is apparent in a stained film.

The mean hemoglobin content per cell is calculated from the red cell count per cu. mm. and the hemoglobin in grams per 100 cc. The normal figure is 30.8 micromicrograms per cell. The hemoglobin concentration per unit volume of cells is calculated by dividing the hemoglobin in 100 cc. of blood by the hematocrit reading for an equal amount of blood. The normal is 34.2 per cent which evidently is the saturation level.

The allowable variation in measurement for normal is 10 per cent. Instead of using absolute figures for cell measurements, it is very convenient to use indices to express the result in terms of normal. An index is calculated by dividing a determined figure by the normal. Thus, if the mean cell volume is 63 cubic microns, and the normal is 90, the



## THE HUMAN RED BLOOD CELL

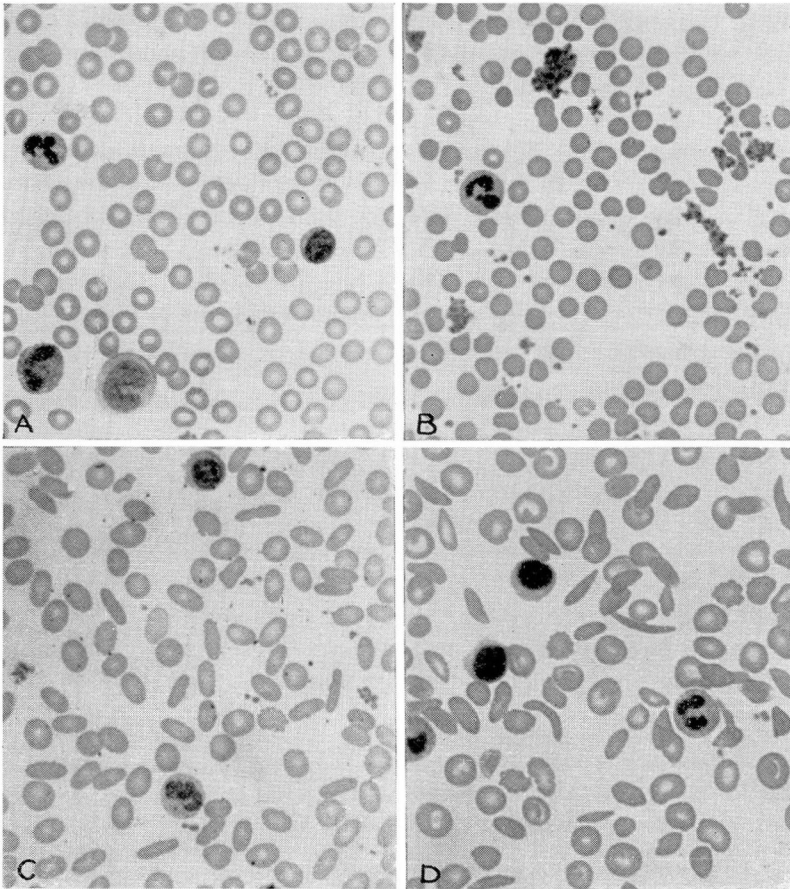


FIGURE 11: Congenital abnormality in shape and structure of the erythrocyte. A., normal biconcave disk. Note the central depression in each erythrocyte and the uniform shape and diameter. B., congenital hemolytic icterus. Note the anisocytosis with predominance of microcytes. The cells stain deeply because they are thicker than normal and have no central depression. C., oval-cell anemia. Note the predominance of oval erythrocytes. D., Sickle-cell anemia. The cytoplasm is abnormal so the cell under certain conditions became sickle-shaped.

volume index is  $63/90$  or  $0.70$ . The normal measurements may be summarized:

	Mean Normal (male-wt. 70 Kg.)
Erythron	2100 cc. (30 cc. per Kg.)
Total hemoglobin	718.2 gms.
Red cells	5.0 million per cm.
Hemoglobin	15.4 gms. per 100 cc. blood
Volume of red cell	90 cubic microns
Volume index	1.00
Diameter	7.7 microns
Thickness	1.95 microns
Mean surface area	135 square microns
Mean hemoglobin content	30.8 micromicrograms
Hemoglobin (color) index	1.00
Mean hemoglobin concentration	34.2 per cent.

Certain congenital anatomic defects may affect the red cell. The cell may be congenitally globe-shaped, as in congenital hemolytic icterus, or oval shaped. The structure of the stroma may be congenitally defective also so that the cell takes on a sickle shape as in sickle-cell anemia, or may be fragile and destroyed easily as in erythroblastic anemia (Cooley's Mediterranean anemia). Cells of abnormal shape and structure are usually removed more rapidly than normal from the circulation so most such patients present an anemia (Fig. 11).

The total red cell mass or erythron has not received the attention it deserves (Fig. 12). With an anemia, the erythron may fall to a very low value. I have observed it as low as 367 cc. in a patient with idiopathic aplastic anemia. In polycythemia vera it always is increased. Here the determination of the total red cell mass helps greatly in differentiating polycythemia vera from the symptomatic type.<sup>35</sup> I have observed the erythron as high as 8,000 cc. in polycythemia vera. In symptomatic polycythemia the red cell mass per kilogram is not significantly increased even with a marked increase of red cells per cu. mm. of blood.

Knowledge of the size, shape, and hemoglobin content of the red cell helps greatly in the study of the anemias. By far the best laboratory classification of anemia is based on the volume and hemoglobin content

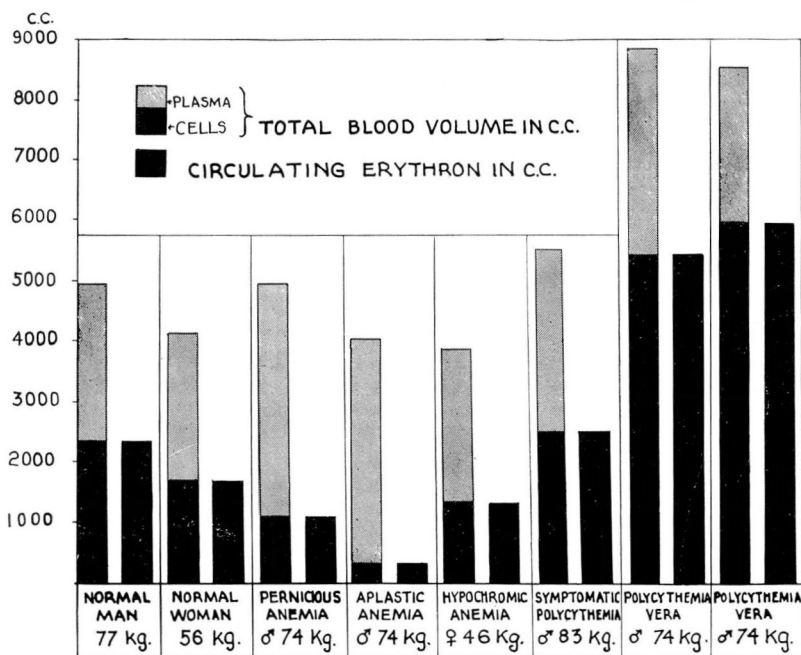


FIGURE 12: The circulating erythron varies greatly in the blood dyscrasias affecting the red cell. Here the erythron is 367 cc. in aplastic anemia and over 5,000 cc. in polycythemia vera.

# THE HUMAN RED BLOOD CELL

## TYPES OF ANEMIA

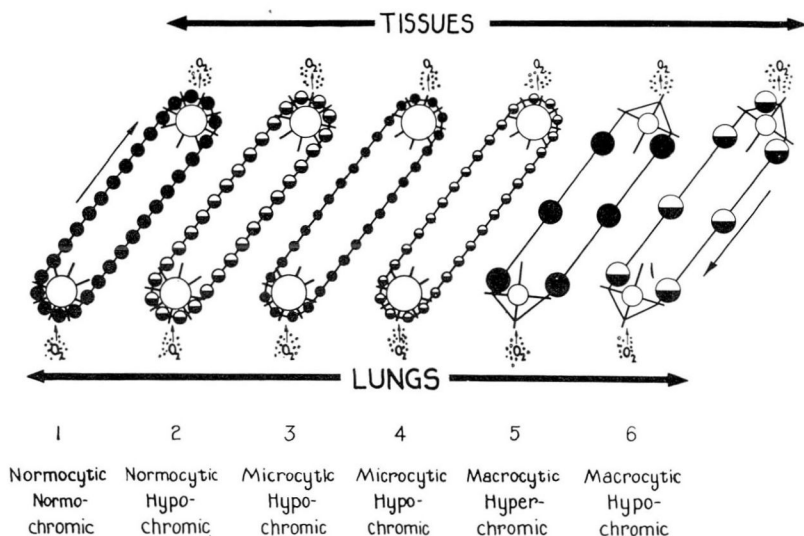


FIGURE 13: Types of anemia represented as varying kinds of endless chain conveyors. Note the great variation in volume and hemoglobin content of the cup (erythrocyte) on the chain.

of the mean red cell. While this is an objective classification it also gives the clinician clues about the cause of the anemia. Thus a microcytosis and hypochromia is never due to pernicious anemia; a hypochromia is never encountered in an iron-deficiency anemia.

The six types of anemia (Fig. 13) classified on the basis of measurements of the red cell are as follows:

1. *Normocytic and normochromic.* The mean cell has a normal volume and hemoglobin content. Volume index and color index, 1.00 ( $\pm 0.10$ ). (Fig. 13, 1)

2. *Normocytic and hypochromic.* The mean cell has the normal volume but is incompletely filled with hemoglobin and so has less than the normal quota. Volume index, 1.00 ( $\pm 0.10$ ), and color index, 1.00. (Fig. 13, 2)

3. *Macrocytic and hyperchromic.* The mean cell has a volume larger than normal and more than the normal amount of hemoglobin. Volume and color index, 1.00. (Fig. 13, 5)

4. *Macrocytic and normochromic.* The mean cell is larger than normal but contains a normal amount of hemoglobin although the concentration is less than normal. Volume index, 1.00, and color index, 1.00 ( $\pm 0.10$ ). (Fig. 13, 6)

5. *Macrocytic and hypochromic.* The mean cell is larger than normal and contains less than the normal quota of hemoglobin. Volume index, 1.00, and color index, 1.00. (Fig. 13, 6)

6. *Microcytic and hypochromic.* The mean cell is smaller than normal and contains less than the normal amount of hemoglobin. Volume and color index, 1.00. (Fig. 13, 3 and 4).

The great value of knowing the shape of the cell or at least the degree of spherocytosis in the study of hemolytic anemia is emphasized in an article on hemolytic anemia.<sup>36</sup> In obstructive jaundice the cells tend to be flattened. A macrocytosis may be observed in conditions other than pernicious anemia. These have already been mentioned but may be summarized:

1. Reticulocytosis from any cause as young cells tend to be larger than normal.
2. Defective absorption of the specific principle of liver (erythrocyte-maturing factor) as in chronic diarrhea or defective use as in myxedema.
3. Cellular liver disease not due to obstruction of the extrahepatic bile ducts. The macrocytosis here seems due to the inability of the liver to store or utilize the erythrocyte-maturing factor.

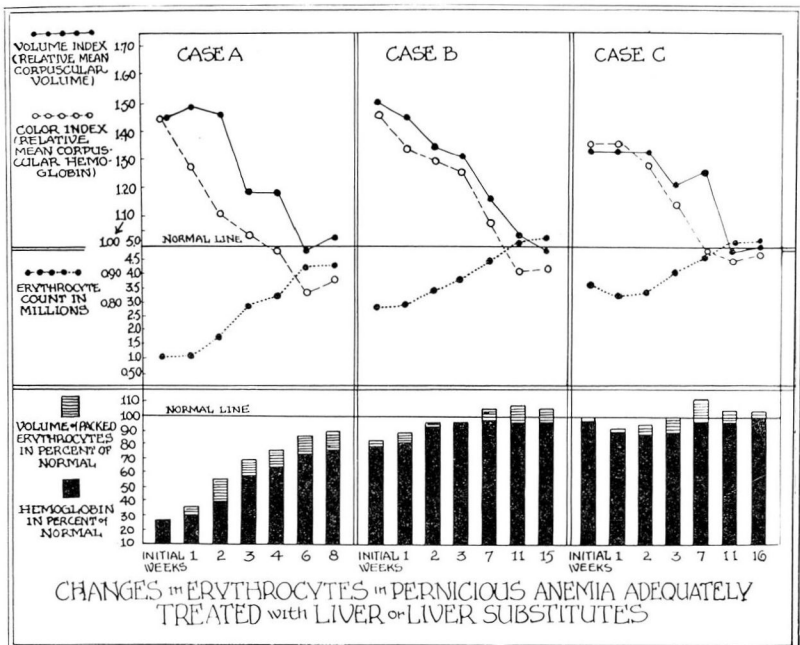
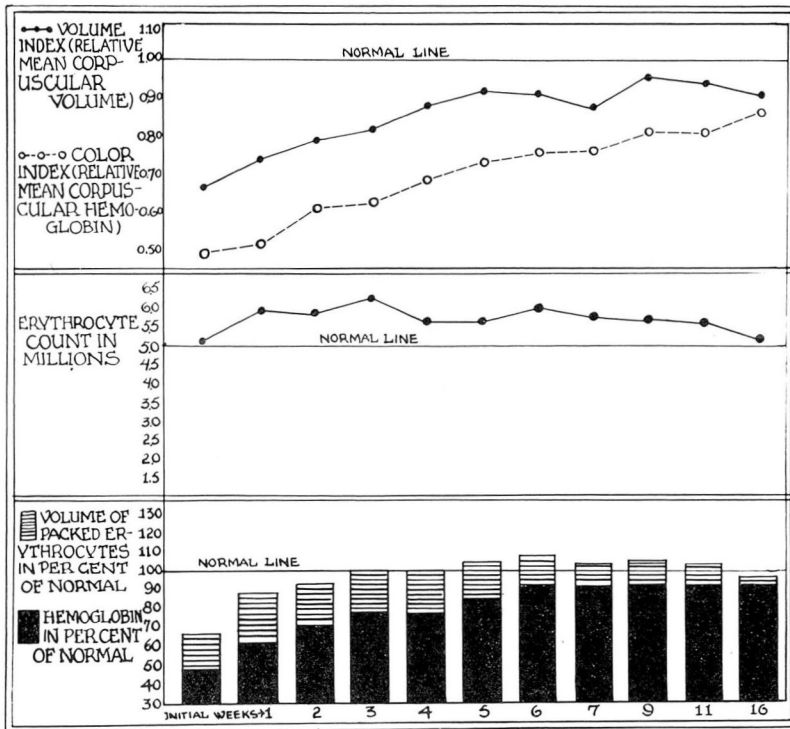


FIGURE 14: Changes in the red cells in pernicious anemia with adequate therapy. Note in every case the large volume of the mean erythrocyte before treatment and the return to normal with treatment. If adequate therapy is continued, the mean cell remains normal.

## THE HUMAN RED BLOOD CELL



### CHANGES IN ERYTHROCYTES IN SIMPLE ACHLORHYDRIC ANEMIA WITH ADEQUATE IRON THERAPY.

FIGURE 15: Changes in the red cells in a patient with iron-deficiency anemia when given adequate amounts of iron. Note the gradual increase in volume of the cell as well as hemoglobin content.

4. Idiopathic aplastic anemia nearly always shows a microcytosis of the red cells. A microcytosis or hypochromia on the other hand is seldom seen except as the result of an iron deficiency. A hypochromia has the same implication.

Knowledge of the volume of the mean red blood cell is necessary in determining the adequacy of treatment of pernicious anemia. With complete therapy the mean cell volume returns to normal (Fig. 14). Likewise in the microcytosis and hypochromia of an iron deficiency, with adequate iron therapy, the cell returns to normal (Fig. 15).

The mechanism of changes in the red cell in an anemia due to a deficient supply of the erythrocyte-maturing factor such as idiopathic pernicious anemia is illustrated in figure 16. Here the hopper supplying the specific factor is poorly filled. The marrow is red because of a great piling up of incomplete cells as shown at necropsy or by sternal puncture. The cells die in the marrow in excessive numbers without reaching

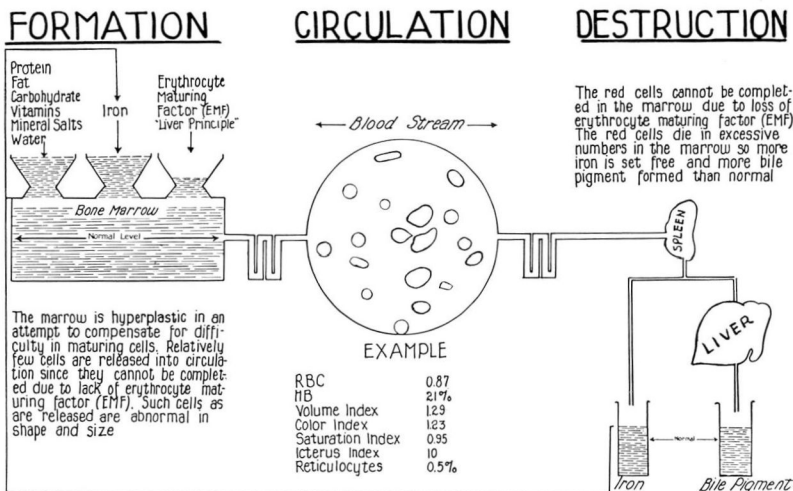


FIGURE 16: Red cells in an anemia due to an idiopathic defect in formation of the erythrocyte-maturing factor (pernicious anemia). Here the marrow is hyperplastic because the cells cannot be completed normally and accumulate. Such cells as are released are not normal as shown by the macrocytosis (volume index, 1.29) which indicates a deficiency of the specific maturing factor (EMF).

the blood stream; hence there is an excessive output of bilirubin, the end-product of hemoglobin destruction. The cells in the circulation are large and often of abnormal shape. If iron is lacking, the picture of the physiological process is exactly opposite (Fig. 17). Here, as the result of a lack of hemoglobin for the cell to carry, the mean cell volume is

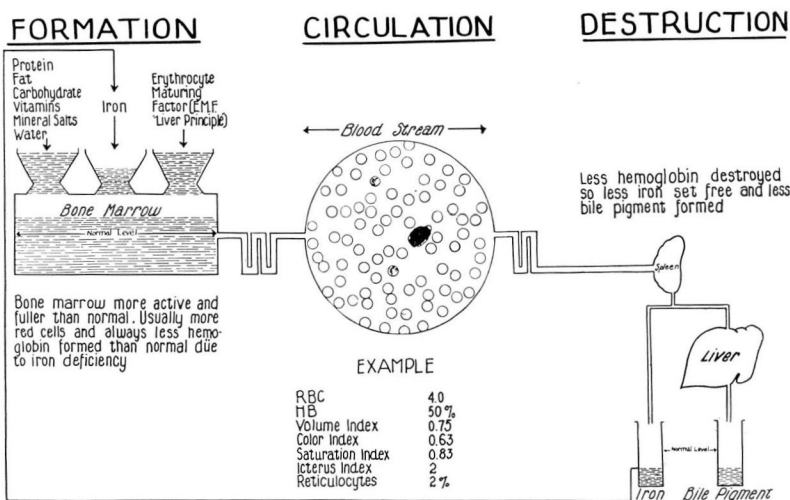


FIGURE 17: The red cells with a deficient intake of iron. As a result of the chronic iron deficiency, the hemoglobin is reduced (color index, 0.63) and the cells become smaller (volume index, 0.75). The icterus index is low because less hemoglobin than normal is discharged.

## THE HUMAN RED BLOOD CELL

less than normal and the stroma is only partly filled with hemoglobin. As less hemoglobin is destroyed, the plasma is pale because of the low bilirubin content.

### SUMMARY

The great interest and importance of the erythrocyte has been emphasized in the preceding pages. The red corpuscle was the first cell seen by the human eye when the microscope unfolded a new world of minute things. Three of the five great early microscopists, Swammerdam, Malpighi, and Leeuwenhoek, independently discovered the red cell and found it an object of exceptional interest.

The structure of the red corpuscle has been determined accurately only recently. The balloon-like structure and the unusual surface membrane seem closely related to the peculiar biconcave shape. This discoidal form is readily altered chemically and physically by injurious agents and mechanical procedures. With injury, the shape tends to become spherical from the play of surface tension forces.

The red cell acts as a cup on an endless chain conveyor whose function is to ferry oxygen from the lungs to the tissues. It is so small that about 60,000 can be placed on the head of a common pin. Each cell makes about 60,000 round trips from lungs to tissues during the average lifetime of thirty days.

All the cells together, the erythron, constitute a vessel for holding hemoglobin by which the oxygen is carried.

The normal red cell is a biconcave disk. There may be congenital variations in shape and structure. Spherocytosis and ovalocytosis are anatomic variations in shape. With certain congenital abnormalities of structure, the cell may become sickle shaped under certain conditions as in sickle-cell anemia, or be destroyed more easily than normal as in Mediterranean (Cooley's) anemia.

All measurements of the red cell are easily made and are of great clinical significance. With accurate measurements the exact total mass of cells in the body is known and the configuration of the mean erythrocyte, the unit of the erythron, can be visualized.

From the laboratory standpoint, the anemias are best classified on the basis of the volume and hemoglobin content of the mean red cell. Such measurements often give a clue to the etiology of the anemia.

With a study of the red cell and bilirubin, the end-product of hemoglobin metabolism, the physiology of the red cell can be determined in any given case.

The treatment of anemias due to a deficiency of the erythrocyte-maturing factor (EMF) of liver, and those due to a lack of iron, is best gauged by a careful study of the mean red cell. If the elements deficient are properly supplied, the cells return to normal.

# RUSSELL L. HADEN

## REFERENCES

1. Hooke, Robert: *Micrographia*, London, 1665, p. 112.
2. Swammerdam, J.: *The Book of Nature*, English edition translated by Thomas Floyd, London, C. S. Seyffert, 1768, p. 31.
3. Malpighi, Marcello: *Opera Omnia, Lugdini Batavorum*, 1687, p. 235.
4. The Collected Letters of Antoni van Leeuwenhoek, Amsterdam, Swets and Zeitlinger, 1939, p. 67.
5. *ibid.*, p. 85.
6. *ibid.*, p. 103.
7. *ibid.*, p. 213.
8. *ibid.*, p. 301.
9. van Leeuwenhoek, Antoni: *Arcana natura detecta, Delphis Batavorum*, 1695, p. 2.
10. Hewson, William: *Collected works published by Sydenham Society*, London, 1794, p. 211.
11. Hunter, John: *A treatise on the blood, inflammation and gunshot wounds*, London, E. Cox, 1812, p. 70.
12. Hoppe-Seyler, F.: *Beiträge zur Kenntniss des Blutes des Menschen und der Wirbelthiere*, *Med. Chem. Untersuch. a. d. Lab.* 1:169, 366, 523, 1867-1871.
13. *ibid.*: *Ueber die oxydation in lebenden Blute*, *Med. Chem. Untersuch. a. d. Lab.* 1:132, 1867-1871.
14. Vierordt, K.: *Zählungen der Blutkörperchen des Menschen*, *Arch. f. physiol. Heilk.*, 11:327-331, 1852.
15. Welcher, H.: *Ueber Blutkörperchenzählung und farbeprüfende Methode*, *Vrtljschr. f. d. prakt. Heilk.*, 44:11-30, 1854.
16. *ibid.*: *Groesse, Zahl, Volum, Oberfläche, und Farbe der Blutkörperchen bei Menschen und bie Thieren*, *Ztschr. f. rat. Med.* 3R, 20:257-307, 1863.
17. Malassez, Z.: *De la numeration des Globules Rouges du Sang*, Adrien Delahyze, Paris, 1873.
18. Preyer, W.: *Die Blut Krystalle*, Jena, Mauke, 1871.
19. Manassein, W.: *Ueber die Dimensionen der Roten Blutkörperchen unter verschiedenen Einflüssen*, August Hirschwald, Berlin, 1872.
20. Hayem, G.: *Recherches sur l'anatomie normale et pathologique du sang*, G. Masson, Paris, 1878.
21. Malassez, L.: *Sur la Richesse en Hemoglobine des Globules rouges du Sang*, *Archiv. de physiologie*, 4:634-655, 1877.
22. Hart, Mrs. Ernest: *Micrometric numeration of the blood corpuscles and the estimation of hemoglobin*, *Quart. J. Micr. Sci.*, 21:132-145, 1881.
23. Isaacs, Rafael: *Personal communication*.
24. Ponder, E.: *The mammalian red cell and the properties of hemolytic systems*, *Protoplasma Monographs*, vol. 6 Gebrüder Borntraeger, Berlin, 1934.
25. *ibid.*: *Kinetics of hemolysis*, *Physiol. Rev.*, 16:19-51, (January) 1936.
26. Barcroft, J.: *The raison d'être of the red corpuscle*, *Harvey Lectures*, pp. 146-163, 1921-1922.
27. Hartridge, H.: *The shape of the red blood cell*, *J. Physiol.*, 51:81, 1920.
28. Jacobs, M. H.: *The exchange of material between the erythrocyte and its surroundings*, *Harvey Lectures*, pp. 147-164, 1926-1927.
29. Ponder, Eric: *The physical structure of the red cell membrane with special reference to its shape*, *J. of Faraday Society*, 33:947-954, 1937.
30. Abramson, H. A., Furchgott, R. F., and Ponder, E.: *Electrophoretic mobility of rabbit erythrocytes and ghosts*, *J. Gen. Physiol.*, 22:545-553, (March) 1939.
31. Bergenhem, B. and Fähraeus, R.: *Ueber spontane Hämolysinbildung in Blut unter besonderer Berücksichtigung der physiologie der Milz*, *Acta path. et microbiol. Scandnav.*, supp. 26, pp. 211-216, 1936.
32. Haden, R. L.: *Technic of determination of relative mass, individual cell volume and volume index of erythrocytes of man*, *J. Lab. & Clin. Med.*, 15:736-746, 1930.
33. Haden, R. L.: *A new instrument for the diffractometric measurement of red cell diameter*, *J. Lab. & Clin. Med.*, 25:399-403, 1940.
34. Von Boros, J.: *Die Behandlung der Anämien*, *Ergebn. d. inn. Med. u. Kinderh.*, 42:635-740, 1932.
35. Haden, R. L.: *The red cell mass in polycythemia in relation to diagnosis and treatment*, *Am. J. M. Sc.*, 196:493-502, 1938.
36. Haden, R. L.: *Studies in Hematology: II. Hemolytic anemia*, *Porter Lectures, Series X*, University of Kansas, 1940.