A DESCRIPTION AND COMPARISON OF NORMAL AND ABNORMAL RED BLOOD CELLS IN MAN

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INTRODUCTION

The purpose of this report is to describe man's red blood cells as seen under normal conditions and then to compare this description with that of man's red blood cells in various abnormal conditions. The material contained here is merely a compilation of the facts, opinions, studies, and theories from the literature in the field of hematology. As is the case in any scientific discipline, new data is constantly being added.

Young (1960) remarks it is a convenience that the red blood cell mass is so easily sampled. This cell repeatedly provides insight into cytochemistry and pathological physiology which may some day be correlated with similar findings in other human cells. Young suggests that recent studies of the red cell should be called prototypes because the future holds great possibilities for application of biochemical and biophysical methods of studying many types of cells. He further states, "One of the reasons the study of medicine becomes more fascinating each year is that we can now begin to sketch some probable courses of events by which abnormalities within certain cells may lead to manifestations of disease in many parts of the body."

Several theories as to the origin of the red blood cells exist. Israels (1939) called the original blood cell material hemocystoblasts and stated that these differentiate to form either leucocytes or proerythroblasts. Each of the latter then forms either a normoblast A, then a normoblast B, then a normoblast C and eventually a normocyte, or, if the anti-anemic liver principle is absent as in pernicious anemia, or not properly functional as a achrestic anemia, a megaloblast A, then a megaloblast B, then a megaloblast C, and eventually a megalocyte. He believed that true megaloblasts occur only when hyperplasia of bone marrow is a result of the lack of, or improper functioning

of, the anti-pernicious anemia liver principle. In all other conditions, normoblastic cells are found. Israels (1941) later indicated that normal erythropoiesis starts from a large cell with basophilic cytoplasm and well marked nuclear structure. As this cell matures, it shrinks to one-half its original size, the cytoplasm becomes less basophilic, and as the red-staining hemoglobin becomes more prominent, the cell passes through the purple and polychromatic stages to be entirely eosinophilic when treated with Wright's stain. Closely correlated with these changes, the nucleus shrinks, the chromatin condenses and appears as a lumpy, darkly staining mass with pale intervening areas, and finally all chromatin coalesces to a dark mass. In the normal maturation process, hemoglobinization of the red blood cell, as judged by the degree of eosinophilia, occurs late in the life history of the cell and when the nucleus has lost its ability to divide by mitosis. Megaloblastic erythropoiesis follows the same sequence except that the nuclear mass is less condensed and the hemoglobinization occurs earlier. Intermediate hemoglobinized megaloblasts are found with slightly polychromatic cytoplasm and mitotic figures, Israels (1941) continues. Israels also notes that this sometimes occurs in normoblasts in hemolytic anemias, when a heavy call for fresh erythrocyte production demands it.

Wintrobe (1956) favors, but not dogmatically, a modification of Downey's neo-unitarian theory of blood cell formation. According to this theory the original substance in the bone marrow, termed reticulo-endothelium, gives rise to megakaryoblasts, large lymphocytes, promegaloblasts or myeloblasts. The latter subsequently give rise to either megakaryoblasts and ultimately blood platelets, or rubriblasts and ultimately red blood cells.

Granick (1949) describes the pronormoblast, which is now termed rubriblast, as being 12-19 micra in diameter, with a large nucleus with thick strands of clumped chromatin, and often prominent nucleoli. The membrane is thin, and there is a thin rim of basophilic cytoplasm. With Wright's stain at this time the stained basophilic granules mask any evidence of the presence of hemoglobin. There is rapid cell division, and the cytoplasm contains much ribonucleic acid. Granick (1949) states the basophilic normoblast, which is now termed prorubricyte, has a nucleus in which the chromatin is course and granular and it may look like a wheel hub and spoke arrangement. The cytoplasm is more basophilic and the amount of nucleic acid is reduced. The rubricyte shows pink granules near the nucleus and this indicates the presence of hemoglobin. The nucleus is smaller, the chromatin is condensed in clumps, and the nucleoli have disappeared. The cell is reduced to 12-15 micra in diameter. The metarubricyte is further reduced to about seven micra, and the nucleus is pyknotic and disappears before the next stage. Cell division has ceased and only a trace of nucleic acid is present, but the amount of hemoglobin has increased from 1-2 to 20%. This cell assumes the biconcave shape. The diffusely basophilic erythrocyte is a large red blood cell with a fine reticulum across the face, or it may appear as small granules or threads. This cell contains fat and has a sticky surface. The reticulum contains some ribonucleic acid. Granick (1949) states that this reticulum is made to disappear by the stomach's erythrocyte maturing factor. These changes have been brought about by nucleic acid metabolism, protein synthesis and differentiation. The diffusely basophilic erythrocyte then further matures to form a red blood cell—normal or abnormal.

NORMAL RED BLOOD CELLS IN MAN

Granick (1949) describes the mature red blood cell as a tiny bag of hemoglobin without a nucleus and hence without the ability to synthesize desoxyribose nucleic acid. As late as 1931 the red blood cell was believed by many to be a homogeneous jelly-like mass with hemoglobin held in meshes of this mass. On the other hand, Key (1921), stated that the red blood cell has a definite membrane within which hemoglobin is held as a hydrophil gel. Wintrobe (1956) states that there is uncertainty regarding the fine structure of the red blood cell, but, "it seems that there is a highly oriented lipid and protein surface ultrastructure surrounding a quantity of hemoglobin which has an orientation more definite than the randomness of a solution.

Size

Wintrobe (1956) states that the mean diameter of normal red blood cells in an adult after careful staining and drying is 7.2-7.9 micra although means within normal persons have been found as low as 6.5 micra. Individual cells as small as 4.75 and as large as 9.5 have been reported, but the greatest usual variation is 3.5 micra. If cells are measured wet they are 0.8-1 micron greater in diameter. The normal mean thickness is given by Price-Jones (1933) as 2.14 micra, and 2.05 micra by Wintrobe (1930). The mean cell volume is given by Diggs (1956) as 87 cubic micra. There are causative variations in red blood cell size, some of which are significant. Erythrocytes from adult females may be 0.14 micron larger in diameter and 2-4 cubic micra greater in volume than those from adult males. Some diurnal variation has been reported but is not significant. Price-Jones (1933) and Smith (1932) claim that the amount of physical exercise of the individual causes variation in red blood cell size.

Hurtado et al (1945) report that low barometric pressure causes an increase in the mean cell size and the red blood cell forming activity. Age of the individual causes very significant differences in cell size. Wintrobe (1956) presents data to show that at birth the mean cell volume is 106-109 cubic micra ±10% and the mean diameter is 8.25-8.63 micra. After two weeks there is a substantial reduction in mean size. After two months, adult values are seen, but the decrease continues until at 12-18 months the mean cell volume may be 65-85 cubic micra. The volume remains below adult values all through childhood until puberty when adult values are reached again. The mean cell hemoglobin varies as does the cell size, but in spite of these changes, the mean cell hemoglobin concentration remains stable at 32-36%.

Shape

Wintrobe (1956) states that the mature red blood cell is round or slightly oval with a thin central area. When on edge it can usually be seen as a quite symmetrical biconcave disc, but forms occur which are somewhat kidney shaped, bell shaped or even spherical. Because of surface tension, red blood cells collect in rouleaux and resemble a stack of coins which have been carefully spilled. Mature red blood cells are elastic and flexible. Flonman and Wintrobe (1938) found 1-15% of the red blood cells in most non-anemic individuals to be oval in shape.

Numbers

4.5 million and 5.0 million red blood cells per cubic millimeter have been repeatedly indicated as the normal values for adult women and men respectively. Wintrobe (1956) provides more exact figures. Adult males have

5.4 ± 0.8 million red blood cells per cubic millimeter, and adult females have 4.8 ± 0.6 million per cubic millimeter. Emigh and co-workers (1957) found after studying 663 normal women that the normal erythrocyte count was 4.37 million/cubic millimeter. Red blood cell number varies throughout an individual's lifetime.

At birth there are 5.1 ± 1.0 million red blood cells per cubic millimeter. Counts as high as 6,800,000 and 9,670,000 have been reported. Values are about 15% higher at birth when the unbilicus is not clamped until the placenta has separated, and counts derived from blood obtained by heel puncture are higher than for blood obtained by venapuncture. At one week, the red blood cell count begins to fall and the volume of packed cells and hemoglobin content fall even more rapidly. At four weeks, these values have decreased significantly. At two to five months the decrease stops. Wintrobe (1956) suggests this is possibly because of the iron supplement to the diet about this time. Today, with iron-fortified infant formulas, the low point of this curve is probably reached earlier than two to five months. At eighteen months the average red blood cell count is 4.5 million per cubic millimeter, hemoglobin is 11.5 grams per 100 milliliters of blood, and the usual volume of packed cells is 35 milliliters ± 10% per 100 milliliters of blood. At two, three or four years a gradual increase in these values begins and continues until puberty when both male and female children have counts equal to those of adult females. Beyond puberty the male count increases rapidly. Many workers report a decrease in red cell count, hemoglobin content and mean cell volume when the individual reaches 60 years of age and suggest that the diet at this age may be the primary cause.

Brown (1946) reports that during a normal day's activities, there is variation in erythrocyte count and hemoglobin content equivalent to 4% of

the mean hemoglobin. During complete inactivity there is little or no variation in count or hemoglobin content. Wintrobe (1956) suggests that muscular activity may increase red blood cell destruction which in turn stimulates production.

Psychic factors play a role in red cell count. Wintrobe (1930) notes that fear brings on an increase in the number of red blood cells and causes "emotional polycythemia". Wintrobe (1956) suggests that a premenstrual decrease in count may be the result of hydremia.

The effect of low barometric pressure on cell size and production has been mentioned earlier. Generally low atmospheric pressure also produces an increase in red blood cell count, quantity of hemoglobin and packed volume. The degree of increase is greater with more frequent and long-lasting trips to high altitudes. There is a limit to the compensating adjustment; that is, the increase in count does not continue indefinitely in direct proportion to the decrease in pressure. Hurtado (1945) reports that when the oxygen saturation in arteries reaches 60-70% there is a decrease rather than further increase in red blood cell count and hemoglobin content. The altitude at which this occurred was about 20,000 feet.

Climate, temperature and season of the year probably have no significant effect on blood cell count. Almost identical values have been found in the United States, East Africa and Malaya.

General Make-up and Chemistry

Mertz (1960) states that whole blood contains 19-23% by volume of solid material and of this amount, 40-50% by volume are the formed elements, i.e., red and white blood cells and platelets in a ratio of about 1 WBC: 100 platelets:

1000 RBC. About one-half of the total solid material in the red blood cell is the chromoprotein hemoglobin, and most of the remainder is plasma protein—mainly albumin and globulin (Mertz 1960). Wintrobe (1956) declares that for a long time the red blood cell was thought of as an inert protoplasmic particle. Now the red blood cell is thought of as a tiny "dynamo of activity" by Bartlet and Marlow (1953) and as a living cell since it does work by transporting oxygen, and it requires nourishment to maintain energy metabolism. Wintrobe (1956) indicates that the red cell has a life span of approximately 110-130 days, it travels 100 miles between the heart and the various body tissues, and it needs energy to maintain the optimum osmotic pressure and the discoid shape. Energy is also required to transport ions across the cell membrane, and to provide a reducing system to reconvert ferric hemoglobin, or methemoglobin to the ferrous state. Granick (1949) believes that small concentrations of a glycolytic enzyme system seem to do this. Wintrobe (1956) indicates that the method by which the hemoglobin is held in the cell is questionable, and how the discoid shape is maintained is unknown, although Granick (1949) states that an antispher-ing protein, a serum albumin with little carbohydrate, plays a part. Wintrobe (1956) points out that when blood is observed flowing through small blood vessels, the red blood cell shows as a flexible, elastic structure. It can be divided without losing its contents which supports the theory that the hemoglobin is contained in a jelly-like mass. The hemoglobin molecules must be very closely packed. Perutz (1948) has estimated that any further increase in concentration of the molecules would seriously affect reaction rates by decreasing the rotation of molecules.

The surface area of the red blood cell is difficult to compute because of its biconcave shape. Wintrobe (1956) estimates that it is of the order

of 140 square micra. In the man of average size, the total red blood cell surface area is 3,820 square meters or more than 2,000 times his body surface area. Only about two per cent of the hemoglobin can be on the cell surface since there are approximately 300,000,000 molecules of hemoglobin in each red blood cell.

Wintrobe (1956) reports the red blood cell contains, in addition to hemoglobin and a glycolytic enzyme system, glucose and its degradation products, other enzymes and lipids, vitamins such as nicotinic acid, riboflavin, and ascorbic acid, sulfhydryl compounds, and minerals such as zinc, iron, and copper. The main cation is potassium, but sodium, calcium, and magnesium are also present. The chief anions are chloride, bicarbonate, hemoglobin, inorganic phosphate and several organic phosphates.

Granick (1949) states that the mature red blood cell cannot synthesize hemoglobin and heme, but contains catalase, an iron protein which protects the heme present from peroxide decomposition. Carbonic anhydrase aids in transporting carbon dioxide as bicarbonate ions by acting as a catalytic agent for this reaction: $H_2O + CO_2 \rightleftharpoons H_2CO_3$ Carbonic anhydrase is a zinc protein. Wintrobe (1956) found a high degree of correlation between the amount of zinc present and the carbonic anhydrase activity. This is significant in all anemias (except pernicious) where both are decreased.

Granick (1949) submits that the stroma or the insoluble framework of the red blood cell makes up 2-5% of the wet weight of the cell. Of this, 40-60% is protein and 10-12% is lipid. He states that in addition to the antisphering protein, catalase, and carbonic anhydrase, the red blood cell also contains elinin, a lipo-protein complex containing the Rh, A and B antigens, hemoglobin, (about 30% by wet weight), hemocuprein, stromatin and phosphomonesterase whose

functions are unknown, cholinesterase similar to the brain type, but different from that found in plasma, and two other unidentified proteins. Young (1960) adds glucose-6-dehydrogenase, 6-phosphogluconic dehydrogenase, phosphohexose isomerase, and aldolase as other red blood cell proteins.

Granick (1949) states that about 40-60% of the lipids are bound to the stroma proteins. This lipid material consists of cephalins in the form of phosphatidyl ethanolamine and phosphatidyl serine, lecithin, cholesterol, cholesterol esters, neutral fats and cerebrosides.

Young (1960) states that the activity of the enzymes decreases as the red blood cells age in circulation and further that this decrease in enzyme activity and the ensuing decrease in energy production likely lead to the death of the red blood cells. He speculates that this decrease in enzyme activity is possibly what happens in the ageing of cells other than red blood cells.

Red Blood Cell Precursors in Circulation

Even in the "normal" blood circulating throughout the blood vessels of "normal" individuals, there are other than mature, normal red blood cells. It will be seen later that these same red blood cell precursors and unusual red blood cells are found in various abnormal conditions as well, although perhaps, in greater numbers. Wintrobe (1956) and Diggs (1956) describe these erythrocyte precursors as follows:

a) Diffusely basophilic red blood cells are found infrequently. They are nearly mature cells without a nucleus but with sparse basophilic cytoplasm remaining. When stained with Wright's stain, the entire cell may appear bluish-gray, but it is more often mingled with pink staining parts.

b) Stippled erythrocytes are other nearly mature red blood cells with round bluish-black granules spread uniformly throughout the cell when stained with Wright's stain. Dark field illumination will enable one to see these also in the unstained condition. These are found in .01% of the cells in normal blood and more frequently in some anemias and heavy metal poisoning. After the cell membrane is punctured, the basophilic ribonucleoprotein is precipitated by the stain.

c) Reticulocytes are found regularly in normal blood. The reticulum may be a dense mass resembling a nucleus, a thin band across the cell, or evenly distributed across the cell. The amount of reticulum appearing decreases as the cell ages and approaches the mature erythrocyte stage. Technique used in staining plays a major role in the appearance of the reticulum. Diggs (1956) states that the reticulum is not visible in unstained preparations nor in dried smears stained with Wright's stain. In the latter case, the cells must be exposed to a supravital stain, i.e., brilliant cresyl blue, which penetrates the membrane of the living cell and stains the reticulum. The cells may then be fixed and counterstained. Wintrobe (1956) states that reticulocytes make up 0.5-1.5% of the red blood cells in normal blood. Friedlander (1929) believes this figure is too high and should be .03-0.2% or not more than 10,000 per cubic millimeter. Friedlander found that there is an increase during the spring months in healthy adults because ultraviolet rays from the sun stimulate reticulocyte production and release. Newborn infants have 2-5% reticulocytes until about five days of age and human embryos have 6-50%. Pregnant women show an increase in reticulocytes. Hemorrhage is a stimulus to blood regeneration and appearance of more reticulocytes. Depending on the amount of hemorrhage, the reticulocyte count reaches its maximum around five days after the

hemorrhage. Then as the red blood cell count reaches normal, the reticulocyte count drops off. Friedlander indicates that the reticulocyte count is a good indicator of whether regeneration will continue or whether transfusion is necessary. Wintrobe (1956) reports that reticulocytes stick together more actively than mature red blood cells and they move more slowly. This may explain their normal tendency to remain in the bone marrow. Wintrobe also reports that reticulocytes probably mature in 2-4 days and normally in the marrow, then enter circulation as mature red blood cells. Key (1921) found that the specific gravity of reticulocytes is lower than that of mature red blood cells and thus they tend to collect in the upper part of cellular suspensions.

d) Macrocytes are generally defined as otherwise normal mature red blood cells which measure more than nine micra in diameter. Wintrobe (1956) reports that these are found infrequently in the circulation of normal blood and are merely recent arrivals which tend to assume a more normal size as they grow older.

e) Issacs (1925) called red corpuscles which contain single refractive bodies about 0.5 micra in diameter granule red blood cells. They occur in about 0.3-0.7% of the cells of normal blood. Wintrobe (1956) states these granules may appear at the edge of the red blood cell or in the center and are red in supravital mixtures and black in stained blood films. Issacs (1925) further states that the granule red corpuscle is the final stage in the maturation of the red blood cell in the bone marrow of man, dog, mouse and rabbit. When increased production of red blood cells is demanded, and the rate of production of mature red blood cells is not adequate, these granule red corpuscles are the first to reach the periphery of circulation. If body

requirements are still not satisfied, reticulated cells appear in circulation. if these do not satisfy the red blood cell requirement and the bone marrow is not diseased, then nucleated erythrocytes appear in increased numbers. Wintrobe (1956) claims that the nature and origin of the granule red corpuscle are unknown, and that they do not take nuclear stain or cresyl blue stain.

f) Diggs (1956) states that siderocytes are red blood cells with small particles of nonhemoglobin iron which appear as blue to black granules extruding from the cell surface when treated with Wright's stain. These bodies have also been called Pappenheimer bodies. Diggs also states that these bodies vary in size from barely visible granules to objects 1-2 micra in diameter, that they are usually round but may be elongated, and that they frequently appear in pairs or small groups. Diggs claims that siderocytes are not found in the peripheral blood of normal individuals, whereas Wintrobe (1956) claims they are found, but not in significant numbers in health. He provides no numbers to clarify "significant". Diggs (1956) submits that these bodies are found in varying numbers in the nucleated red cells in bone marrow smears of normal individuals. Wintrobe (1956) has found that the administration of phenylhydrazine to normal individuals causes an increase in siderocyte count which is equal to the decrease in normal erythrocyte count. He further suggests that the siderocyte is an ageing erythrocyte and that a siderocyte count is an indicator of the severity and progress of hemolytic conditions.

ABNORMAL RED BLOOD CELLS IN MAN

General Considerations

When red blood cells are found to deviate significantly from normal as described in the previous pages, it is obvious that they would be termed

abnormal. The literature records many disagreements as to what is normal and what is abnormal. Wintrobe (1956) claims that close inspection of normal blood shows that not all red blood cells are identical even though great care has been taken in preparing the smears. When few unusual red blood cells are found, the patient's condition can be considered normal or near normal; however, when unusual cells are found in quantity, they are commonly associated with an abnormal condition. Erythrocytes become unusual when they deviate significantly from the normal in size, shape or number.

Jandl and co-workers (1961) have found that physically abnormal red blood cells found in certain anemias can be filtered and thus segregated from normal cells by an inert filter. This is done on the basis of size, shape, viscosity, applied pressure, and size of the filtering apparatus. They state that the liver, spleen and capillaries act as the filtering apparatus in a similar segregation of normal and abnormal red blood cells during maturation and circulation.

Size

Anisocytosis is an exaggeration of the variation from normal size in red blood cells. The mean diameter has been given as 7.2-7.9 micra, and a cell this size is considered a normal erythrocyte. A microcyte is a cell whose diameter is less than 6 micra, and a macrocyte is a cell whose diameter is more than 9 micra. Wintrobe (1956) claims that size variation is the most common alteration in disease.

Shape

Poikilocytosis is a condition of variation from the normal shape of red blood cells. Wintrobe (1956) points out that in identifying poikilocytes it is important to see that preparations are handled and stained with care. It is possible to damage cells and cause crenation or hemolysis. He further states that confusion may arise from observing degenerating erythrocytes and thinking they are poikilocytes or normal cell inclusions. Maragliano bodies are round or oval bodies appearing as vacuoles in the red blood cell. These bodies are evidence of erythrocyte degeneration.

Numbers

Polycythemia vera is a condition characterized by an abnormally increased number of red blood cells in circulation. Oligcythemia is a condition in which there are fewer red blood cells than normal in circulation.

CONDITIONS ASSOCIATED WITH ABNORMAL RED BLOOD CELLS IN MAN

Ovalocytosis

Some red blood cells deviate from the normal shape and appear more oval or elliptical. Wyandt and co-workers (1941) found that no patients had more than 15% elliptocytes unless anemia was present. The same publication quotes Gunther as stating that a normal person should have no more than 12%, or, in rare instances, a 22% sum of elliptical and rod shaped cells. Florman and Wintrobe (1938) indicate that less than 40% elliptocytes and 10% rod shaped cells may be considered normal. Stephens and Tatelbaum (1935) state that this condition is similar to the sickling of cells seen in sickle cell anemia,

but differs in that ovalocytes round up and appear normal in a sealed fresh blood preparation. Ovalocytes and elliptocytes appear frequently in anemias. Florman and Wintrobe (1938) report that in macrocytic anemia, more than 25% of the red blood cells are elliptical, and in hypochromic microcytic anemia, 6% are oval, while in simple microcytic anemia, 8% of the red blood cells are oval. These percentages of ovalocytes in normal and anemic individuals represent conditions known as symptomatic ovalocytosis. When the percentage of ovalocytes and rod forms rise to 50-90%, a condition of hereditary elliptocytosis is likely. Wintrobe (1956) states that this is estimated to be present in 0.4% of the general population, and is especially common in the Dutch, German and Italian groups. It is also found in Negroes, Scotch-Irish and Jewish peoples. Diggs (1956) states the trait is transmitted genetically as a simple Mendelian dominant affecting both males and females. Penfold and Lipscomb (1943) state that elliptocytosis is usually harmless, but the condition has been found in association with evidence of increased blood destruction in about 12% of those individuals carrying the trait. Wintrobe (1956) classifies hereditary elliptocytosis according to its severity as follows:

1. Cases which show no signs of hemolysis.

2. Cases which show hemolysis but no anemia probably because increased production compensates for the increased destruction.

3. Cases in which there is hemolysis and anemia.

Stephens and Tatelbaum (1935) studied fifteen members in one Italian family and found eight had elliptical red blood cells, while the remaining members had normally shaped erythrocytes. This anomaly has been found in two Maltese families and reported on by Grech and co-workers (1961) who claim that theirs is the first report of elliptocytosis among the Maltese peoples. Other

Isolated instances of elliptocytosis or ovalocytosis in human beings have been recorded by Motulsky (1953), Fadem (1949), Wagner (1945), Terry (1932), and others. There seems to be general agreement that this disorder is usually not incompatible with health and it has not been proven to be related to any disease. Terry and associates (1932) further state that it is found in all four ABO blood groups in almost equal proportion.

Average ovalocytes are 8.1 micra long and 5.3 micra wide although the extremes are given by Wintrobe (1956) as 12.2 micra long and 1.6 micra wide. Most of the above cited reports fail to state findings in total red blood cell number although Wintrobe (1956) indicates they may be slightly higher than normal. Stephens and Tatelbaum (1935) state that their eight subjects showed an increase in the number of red blood cells per cubic millimeter and a definite decrease in mean corpuscular volume. The red cell count is inversely proportional to the mean cell volume. Grech and co-workers (1961) found in 35 members of the two Maltese families that 17 had elliptocyte counts of 30-100%, two had 20-25%, and 16 had 0-1%. They believe that any individual who has an elliptocyte count above 20% whose near relatives show definite evidence of elliptocytosis constitutes the full carrier state.

The abnormal shape of these cells appears first in the reticulocyte stage states Wintrobe (1956). Grech and co-workers (1961) claim the cell contour changes in the late reticulocyte stage. Diggs (1956) reports the elliptical shapes develop days after the cell enters circulation; nucleated cells and reticulocytes rarely show the oval shape. Wintrobe (1956) and Terry (1932) agree that these cells round up and appear normal when they are subjected to hypotonic salt solution; however, Wagner (1945) believes that in true ovalocytosis this is not so. He describes an experiment done on a Negro with 86%

elliptocytes, a few macrocytes and microcytes and an occasional poikilocyte. He suggests that this individual had an intermediate type of ovalocytosis since these elliptical cells all rounded up after 24-56 hours in a sealed isotonic saline preparation. When the owner's plasma was added, the cells reverted to their elliptical shape, and when he added plasma from blood of the same type, the rounded cells reverted to their original elliptical shape. He notes that the elliptocytes showed central achromia except when they rounded up, and that similar cases have been studied by Terry and associates and by Cheney. Wintrobe (1956), Motulsky (1953) and Fadem (1949) state that the concentration of hemoglobin is normal; Stephens and Tatelbaum (1935) found the mean cell hemoglobin inversely proportional to the red blood cell number. Motulsky and associates (1953) have reported on three cases of elliptocytosis. The life span of the elliptocytes in two of these cases was 103 and 117 days, or near normal. The life span of elliptocytes in the third case was 45 days, and this patient showed evidence of hemolysis without anemia. This report also states that the elliptocytes from the patient with hemolytic elliptocytosis were morphologically indistinguishable from those which showed a normal life span.

Wintrobe (1956) reports the anomalous shape of the cell appears to be the result of a property within the cell rather than some unique environmental influence. He further claims it has been suggested that elliptocytes are atavistic forms which represent structural adaptations to some unknown constitutional factor.

Fadem (1949) describes a Negro, aged 25, who indicated he had a chest pain and felt weak. A blood examination revealed that he not only had 65-84% elliptocytes, but also 3-11% sickled cells, and some normal red blood cells.

Other counts and indices were normal. Apparently elliptocytosis can be complicated by other disorders. Wintrobe (1956) mentions that elliptocytosis has also been found in association with another hereditary disorder, hemorrhagic telangiectasia or excessive dilatation of the capillaries.

Heinz-Erhlich Bodies

Wintrobe (1956) found these refractile irregularly shaped bodies are seen in wet unstained preparations lying near the edge of the red blood cell. They can be distinguished from other bodies by staining in methyl violet. These bodies stain a deep purple, Howell-Jolly bodies and Pappenheimer bodies stain almost black with a bluish tint, and the reticular filaments of reticulocytes stain a pale blue. He describes the Heinz-Erhlich bodies as ranging in size from minute particles to three micra in diameter, and their shape as irregular. Several may occur in a single cell, but if the body is especially large it is usually alone.

Harley and Mauer (1961) state that Heinz-Erhlich bodies contain protein, but exactly how these bodies are formed is debatable. They say that the bodies are primarily composed of the degradation products of hemoglobin. They found a close relationship between the degree of Heinz-Erhlich body formation and intact hemoglobin destruction. Therefore, they claim, the Heinz-Erhlich body formation is probably due to certain drug action on intracellular hemoglobin.

Wintrobe submits that a primary reaction takes place between the red blood cell and one of these toxic agents: several aromatic nitrogen compounds of the nitrobenzene series, aniline derivatives, aliphatic nitro-compounds, hydroxylamine, sodium nitrate, sodium chlorate, or naphthalene. These produce semi-fluid bodies which fuse and form semisolid bodies. At the same time,

the hemoglobin in the mature cell diminishes. Reticulocytes do not seem to be affected. Wintrobe (1956) reports that if man consumes acetanilid or phenacetin continuously, a few Heinz-Erhlich bodies may be found in the red blood cells. These bodies may be formed without anemia, but large doses of these drugs will cause hemolytic anemia. Young (1960) found available evidence indicates a deficiency of glucose-6-phosphate dehydrogenase activity as the fundamental abnormality in these hemolyzed red cells. Wintrobe (1956) reports Heinz-Erhlich bodies are also formed when phenylhydrazine or acetylphenylhydrazine are given to polycythemia patients. Primaquine and pamaquin cause hemolytic anemia in man and more especially the Negro. The older red blood cells seem to be more sensitive than younger ones, and they form Heinz-Erhlich bodies more readily. The degree of oxygen availability seems to increase Heinz-Erhlich body formation. The sensitive cells contain two-thirds the amount of glutathione as the nonsensitive cells do. From this, Wintrobe (1956) speculates that glutathione acts as an anti-Heinz-Erhlich body forming factor.

Polycythemia

Wintrobe (1956) defines polycythemia generally as an abnormal increase in the number of red blood cells in circulating blood; however, several specific types of polycythemia should be mentioned. Relative polycythemia is an increase in blood cell number compared to blood volume as a result of reduced intake of water, loss of body fluid through copious sweating, vomiting, or diarrhea, or loss of blood plasma. Transient polycythemia is an increase in the number of red blood cells as a result of stimulation of the spleen by epinephrine injection or an emotional response. "Emotional polycythemia" is an example. This is found more often in middle aged over-weight men in a state of anxiety

than it is in women. Absolute polycythemia is a term applied to an increase in the total red cell mass. Erythremia is polycythemia resulting from an unknown cause; erythrocytosis is polycythemia in response to one of these known causes:

1. Decreased oxygen saturation of arterial blood due to decreased atmospheric pressure or poor oxygenation of venous blood in the lungs.

2. Defective circulation as in congenital or chronic acquired heart disease or A-V aneurysm.

3. Formation of abnormal pigment such as methemoglobin or sulfhemoglobin resulting in ineffective red blood cells.

4. Brain tumors, overactive kidney tumors and Cushing's syndrome.

High altitude and the accompanying low atmospheric pressure produce transient erythrocytosis in individuals who do not stay in the environment long enough for their bodies to adjust to it. Wintrobe (1956) reports their symptoms are dizziness, nausea, ringing in the ears, vomiting and chills, but if they remain in low pressure areas for extended periods of time, apparently physiological readjustment produces an absolute polycythemia, for natives in the Peruvian Andes have been shown to have red cell counts of 7.5-8 million per cubic millimeter, and are apparently otherwise normal. Albritton (1952) reports Peruvian residents at an altitude of 4.5 kilometers have counts of 6.15 million cubic millimeter. In persons with transient erythrocytosis, the red blood cells are normal in size and shape. Albritton (1952) presents evidence of sojourners to Nauga Parbat, India, at an altitude of 7.0 kilometers, having erythrocyte counts of 8.10 million/cubic millimeter. Monge (1943) reports counts of 7 million are common and he reports one case of a count of 9.6 million red blood cells. Hurtado (1942) reports the packed volume of red

blood cells is slightly increased, the hemoglobin is increased, the mean cell volume is normal or slightly increased, and the mean cell hemoglobin concentration is normal. Merino (1950) found cell volume is markedly increased to 88-95 milliliters compared with the normal of 29.9 milliliters/kilogram of body weight. Wintrobe (1956) reports that departure from high altitudes relieves the condition, and although the specific cause of this polycythemia is unknown, probably there is overcompensation in response to the reduced amount of oxygen in the air.

Congenital heart disease as a result of pulmonary stenosis, defective auriculoventricular septum, patent foramen ovale or ductus arteriosus brings on erythrocytosis. The red cell mass is so increased that the total blood volume is higher than normal. Wintrobe (1956) reports red blood cell shape is not abnormal, but counts of 7-8.5 million per cubic millimeter are usual, and there have been counts of 10 million and 13.9 million. The serum bilirubin and iron are increased above normal but not more than should be expected with the increased hemoglobin metabolism.

Wintrobe (1956) claims that hemoglobin pigments such as methemoglobin and sulfhemoglobin are produced by excessive use of coal tar derivatives and cobalt and other chemicals. This causes inefficient red blood cells, and the body struggles to produce more, resulting in polycythemia. Caffeine, nicotine, iron, mercury, manganese and radium salts may have the same effect.

Erythrocytosis has been reported in many cases involving disorders of the central nervous system, especially brain tumors.

Erythremia is a chronic polycythemic disease of unknown origin and cause. It is also called polycythemia vera, splenomegalic polycythemia, polycythemia rubra, and many more names according to Wintrobe (1956). He also states that

there is an absolute increase in the number of red blood cells and total blood volume and hyperplastic bone marrow. Usually the skin has a purplish red color and the spleen is enlarged. Lucas (1912) found it is more common in men than women and has high incidence in Jewish people, low in Negroes. The blood is seen as dark in color and very thick. Wintrobe (1956) found red blood cell volume is usually around 80 cubic micra or seven cubic micra below normal, and the volume of packed cells is very high at 80-86 milliliters/100 milliliters of blood. Cell shape is usually normal but red blood cell counts of 7-10 million/cubic millimeter are common. Cases have been reported in which there are 12-15 million red blood cells/cubic millimeter. Wintrobe (1956) questions this by stating: 'When red corpuscles are normal in volume (87 cubic micra) there is 'standing room' only for about 11,500,000 cells and it is inconceivable that anyone could live with the blood consisting of all cells and no plasma." He also states hemoglobin content is increased to 18-20 grams/100 millimeters blood. Rubricytes and prorubicytes and occasionally a metarubricyte may be found and the reticulocyte count is near normal. Rosenthal and Bassen (1938) report the leukocyte count may be five times normal, and Wintrobe (1956) claims the number of platelets is 5-10 times normal and the blood itself is 5-8 times as viscous as normal blood because of the increased number of cells. The serum alone shows less than normal values in viscosity and specific gravity. The sedimentation rate is greatly delayed, the total blood volume is greatly increased, and the plasma volume is not found to be normal or above. Wintrobe (1956) reports the cause of erythremia is really unknown although the following have been suggested:

1. Some defect resulting in a lack of oxygen availability.
2. A reduced capability of the red blood cells to absorb oxygen.

3. Increased capillary dilation so that the red blood cells cannot shed oxygen and this stimulates more red blood cell production.
4. An increased number of megakaryocytes plugging long capillaries and producing oxygen want.
5. Compensation for anoxemia in the bone marrow.
6. Result of a combination of increased red cell production, decreased red cell destruction, and longer life span of red blood cell.
7. May be the antithesis of pernicious anemia i.e., overproduction of cells results in excessive stomach blood cell forming factor.
8. May be a relationship between erythremia and chronic myelocytic leukemia called erythroleukemia.
9. Related to abnormalities of the endocrine glands and resulting imbalance in hormone control of hematopoietic activity.
10. May be a constitutional basis because it is rarely found in stout or overweight people.
11. The spleen may play a major role in erythremia by decreasing hemolytic activity, by not regulating the increased activity of the bone marrow, and then tubercle bacilli causing myeloid tissue to replace the lymphoid tissue in the spleen.

ANEMIAS

General Considerations

Wintrobe (1956) reports the term anemia indicates a reduction below normal in the number of red blood cells per cubic millimeter, the quantity of hemoglobin, and the volume of packed red blood cells per 100 milliliters of blood. Wintrobe (1956) specifies that oligemia is a reduced total amount of blood, oligcythemia is a reduced number of red blood cells and oligchromenia is a reduced quantity of hemoglobin. A review of the literature shows anemias have been classified in many ways for purposes of study, identification and treatment. An old and outdated classification placed those with a basic disorder of the hematopoietic system in a primary category, and those anemias which were symptomatic of something else in a secondary category. It is now believed that all anemias are symptomatic, thus secondary, and this classification is valueless.

Wintrobe (1934) provides a classification of anemias on the basis of morphology.

1. Macrocytic anemias are those in which there is a greater decrease in red blood cell count than in the amount of hemoglobin and volume of packed cells because the cells are larger than normal.

2. Microcytic anemias are those in which there is a greater decrease in the amount of hemoglobin and volume of packed cells than in red blood cell count because most of the cells are smaller than normal.

3. Normocytic anemias are those in which there is a proportionate decrease in the number of red blood cells, amount of hemoglobin, and volume of packed cells.

4. Hypochromic anemias are those in which there is a greater reduction in quantity of hemoglobin than in volume of packed red blood cells because most of the cells contain less than the normal amount of hemoglobin for their size. The cells in this type of anemia are usually also reduced in size.

The anemias described in this report are discussed primarily according to the morphological classification.

Macrocytic Anemias

Macrocytic anemias are sub-divided into two main categories. One category includes all those which are the result of a lack of, or imperfect utilization of, vitamin B_{12} or folic acid, and in which the bone marrow is megaloblastic. The second main category of macrocytic anemias includes those arising from hyperplastic but non-megaloblastic bone marrow. This latter category will be discussed following representatives of the first group.

Pernicious Anemia. An example from the first group of macrocytic anemias is pernicious anemia. Wintrobe (1956) describes this condition as one in which the majority of the red blood cells are macrocytes, i.e., greater than nine micra in diameter, and as the anemia becomes more severe, the number of macrocytes increases. Occasionally round or oval cells twice the normal size with the blue-gray tint of stained prorubricytes may be seen. Price-Jones (1929) states diagnosis is more dependent upon the variation in the diameter of cells than upon the number of macrocytes. When the diameters are measured and plotted on a frequency curve (the Price-Jones curve), it can be seen that there is more variation in diameter than normal and that the majority of cells are larger than normal. In other words, there is a "shift to the right" in the Price-Jones curve. Wintrobe (1956) reports advanced stages of pernicious anemia usually show marked poikilocytosis and all shapes of cells are seen--"dumbell, anvil, cocked hat, hand mirrors etc." Many of these are smaller than normal red blood cells. Often there are 2,000,000 or fewer red cells/cubic millimeter. Cases have been reported with less than 1 million. Wintrobe (1956) writes these cells must be well filled with hemoglobin, or life would be impossible. Normally there should be no central pallor in these cells because they are thicker and darker, unless an iron deficiency accompanies the case and this is extraordinary. He further states the hemoglobin content increase is proportional to the cell size increase. The mean cell hemoglobin weighs 33-38 micromicrograms in moderate anemia, and 33-56 in severe cases, as compared with normal values of 27-31 micromicrograms. The color index of hemoglobin is also increased. Wintrobe (1956) reports these cells are not necessarily supersaturated with hemoglobin, as they appear dark when stained because of their increased thickness. The concentration of hemoglobin is normal (32-36%).

The blood picture of pernicious anemia cases can be described as generally containing all of the abnormalities of red blood cells except hypochromia. Prorubricytes, rubricytes, fine or coarsely stippled cells, Howell-Jolly bodies, chromatin particles, Cabot's rings, and pyknotic nucleated red cells are all found. Reticulocyte count is usually normal unless treatment has been started. Leukocyte count is low, and the plasma looks darker than normal. The cause of pernicious anemia is a lack of the intrinsic factor from the gastric juice. Wintrobe (1956) states this unidentified factor is not the hydrochloric acid or pepsin or renin, but it may be a hexosamine or a polypeptidase or something else which needs Vitamin B_{12} to combine with.

Sprue, Idiopathic Steatorrhea and Celiac Disease. Wintrobe (1956) describes sprue as a chronic wasting disorder found mainly in the tropics accompanied by diarrhea, glossitis, light colored, bulky, frothy stools and usually macrocytic anemia. He states that idiopathic steatorrhea or non-tropical sprue is a similar disease which is also accompanied by tetany, osteomalacia and osteoporosis due to calcium deficiency. Celiac disease is a similar disease appearing in infants. The blood picture of sprue shows a macrocytic anemia identical to that in pernicious anemia and often shows leukopenia and relative lymphocytosis. Macropolycytes and giant metamelocytes with broad vacuolated nuclei have also been reported. In idiopathic steatorrhea the anemia is commonly macrocytic but may be microcytic. In celiac disease, the anemia is more likely to be hypochromic microcytic. There may also be a large number of normoblasts in these two disorders, and especially in celiac disease. These two also show an iron deficiency as well as a vitamin B_{12} and folic acid deficiency more often than pernicious anemia does. These three disorders seem to be caused by impaired or inefficient absorption of vitamin B_{12} or folic

acid from the gastro-intestinal tract more than by a basic deficiency of these materials according to Wintrobe (1956).

Nutritional Anemias. There are several nutritional macrocytic anemias. Tropical macrocytic anemia as reported by Wills and Evans (1948) is found in tropical and sub-tropical regions and resembles pernicious anemia. It is probably the result of a deficiency of folic acid rather than vitamin B_{12}. Groen and Snapper (1937) report cases of a temperate zone macrocytic anemia which is similar to the tropical variety and pernicious anemia, but not nearly so common. Again it is the result of a folic acid deficiency more than a lack of vitamin B_{12}. Wintrobe (1956) reports pellagra is a clinical syndrome resulting primarily from a dietary deficiency of meat, eggs, dairy products, vitamins, and proteins. Chronic alcoholism with a poor diet, and food fads have also been known to bring on cases of pellagra. Various anemias have accompanied pellagra, and the type apparently depends upon the acuteness of the disorder. Although macrocytic anemia has been reported in association with pellagra, it is probably more rare than normocytic or microcytic types. The macrocytic anemia of pregnancy or pernicious anemia of pregnancy is not due to a dietary deficiency alone. Wintrobe (1956) suggests some cases have revealed that toxemia and infection interfere with the use of hematopoietic factors, while other cases reveal that numerous pregnancies and hemorrhages increase the demands on the body while a poor diet and/or faulty gastro-intestinal tract absorption decrease the supply or use of these factors.

Macrocytic Anemia Associated with Surgery. Macrocytic anemia associated with surgery or organic diseases of the gastro-intestinal tract has been recorded. Carcinoma of the stomach has been found in cases treated for pernicious anemia. It seems debatable which occurs first, but Wintrobe (1956) suggests

it is more common for the anemia to lead to development of the tumor. Total gastrectomy impairs absorption of vitamin B_{12} more than partial gastrectomy does and sometimes macrocytosis develops. In other cases macrocytic anemia is the result. Patients with intestinal strictures, mainly in the ileum, have developed a macrocytic anemia in which the blood picture, as described by Wintrobe (1956), is identical with that in pernicious anemia both in morphology and amount of reduction in red cell count. Watson and Witts (1952) theorize this anemia may be due to the presence of abnormal bacteria in the intestine which somehow divert folic acid and vitamin B_{12} from the host. Chronic dysentery, regional ileitis and pancreatic disease have been accompanied by macrocytic anemia in some instances, but Wintrobe (1956) states little detailed information is available concerning this subject.

Macrocytic Anemia Associated with Liver Disease. Macrocytic anemia may be encountered in conjunction with disease of the liver. This type of anemia is similar to that of pernicious anemia but not so severe. Red blood cells number 2.5-3.5 million/cubic millimeter and most are oval macrocytes. Poikilocytes, rubricytes, and nucleated red blood cells are rare. Macrocytic anemia is not the only type found in association with liver disease. Wintrobe (1936) studied 132 cases and found no anemia in 22.7%, macrocytic anemia in 32.6%, normocytic anemia in 30.3%, and microcytic anemia in 14.4%. Macrocytic anemia was found only in those patients who had had liver disease for a long time and had extensively diseased tissue. Wintrobe (1936) suggested that this macrocytic anemia is brought about by defective storage or metabolism of the anti-anemic principle in the liver; the picture is complicated at present and the causes could be numerous. Diet, intrinsic factor, production, storage, metabolism and red blood cell destruction by extracellular mechanisms may all

play a part. Bingham (1961) has reported on thin, thick, and target macrocytosis in 222 patients with hepatic and biliary tract diseases. 62% of these patients had some form of macrocytosis having mean cell diameters of 7.60 micra or higher. Thin macrocytes are produced in the bone marrow after hepatic disease involving damage to the parenchymal cells. This thin macrocytosis is eliminated only when the diseased cells are repaired. These cells are unusually thin but have normal mean cell volumes. Thick macrocytes are caused by a nutritional deficiency. Patients who have pre-existing hepatic disease and thin macrocytosis are especially susceptible to nutritional deficiency and its resultant thick macrocytosis. Target macrocytosis is caused by sustained biliary tract obstruction. These cells are actually thin macrocytes which are even more thoroughly flattened and in which the hemoglobin has been rearranged. Bingham (1961) has furnished some comparative cellular dimensions in his report, and they are reproduced here for clarification.

		Macrocytes		
Mean cell diameter (micra)	Normal red cells 7.14	Thick 8.17	Thin 7.94	Target 7.91
Mean cell thickness (micra)	2.30	2.27	2.01	2.00
Mean cell volume (cubic micra)	95.0	123.0	99.0	101.0

Macrocytic Anemia Associated with Fish Tapeworm. Fish tapeworm macrocytic anemia is caused rarely by the parasite Diphyllobothrium latum which inhabits certain fish eaten by man. Wintrobe (1956) believes that somehow this parasite causes anemia in individuals who are constitutionally predisposed to be susceptible. The anemia is rarely encountered in persons infested with the tapeworm. In a household in which all members harbored the tapeworm, macrocytic anemia would be found in related persons, but not among the servants

according to one student of this disease. In Finland where tapeworm infestation is heaviest only 0.1-0.2% of the people have macrocytic anemia. It so closely resembles pernicious anemia and is found so infrequently in individuals with the tapeworm, that it may be only an accidental occurence of two separate entities in some individuals. Precisely how the worm causes anemia, or if it does, is not at all clear.

Macrocytic Anemia Associated with Hypothyroidism. Macrocytic anemia has also been found in cases of hypothyroidism as reported by Stern and Altschule (1936). In this anemia the cells are normally shaped, rarely number less than 3.5 million/cubic millimeter, but they are uniformly macrocytes. The mean cell volume is increased to 95-120 cubic micra, the mean cell hemoglobin concentration is normal, and there is a shift to the right in the Price-Jones curve. Wintrobe (1956) reports the cause of this anemia is not clearly understood. Stern and Altschule (1936) indicate the bone marrow is hypoplastic, but there is no direct evidence that hypothyroidism inhibits cell maturation; however, administering thyroid extract seems to bring about a normal blood picture in 3-9 months.

Refractory Megaloblastic and Achrestic Anemia. Refractory megaloblastic and achrestic anemias are macrocytic and Wintrobe (1956) states the Price-Jones curve is shifted to the right as in pernicious anemia. Nucleated red blood cells, rubricytes, and an occasional prorubricyte are reported, but reticulocytes are scarce except after intensive liver extract injection therapy. It has been suggested by Israels and Wilkinson (1940) that achrestic anemia is a result of failure to use the anti-anemic principle. This anemia responds poorly, if at all, to liver therapy. Refractory megaloblastic anemia has been reported in a few instances by Davidson (1948). Several cases which did not

respond to liver therapy as the name implies, were identified in association with pregnancy or sprue. They responded to treatment with proteolysed liver or folic acid. Wintrobe (1956) states the pathogenesis of these anemias is unclear at this time. Most, but not all, cases seem to respond to folic acid treatment.

Normocytic Anemias

Wintrobe (1956) defines normocytic anemias as those in which the mean corpuscular volume and mean corpuscular hemoglobin content do not deviate significantly from the normal. Specifically, mean cell volume is 82-92 cubic micra in contrast with the normal of 87 ± 5, and the mean cell hemoglobin concentration is 32-36% in contrast with the normal of $34 \pm 2\%$. This should not be construed to mean that the red blood cells must be all uniform in size and shape or in hemoglobin content, but here again there are slight variations. Temporary exceptions to these rules may be seen and will be discussed under the specific anemia involved. In addition, several other anemias are permanent exceptions to these rules for normocytic anemia, but they are nevertheless classified as normocytic. Normocytic anemias are found to result from a sudden blood loss, increased destruction of blood cells, decreased production of blood cells, or increased dilution of the blood by body fluids. Wintrobe (1956) reports the latter is only an apparent anemia and should be more accurately called hydremia. Representatives of these four categories of normocytic anemia will now be discussed by specific title.

Acute Posthemorrhagic Anemia. Acute posthemorrhagic anemia is caused by a sudden and acute loss of blood. This in turn may be caused by trauma or shock, ruptured ulcer, or a complication of several unrelated disorders.

It may be also a symptom of a blood disorder such as hemophilia, acute leukemia, aplastic anemia or purpura hemorrhagia. The red blood cell size is usually near normal although if the hemorrhage is very severe, macrocytes may be seen immediately as increased red blood cell production occurs. Wintrobe (1956) claims the shape of the cell is normal. A count of 1-4 million red cells/cubic millimeter is noted by Selverd (1958). There is no change in the make up of the erythrocyte in posthemorrhagic anemia. The blood picture shows an immediate rise in platelet count to around 1,000,000/cubic millimeter and there is a shortened coagulation time. Polymorphonuclear leukocytosis occurs within 2-5 hours with counts usually 10,000-20,000/cubic millimeter. There is a reticulocyte increase after one to two days as this count reaches 5-15%. Wintrobe (1956) reports that red blood cell count, hemoglobin content and volume of packed cells are all high at first because the blood vessels constrict to redistribute the cells in the fluid that remains. Then these values decrease slightly for several days after this blood loss because of dilution of the blood by body fluids. Rubricytes and metarubricytes appear within a few days if the hemorrhage has been severe. Selverd (1958) expresses a different view of the blood picture in acute posthemorrhagic anemia. He states that the blood picture immediately after administration of more fluid, which could be done by nature or man, shows decreased hemoglobin content and low red blood cell count because of this dilution. The body tries to compensate for the blood loss by hurrying immature erythrocytes into circulation. These are hypochromic microcytes and are characteristic of this condition. Again Wintrobe (1956) points out that macrocytosis resulting from unusually great bone marrow stimulation is seen here for several days. In another publication, Wintrobe (1934) states that in the otherwise healthy normal individual with a satisfactory

diet and in whom the stores of blood building materials are adequate, significant microcytosis does not develop. If it does, the patient may have had previous hemorrhages.

Refractory Anemias. Wintrobe (1956) defines primary refractory anemias as those characterized in most cases by poorly functioning bone marrow, by way of granulocytopenia, which are refractory to any treatment except transfusion, and are not associated with infection, malignancy, disease, or malnutrition. In secondary refractory anemia, there is an association with some variety of disorder and the anemia is just one part of the condition. Within the primary grouping a further breakdown is possible. One sub-division is for anemias associated with an exposure to a physical or chemical agent which predictably causes bone marrow aplasia. Another sub-division includes those anemias in which certain agents can occasionally cause bone marrow aplasia or hypoplasia. Another group is reserved for those cases in which no chemical or physical agent can be blamed. The secondary refractory anemias are associated with infection, malignancy, and diseases. The red blood cell size is usually normal, although in some instances there is anisocytosis. The shape is also usually normal, although moderate poikilocytosis is reported by Thompson and co-workers (1934). The red cell count may be two million/cubic millimeter or even lower when the patient is first seen. Wintrobe (1956) reports the free protoporphyrin content in the erythrocyte is usually increased and plasma iron is usually increased, but other factors appear to remain normal. Selverd (1958) reports the hemoglobin content is greatly reduced to 1-7 grams compared to the normal of 12-17 grams. In the blood picture usually the red blood cells appear normal in spite of the severity of the anemia. The reticulocyte count is very low or zero. Rubricytes, prorubricytes, and nucleated red blood cells

are not usually present. Wintrobe (1956) reports there is a reduced number of leukocytes and thrombocytes, the former being 150-1500/cubic millimeter. The leukocytes in the bone marrow are affected and a marrow smear may be 70-90% lymphocytes. When immature erythrocytes are found in the blood, occasionally an immature myeloid leukocyte is present also. A congenital variety of refractory anemia has been identified and is known to show an increase in reticulocytes to 6-10%. In cases of pure red cell aplasia neither leukopenia nor thrombocytopenia is present. Wintrobe (1956) submits that the cause of refractory anemias is generally a bone marrow failure which is not due to a recognized deficiency such as is seen in pernicious anemia or in iron deficiency anemia. Other causes, however, have been identified. Benzol inhibits cell division and maturation of cells past the primitive reticular stage. Organic arsenicals possibly do the same. Ionizing radiation disrupts mitosis by disturbing the synthesis of desoxyribose nucleic acid in the nuclei of the cells. Familial infantile pernicious-like anemia is known to be associated with fatty hypocellular bone marrow. This condition is thought to be inherited through a recessive gene primarily; however, spontaneous cases may arise through mutation. In red cell aplasia the bone marrow is hypoplastic and there is an almost complete absence of nucleated red blood cells. Wintrobe (1956) notes that it has been suggested that this is due to action by autoantibodies and certain toxic agents.

Simple Chronic Anemia. The anemia associated with infectious and chronic systemic diseases is called simple chronic anemia. Wintrobe (1956) states the blood picture resembles that of normal blood. This anemia is long lasting and is probably the most common type of anemia encountered. It is usually relatively mild and may go unnoticed and untreated for some time. The erythrocytes

are nearly normal in size with some anisocytosis. The volume of packed cells is 25-36 milliliters/100 milliliters of blood and in severe cases it is 18 milliliters/100 milliliters of whole blood. The mean cell volume is 70-93 cubic micra, the mean cell hemoglobin is 22-35 micromicrograms, and the mean cell hemoglobin concentration is 30-35%. The most severe cases showing the smallest values are a result of kidney disease and are cases in which nitrogen is retained by the body. The red blood cell count is about one million/cubic millimeter in these cases. In less severe cases the red blood cell count ranges between 2 million and 4.79 million/cubic millimeter. Wintrobe (1956) records an average count of 3,880,000 red blood cells/cubic millimeter. The blood picture shows that frequently the number of cells, the volume of packed cells, and the amount of hemoglobin are equal in degree of variation from normal blood values. Sometimes the decrease in hemoglobin and the volume of packed cells is greater than the decrease in the number of erythrocytes, so there is microcytosis without hypochromia. Since rubricytes and nucleated red blood cells are not found, Wintrobe (1956) suggests there is no attempt to regenerate blood. Cartwright and co-workers (1954) report reticulocytes are found in about the normal amount, i.e., 1%, or are reduced in number; however, Saifi and Vaughan (1944) have found an increase to 9.4% reticulocytes in many cases of active infection with anemia. Red blood cell shape is usually near normal or with slight poikilocytosis. The leukocyte picture varies with the cause of the disorder. There are several specific causes of chronic anemia. Wintrobe (1956) reports chronic inflammatory diseases, kidney diseases and nitrogen retention disorders probably shorten the life span of the erythrocytes and somehow bring about a deficient red bone marrow response to cell production. Malignancy is not a cause of anemia in itself, but it causes a loss of appetite and

possibly may cause gastro-intestinal bleeding. Chronic liver disorders, Gaucher's disease, and Hodgkin's disease also tend to reduce the life span of the erythrocyte. Parasite infestation does not really produce simple chronic anemia except through the gradual loss of blood it may cause. The hookworm is specifically to be mentioned in this connection. Removal of the anterior pitultary gland causes anemia, but the mechanism is not clear. Several other endocrine glands exert a controlling influence over the blood forming system. Many vitamins play a role in blood cell production, but the precise roles are not clear. Wintrobe (1956) relates it is known that pyridoxine, pteroylglutamic acid, B_{12}, riboflavin, and nicotinic acid are involved. Other vitamins involved in metabolism and hematopoiesis are pantothenic acid with Coenzyme A as reported by Daft and co-workers (1947), thiamin reported by Rinehart and co-workers (1948), and biotin reported by Ruegamer and co-workers (1945). Wintrobe (1956) states the lack of vitamin C is not clearly a cause of simple chronic anemia as was once thought, and the simple chronic anemia of pregnancy is not a true anemia since there is usually only an increase in plasma volume. There may also be a degree of iron deficiency because of increased demands unless more iron is supplied through the diet, or the store of iron in the body is particularly good.

Myelophthistic Anemia. This anemia is associated with bone marrow disorders in which the marrow shows large spaces. Wintrobe (1956) reports it is characterized by the presence of immature leukocytes of the myeloid series and immature red blood cells in circulation, and for this reason, this anemia is sometimes called a leuko-erythroblastic anemia. Other names are leuko-erythroblastosis, myelopathic anemia, and osteosclerotic anemia. The red blood cell size is normal to slightly enlarged because of the immature cells. Chapman

(1933) reports that tear drop shaped erythrocytes are especially common. The blood picture shows that the enormous number of nucleated red blood cells in circulation is characteristic, and is out of proportion with the seriousness of the anemia. Vaughan (1936) reports most of these nucleated cells are metarubricytes although some primitive forms may be found. In relatively mild cases as many as 53 nucleated red cells/100 leukocytes have been found. Reticulocytes are also usually increased in number, and rubricytes may be found. Leukocytes have been shown to be decreased, normal, or increased in number. Wintrobe (1956) states that myelocytes and infrequently myeloblasts are found, and blood platelets are found in normal or reduced numbers. Thrombocytopenia may be found even when the leukocytes are normal in number. Giant platelets, megakaryocytes and megakaryoblasts, which are young platelets, are also described. There is usually no icterus, or jaundice, or evidence of excessive blood cell destruction. Wintrobe (1956) reports the cause of this kind of anemia is generally assumed to be mechanical limitation of the blood cell forming tissue by malignant tumors entering the bone marrow; however, Vaughan (1936) claims superfluous amounts of red marrow are sometimes found, so it is suggested that some unknown factor stimulates red cell production in compensation for the red marrow destruction by an unknown toxic agent. Hutt and co-workers (1953) further suggest that a similarity exists between myelocytic leukemia, megakaryocytic myelosis and polycythemia vera in that they all are "myeloproliferative disorders". Wintrobe (1956) states that metastatic carcinoma in the bone marrow is probably the most common cause of this type of anemia. Tumors of the prostate gland, breast, thyroid, lungs and adrenals are the most likely tumors to spread to bones in which the marrow is normally active during life. Myelofibrosis or myelosclerosis, an erratic increase in the

amount of fibrous or bony tissue in the bone marrow, may cause progressive myelophthisic anemia. Some cases have arisen which have been accompanied by enlarged spleens or enlarged livers and Wintrobe (1956) suggests that these are cases in which these organs try to form more blood cells when there is a production deficiency in the bone marrow.

Rosenthal and Erf (1943) describe marble bone disease or osteopetrosis as an hereditary disease which may produce myelophthisic anemia. Vaughan (1936) reports that this anemia is only seen in about one-fourth of the cases of osteopetrosis. In this disorder the bones become very compact as the spongy areas and medullary canal fill in.

Hemolytic Anemias

Hemolytic anemias are those which are characterized by excessive blood cell destruction. They arise from various causes and have various degrees of severity. As mentioned earlier, they are grouped with the normocytic anemias although they are exceptions to the rule that the hemoglobin content in the red blood cell is near normal as in normocytic anemias. In classifying the hemolytic anemias, Wintrobe (1956) suggests two general groups. Intracorpuscular anemias are caused by faulty erythrocyte production in the bone marrow and are chiefly the hereditary and familial disorders. The extracorpuscular anemias are caused by some external agent affecting normal red blood cells and are mainly acquired disorders. In all cases of hemolytic anemia there is marked variation in the size of the red blood cells. Some are microcytes, some are normal, and some are macrocytes. The shape of the cells is normal in most cases; however, sickle cell anemia is an exception. There is a reduction in the number of normal red blood cells, but an increase in the number of reticulocytes

and immature erythrocytes. Vaughan and Haslewood (1938) report the bilirubin in the blood plasma is increased from the normal amount of 0.5-0.8 milligrams/ 100 milliliters of blood, and Wintrobe (1956) points out that the amount of increase depends upon the degree of blood cell destruction. If the hemolysis is very severe, free hemoglobin and methemoglobin, a heme pigment, are found in the plasma. Methemoglobin is a composite of albumin and hematin - the iron complex of protoporphyrin. Hemolysis is not often this severe, in which case only increases in the icterus index and the serum bilirubin are noticed. The blood picture shows a marked increase in reticulocyte count. Wintrobe (1956) reports 10-25% reticulocytes are found in chronic hemolytic anemias, and 60% or more in acute cases. There are also more than the usual number of rubricytes and metarubricytes. There is also leukocytosis with some myelocytes and rarely myeloblasts. The leukocytes may number 132,000/cubic millimeter. Platelets are more numerous and are abnormally large even in the less severe chronic cases. Spherocytes, which stain red with no central paleness, are present especially in the familial disorders. These form unusual looking rouleaux in that they are short chained and rarely straight. Schistocytes, or erythrocyte fragments, and shrunken distorted cells are seen. Wintrobe (1956) states it is possible to see some red cells phagocytized by monocytes and neutrophils. Heinz-Erhlich and Pappenheimer bodies may be found in some cells.

The causes of hemolytic anemia are numerous. Wintrobe (1956) points out that in malaria, anemia is caused by the protozoan Plasmodium sp. which develops inside the erythrocytes and destroys them. The anemia may also be due in part to inhibition of the bone marrow activity. In blackwater fever an acute anemia is brought on by Plasmodium falciporum infesting the red blood cell. Beritic and Vandekar (1956) report that some people who have taken quinine irregularly

to combat repeated attacks of malaria have been victims of blackwater fever. Wintrobe (1956) observes that often the attack seems to be brought on by the taking of quinine, and that this has been interpreted as suggesting the disease results from an association of quinine with erythrocytes producing an antigen, thereby encouraging antibody formation. Wintrobe, however, adds that these antibodies have not been observed. Hemolytic anemia is also associated with oroya fever caused by Bartonella bacilliformis, a flagellated bacillus blood parasite carried by the sand fly. Viruses accompanying primary atypical pneumonia and infectious mononucleosis play a part in hemolytic anemia, as do bacteria accompanying cholera, typhoid fever, endocarditis in children, tuberculosis and other diseases. Phenylhydrazine, trinitrotoluene, benzene, lead, and other chemical agents may unite with red blood cells forming an antigen which causes auto-immunization. Hypersensitivity to certain drugs such as sulphanilamide may result in anemia. Moore and co-workers (1946) report physical agents such as burns and ionizing radiation are known to be associated with hemolytic anemia. Wintrobe (1956) reports several vegetable and animal poisons cause this anemia as well. Fava beans and inhalation from the blossoms of other bean plants by sensitive persons are two examples. Castor beans contain ricin, a powerful hemolytic agent. Wilbur and Collier (1943) explain that snake venoms contain a lecithinase which converts lecithin to lysolecithin, an agent which causes red blood cells to swell. Wintrobe (1956) discusses isoagglutinins as a possible cause of severe hemolysis in mismatched blood.

Paroxysmal Cold Hemoglobinuria. Wintrobe (1956) explains paroxysmal cold hemoglobinuria as the sudden passage of hemoglobin into the urine from the blood due to an autohemolysin in the blood during exposure to cold temperatures. The autohemolysin unites with the red blood cells during the exposure to a cold

environment, and then after the blood is warmed again, the blood cells rupture and free the hemoglobin.

Symptomatic Hemolytic Anemias. Under the term symptomatic hemolytic anemia, Wintrobe (1956) groups those overt cases in which the symptoms of anemia seem to arise first and not as a subsequent feature of some other infection or disease. Examples of this type of anemia are seen in Hodgkin's disease and chronic lymphomatic leukemia as reported by Wasserman and co-workers (1955) and in association with ovarian tumors as reported by Jones and Tillman (1945). Idiopathic acquired hemolytic anemias are those for which no cause can be found. A significant number of hemolytic anemia cases are classified as such, and there are several varieties. Wintrobe (1956) claims most of them involve antibody formation. The majority of cases have a "warm" antibody which becomes activated at temperatures above 37° Centigrade, while a minority of cases show the presence of a cold antibody which becomes active at temperatures below 37° Centigrade.

Chronic Hemolytic Anemia with Paroxysmal Nocturnal Hemoglobinuria. Wintrobe (1956) describes this disorder as a rather uncommon, gradually-developing, long-lasting disease characterized by signs of hemolytic anemia and sudden attacks of hemoglobinuria at night. Red blood cells often number less than 2,000,000/cubic millimeter, and they are usually macrocytic with great variation in size. There are no spherocytes, abnormal leukocytes or remnants of phagocytosis of erythrocytes. Reticulocytes usually number 10-20% and there are usually rubricytes and metarubricytes present. Ham and Horack (1941) claim that free hemoglobin in the plasma makes it look brownish in color. Methalbumin is also found in the plasma. Wintrobe (1956) reports leukopenia, a lack of leukocytes, is common and the number may be as low as 2500/cubic millimeter;

thrombocytopenia is also common. Ham (1939) observes that this sudden passage of hemoglobin into the urine occurs whenever the patient is asleep. This observation brought forth a suggestion that an accumulation of carbon dioxide and the subsequent lowering of the hydrogen ion concentration of the blood promotes red cell destruction.

Hereditary Spherocytosis. Wintrobe (1956) points out that this form of jaundice is, as the name denotes, a familial condition characterized by spherically shaped red blood cells. Though these cells vary widely in size, the mean cell diameter is usually reduced because of the spherical shape of the cell. Figures of 5.4-7.6 micra represent common diametric dry smear findings, and Wintrobe (1956) states that spherocytes 4.0 micra in diameter have been reported. In wet film, diameters are reported as 0.5-1.0 micra larger, but even this is below the normal value. Vaughan (1937) states that spherocytes are especially thick, being 2.2-3.4 micra instead of the normal 2.0 micra. This explains the low mean cell diameter and yet normal or only slightly reduced mean corpuscular volume of 77-87 cubic micra. These red blood cells are described by Diggs (1956) as spheroid or globe-like rather than biconcave discs. This condition represents only a moderate anemia and Wintrobe (1956) has said "patients are generally more yellow than sick". Three to four million red blood cells/cubic millimeter are most commonly reported. During a crisis this number decreases to 1-2 million/cubic millimeter.

As regards chemical make up of the blood, mean cell hemoglobin values are usually proportional to the reduced mean cell volume, but Vaughan (1937) states sometimes mean cell hemoglobin concentration is as high as 37-39%. Selverd (1958) reports spherocytes are usually more fragile than normal red blood cells in hypotonic saline solution. Wintrobe (1956) states serum iron

may be normal or increased. Diggs (1956) reports that in very severe anemia, macrocytosis is common. Reticulocytes are more prevalent with Wintrobe (1956) reporting commonly 5-20%. Seiverd (1958) claims reticulocyte counts of 10-30% are commonly reported. Rubricytes, metarubricytes and microblasts are found in varying degrees, but are particularly high after the patient has had a crisis. Wintrobe (1956) claims that structures like fragmented red cells may also be seen. Leukocytes are usually found to be normal in number or slightly higher after a crisis. During the chronic stage of anemia the lymphocytes, basophils and plasma cells are increased, but the platelet count is near normal. The reticulocytes are also less disc shaped than normal and as these cells mature they assume a more spheroid shape.

Hereditary spherocytosis is inherited as a Mendelian dominant. These spherical cells possess an abnormal intracellular carbohydrate metabolism mechanism. Wintrobe (1956) summarizes investigators' reports by stating "It appears that an adequate rate of supply of energy-rich phosphate bonds is necessary for the maintenance of the biconcave shape of the red corpuscle. Energy production may be defective as a result of genetically controlled enzyme deficiencies or functional inadequacies."

Sickle Cell Anemia. This is an inherited, familial, chronic hemolytic anemia found mostly in Negroes according to Wintrobe (1956) but Mourant (1954) reports that this condition is found sporadically in the area from the Mediterranean to India. This anemia is characterized by sickle or oat shaped red blood cells and evidence of excessive blood cell destruction. This anemia is the consequence of inheritance of a genetic trait for sickling. If an individual is heterozygous for this trait, there is little evidence of its

presence; but if the individual is homozygous for sickling, the condition is exaggerated and sickle cell anemia is the result.

Wintrobe (1956) reports the cell size is variable in sickle cell anemia, and small, medium, and large cells are found. Diggs and Bibb (1939) explain that the reduction in hemoglobin and volume of packed cells is often proportional so that normal values for mean cell volume and mean cell hemoglobin concentration are found. Selverd (1958) states flatly that the hemoglobin is reduced in amount. If the anemia is especially severe, it may be macrocytic, yet in some cases the mean cell volume is as low as 65-75 cubic micra. The mean cell diameter is often increased. The shape of the cell depends on the degree of oxygen saturation. In stained smears most cells are round or oval. A few cells are elongated and narrow with pointed ends, and may measure 20-50 micra long by 1-4 micra wide. Diggs (1956) reports only a few cells in this anemia are sickle shaped and one cannot tell from the blood picture that sickle cell anemia is represented; however, these are characteristic and can be seen when a drop of blood is sealed under a cover slip. In two to six hours bizarre shapes can be seen. Wintrobe (1956) explains the cell thickens on one side and thins out on the opposite, corresponding side and forms the characteristic sickled shape. The number of red corpuscles in sickle cell anemia is often severely reduced to one to two million erythrocytes/cubic millimeter.

Cell resistance to hypotonic saline solution is increased in sickle cell anemia; however, cell resistance to mechanical shock is decreased. There is a tendency for rouleaux not to form because of the erratic shape. Also the lack of rouleaux formation may cause the sedimentation rate to be very slow.

Wintrobe (1956) claims hypochromia is not commonly seen in this anemia, but frequently one can see cells with a dark center and a lighter colored

ring similar to the Mexican hat or target cells. Nucleated normoblasts are found in numbers of 1-10/100 leukocytes. Rubricytes, basophils, and cells with Howell-Jolly bodies are found. Reticulocytes are increased to 5-25% and they are usually round or oval and not sickle shaped. Leukocytosis is quite common in sickle cell anemia. When active blood cell destruction is going on, the leukocyte count may be 10-30 thousand/cubic millimeter because of an increase in myeloid cells. Wintrobe (1956) further reports blood platelets are also increased to 300,000-500,000/cubic millimeter and very odd shapes can be seen. Bleeding and coagulation times are normal.

The cause of sickle cell anemia is believed to be intracorpuscular. Sherman (1940) suggests there is an oxygen deficiency and a lowering of the cellular pH which cause the cells to assume the sickle shape. This does not occur to a significant degree in an alkaline environment, and exposure to oxygen causes these sickle cells to "round up". The globin protein of the hemoglobin of sickle cell anemia has two to four more net positive charges per molecule than normal hemoglobin. This is because of a difference in coiling of the polypeptide chains and it accounts for adding or removing charged groups on the chain from action.

Homozygous C Disease. Wintrobe (1956) states this is a blood anomaly found mostly in the Negro. Reports show it is possible to have a mild normocytic hemolytic anemia or no anemia at all. The erythrocyte size is normal, the shape is near normal, and the number is near normal. In blood smears 40-100% of the red blood cells are target cells, and some nucleated red cells are present. Reticulocytes number about 2-7%. Thomas and associates (1955) report cellular osmotic fragility is decreased, and resistance to mechanical shock is decreased in some cases. Serum iron content is normal. Homozygous

C disease is caused by inheritance of a special hemoglobin C trait from each parent. This hemoglobin is characteristically different from the normal type and is found in 2-3% of the American Negro and in 12% of the West African Negro. This hemoglobin does not cause sickling.

Mention should be made of other minor hemoglobin differentiations which are genetically passed through families and are seen to show the presence of target cells. Jonxis and Huisman (1958) report that hemoglobins A, S, C, D, E, F, G, H, I, J, K, L, and M have been identified thus far.

Thalassemia. This is another familial red blood cell disorder. Wintrobe (1956) reports the condition is characterized by a chronic progressive anemia early in life with well marked erythroblastosis and flat target cells. It is found mostly in the Mediterranean area or in people who lived in that area. Thalassemia minor refers to the characteristics which result from possession of genes heterozygous for this trait and thalassemia major denotes the homozygous condition. Variations in degree of anemia are thought to be due to the action of modifying genes. Very characteristic of thalassemia is the variation in red blood cell size from 3-15 micra in diameter. Poikilocytosis is common, many cells being unusually flat often with an edge doubled under the remainder of the cell. These cells contain so little pigment that they sometimes resemble an almost colorless membrane. Red blood cells in thalassemia number 2-3 million/cubic millimeter while extremes of variation are reported by Wintrobe (1956) as 1-4 million/cubic millimeter. The hemoglobin reduction and volume of packed cells are completely out of proportion to the reduction in the number of cells and the anemia is actually hypochromic microcytic. Cell fragility is normal or perhaps decreased. Cartwright and associates (1948) report the protoporphyrin content of the cell may be normal or increased,

plasma copper is increased and serum iron is high. The serum shows no free iron binding ability as do other anemias which involve red blood cell destruction.

Wintrobe (1956) describes the blood picture of thalassemia major as showing most erythrocytes are target cells, although some show a bridge between the inner circle and outer circle. Nucleated red cell number is characteristically high or may be 200 for each 125,000 white cells. The majority of the nucleated red blood cells are typical normoblasts or microblasts, and most of the remaining group are very immature and large normoblasts. The latter are round or oval and measure 15 micra in diameter. Diggs (1956) reports the cytoplasm of these primitive normoblasts stains a deep blue color and granular nuclear protein can be seen. These cells are not megaloblasts as seen in pernicious anemia. The reticulocyte count increases to 5-15%, and rubricytes, stippled erythrocytes, and occasionally cells containing Howell-Jolly bodies are found. Leukocytes are frequently more numerous than normal as Wintrobe (1956) reports them to be 10,000-25,000/cubic millimeter.

Thalassemia minor is a condition similar to thalassemia major, but it may go unnoticed for some time. A tired feeling is the most common overt symptom. The blood is similar to that in the major anomaly except that nucleated red cells are not found and the red cell count may be higher than normal. Wintrobe (1956) claims changes in cellular morphology are considerably out of proportion to the degree of anemia exhibited.

Pearson and co-workers (1960) studied nine individuals with the thalassemia trait. They found by tagging red blood cells with Cr^{51} and Fe^{59} that the plasma iron turnover was increased from normal, that the iron was poorly utilized in the circulating red cell mass, and that the survival time of the

red blood cells, was slightly reduced below the normal. The mean red blood cell survival for this group of 9 patients was 23 days. This was determined by using Cr^{51} and calculating half-lives by gamma ray spectrometry. It is generally believed that the thalassemia syndromes are associated with abnormal genes which interfere with the synthesis of adult hemoglobin. Pearson and co-workers (1960) state the normal hemoglobin molecule can be characterized by a_2b_2 which represents two sub-types or two polypeptide chains. If one of these sub-types is faulty, an out of balance condition exists and this is termed faulty hemoglobin.

Pearson's study supports the theory that "ineffective erythropoiesis" is important in the pathenogenesis of minor and major thalassemia. Although in their studies there was evidence of red cell activity in the bone marrow and rapid iron turnover, there was a decreased ability of these patients to produce circulating red cells.

Hypochromic Microcytic Anemia

General Considerations. Hypochromic microcytic anemia is characterized by a greater reduction in the amount of hemoglobin and the volume of packed cells than the reduction in red blood cell count. Most of the red blood cells in this condition are smaller than normal and are poorly filled with hemoglobin. The mean cell hemoglobin concentration is particularly reduced, below 30%, and this is the most significant quantitative value shown. Wintrobe (1956) states this anemia is also called nutritional hypochromic anemia, idiopathic hypochromic anemia, chloranemia, chlorosis, asiderotic anemia, and hypochromic anemia of pregnancy.

The size of red blood cells varies considerably. Even in severe cases there are some normal cells, well-filled with hemoglobin. Some macrocytes are seen and explained as attempted red blood cell regeneration; however, most red blood cells are decreased in diameter as Wintrobe (1956) indicates this mean value is 6.2-6.7 micra. The mean cell volume is reduced to 50-80 cubic micra, the Price-Jones curve is broadened at the base and is swung to the left of normal.

The red cells in hypochromic microcytic anemia are mostly normal in shape although there may be a moderate number of elongated poikilocytes. Wintrobe (1956) also reports most of the red blood cells are extremely thin. The number of red cells is near normal or may even be above, but is more likely below normal. Wintrobe (1956) found that 90% of his cases showed between 3 million and 5.15 million red blood cells/cubic millimeter. Extreme reductions are not found.

The hemoglobin content is so reduced that 6-10 grams were found in 74% of Wintrobe's cases and most of the erythrocytes appear as mere rings. In 83% of Wintrobe's cases, the mean cell hemoglobin concentration was 25-30%. Daland and Worthley (1935) report the fragility may be normal but more often there is increased resistance to cell destruction in hypotonic salt solutions. The blood plasma is very pale and the protein content is often reduced. The iron content in the plasma is very low - less than 35 micrograms per cent. Serum copper is high and red cell protoporphyrin content is increased to as much as 500 micrograms/100 milliliters of red cells.

Wintrobe (1956) states the reticulocytes are normal or slightly reduced in number; however, they increase after a hemorrhage. Normoblasts and microblasts are found in severe cases, but stippled cells are rarely found.

Rubricytes can sometimes be distinguished. Leukocytes are either normal or may be decreased in number, but a severe hemorrhage may cause an increase in the number of leukocytes, especially neutrophils and an infrequent myelocyte. Platelets are usually small but normal in number.

Basically this anemia is caused by a body deficiency in iron. This could be the result of a dietary deficiency, an increased requirement such as pregnancy, faulty absorption of the iron that is available, imperfect use of what is available, or excessive elimination of iron. Wintrobe (1956) claims cases reported usually show a combination of these ultimate causes rather than any single one; however, cases have been recorded in which a singular cause was responsible.

Associated with Achlorhydria. Frequently achlorhydria accompanies this anemia and it appears to precede the anemia rather than be caused by it. Although Poland (1933) reports many normal individuals have achlorhydria, but do not show anemia, and achlorhydria is characteristic of pernicious anemia, while iron deficiency is not, Wintrobe (1956) states chronic hypochromic anemia following total gastrectomy is not uncommon. This anemia is rare in men otherwise.

RECAPITULATION

Table of comparative erythrocyte values reported under various conditions (from various sources.)

Red blood cell associated with this condition	Mean cell volume in cu. micra	Mean cell thickness in micra	Mean cell diameter in micra	Relation of thickness to diameter	Total number of RBC in million/cu.mm
Normal	90	2.0	7.70	1:3.9	4.5-5.0
Pernicious anemia	135	1.6	8.89	1:4.0	1.5-2.0
Simple microcytic anemia	63	2.2	7.07	1:4.4	3.5-5.0
Chronic hemolytic anemia	90	3.0	6.18	1:2.0	4.5-5.0 (high % of reticulocytes & immature forms)
Obstructive jaundice	92	1.6	8.57	1:5.4	2.5-3.5
Hereditary spherocytosis	82	2.3-3.4	5.4-7.6	1:2.3	3-4 (1-2 in crisis)
Sickle cell anemia	near 90	near 2	near 8	near 1:4	1-2 (only a few sickle cells)
Polycythemia vera	80	near 2	near 7.70	near 1:3.9	7-10
Simple chronic anemia	70-93	near 2	near 7.70	near 1:3.9	1-4

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A DESCRIPTION AND COMPARISON OF NORMAL AND ABNORMAL RED BLOOD CELLS IN MAN

by

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Major Professor

The purpose of this report is to describe man's red blood cells as seen under normal conditions and then to compare this description with that of man's red blood cells in various abnormal conditions. The material contained here is merely a compilation of the facts, opinions, studies, and theories from the literature in the field of hematology. As is the case in any scientific discipline, new data is constantly being added.

After comparing several theories as to the origin of red blood cells, the report describes normal red blood cells in man by size, shape, numbers, and general chemical make-up. This is followed by a discussion of various immature erythrocytes normally found in circulation.

Close inspection of normal blood shows that not all red blood cells are identical even though smears have been prepared very carefully. When unusual erythrocytes are found in quantity, they are commonly associated with an abnormal condition. Red blood cells are considered unusual when they deviate significantly from the normal in size, shape or number. Definitions of these deviations are included in this section of the report.

Erythrocytes associated with ovalocytosis, Heinz-Erhlich body formation, and polycythemia are described in this report by size, shape, number, and chemical make-up. Causes of these conditions are also discussed.

After a general description of anemias, which includes classification and definition of terms, the report describes various specific anemias under the headings of macrocytic anemias, normocytic anemias, hemolytic anemias, and hypochromic microcytic anemia. An attempt is made to describe each of twenty-three types of anemia in terms of red blood cell size, shape, number and chemical make-up, and the basic cause responsible for the condition.

A table of comparative erythrocyte values reported under various conditions is included in this report as a recapitulation of the information presented.