Red Cell Production and Survival

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ALTERATIONS in the circulating red cell mass (erythron) are part of the daily experience of the anesthesiologist, ranging from the patient with acute hemorrhagic shock to the plethoric child with congenital cyanotic heart disease. In many instances during a surgical procedure, the anesthesiologist is not only closest to detecting such disturbances, but he is often responsible for the administration of fluids, blood cells, and drugs, as well as gases, that may have profound effects on the red cells and their oxygen-carrying capacity.

Concept of the Dynamic Erythron

Insight into the mechanisms of disorders of the circulating erythron has been greatly facilitated by the use of radioactive isotopes in erythrokinetic measurements.[1, 2] Thus, the vascular red cell mass, as shown in figure 1A, may be considered to be a function of two opposing forces: production versus removal of red cells. The circulating erythron in the 70 kg. normal adult is about 2,000 g. Each day, about 17 g. of cells enter this compartment from the erythroid marrow, which measures about 200 g. Once in the blood stream, the cell lives about 120 days before dying of senescence. Under normal, steady-state conditions, the volume of effete red cells leaving the circulation equals the input of young cells from the marrow.

Total red cell production may be readily assessed clinically by morphologic study of a marrow sample (assuming no significant ectopic erythropoietic foci), and calculation of plasma iron turnover. Entrance of cells into the blood may be gauged by the reticulocyte determination and the per cent incorporation of radio-iron into newly-formed and released erythrocytes. A convenient index of total destruction of red cells, including a small fraction of marrow precursors, is the fecal excretion of the hemoglobin-breakdown product, urobilinogen.† The rate of removal of circulating red cells is simply measured by using an isotope tag, such as ^{51}Cr.

With these and other aids, one can determine whether a reduction in the circulating red cell mass, *i.e.*, anemia, results from underproduction or accelerated removal of erythrocytes. A rapid shrinking of the blood erythron can mean only massive hemorrhage and/or hemolysis, for it would be weeks before impaired erythropoiesis became evident by changes in blood hemoglobin or hematocrit values.

On the other hand, expansion of the circulating erythron, *i.e.*, erythrocythemia, results only from enhanced production of red cells; for supernormal prolongation of red cell life-span doses not occur.[3]

With acute diminution or enlargement of the red cell mass, homeostatic mechanisms come into play that tend to restore the norm. An example is acute hemorrhage, not of sufficient magnitude to require immediate cell- and fluid replacement, and without depletion of marrow iron stores. In such an instance, there follow prompt hyperplasia of the erythroid marrow and restoration of the vascular erythron to normal dimensions within a few to several weeks. In cases of chronic, sustained deviation of the red cell volume, however, such as occurs in a child with the tetralogy of Fallot, hemoglobin concentration values may

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† Recent studies of S. H. Robinson *et al.* reveal that, at least in the rat, a small, but significant fraction of fecal bile pigment is of hepatic, and not of erythroid, origin. (J. Lab. Clin. Med. 66: 1015, 1965, Abstract.)

remain elevated at, say, 20 g./100 ml., for years, although daily production and destruction of red cells are equal and only slightly above normal rates. Similarly, in an instance of chronic hemolytic anemia due to sickle cell disease, hemoglobin concentration may remain fairly constant at low levels of 8 g./100 ml. for long periods. In such a case, although hemolysis is relentless, marrow production of red cells may attain rates as high as 8 times normal and be roughly equivalent to the rate of destruction.[4] In such instances, however, compensation is not of sufficient degree to bring hemoglobin values up into the normal range.

These observations indicate that homeostatic regulation is directed primarily at erythropoiesis, rather than at adjustment of red cell survival. The extraordinary constancy of the circulating red cell volume in normal subjects under nonstressful conditions means that this control system must be exquisitely sensitive to moment-to-moment shifts, which for an entire day amount to the removal and replacement of less than 1 per cent of the blood erythron.

Regulation of Erythropoiesis

Current evidence suggests that the hormone erythropoietin plays a key (although perhaps not exclusive) role in this regulation. The clear-cut demonstration of a humoral erythropoietic factor, beginning with Reissman [5] in 1950, and the development of sensitive methods of assay of this material by Jacobson and his colleagues [6] starting in 1955, have provided the impetus for extensive studies, which have not only contributed immensely to our understanding of the detailed mechanisms of erythropoiesis, but have also served as models for the basic biologic processes of cytoproliferation and cytodifferentiation.

According to the hypothesis proposed by Fried *et al.*,[7] and schematized in figure 2A, alterations in the relation of oxygen supply to body requirement somehow signal graded elaboration of erythropoietin. Accumulated evidence indicates that the kidney more than any other tissue is essential for the appearance of the hormone and thus, presumably, this

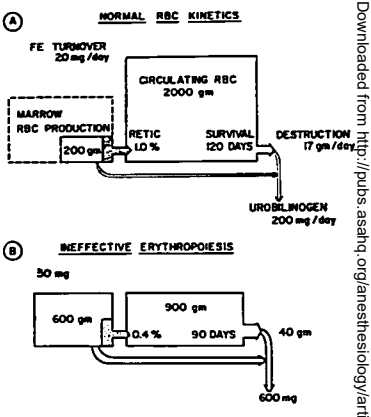

FIG. 1. Characteristic erythrokinetic relationships in (A) a normal adult, and in (B) a patient with Addisonian pernicious anemia exhibiting ineffective erythropoiesis. Illustrated are the capacity of the erythroid marrow to expand, the reticulocyte count as an index of red cell delivery into the circulation, the equality of erythrocyte production and destruction under steady-state conditions, the reutilization of iron as an expression of erythropoiesis, and urobilinogen excretion as a reflection of total red cell destruction. When ineffective erythropoiesis is severe, as is shown here, although red cell synthesis is increased, there is failure of adequate release of cells into the blood, and accelerated breakdown of circulating red cells and marrow precursors.

organ is the major site of origin of the material. The hormone stimulates marrow production of red cells. The oxyhemoglobin of the circulating cells constitutes the "supply" of the monitoring system. This completes the cycle of the delicately-balanced feed-back mechanism.

Although considerable evidence supports this scheme, other data suggest that it represents an oversimplification.[8, 9] Ignorance centers on the precise mechanism whereby the postulated oxygen supply-demand monitor conveys its message to the as-yet unidentified responsive cells in the kidney. Further, technical difficulties have made elusive the precise chemical nature of the hormone, so that measurements of activity have had to depend upon tedious methods of bioassay.

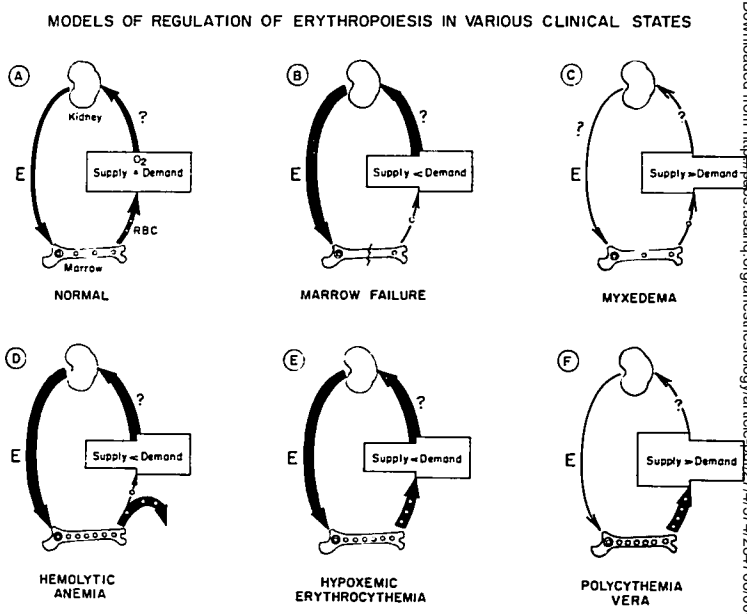

FIG. 2. In (A), the normal subject, oxygen supply and demand are in equilibrium. Through unknown mechanisms, the kidney elaborates normal amounts of erythropoietin, E, which stimulates marrow red cell production at a rate equal to body requirements. In (B), marrow failure leads to inadequate oxygen supply and, thus, augmented erythropoietin secretion. In myxedema (C), the hypometabolic state presumably results in reduced erythropoietin production, but this has not been conclusively demonstrated. Hemolysis (D) of sufficient degree to result in anemia, also reduces oxygen supply and thus, leads to hypererythropoietinemia. In (E) hypoxemia, such as in congenital cyanotic heart disease, marrow erythroid production is increased by erythropoietin stimulation. In (F), the marrow proliferative defect of polycythemia vera is presumed to be responsible for a reduction of plasma erythropoietic activity, an observation reported by some investigators.

Mechanisms of Anemia

Nevertheless, in spite of crude assays of erythropoietin, their relative values have permitted more precise appreciation of the pathophysiologic mechanisms of anemia. These may be classified as in table 1. Specific models are depicted in figure 2B, C, and D. It is apparent that the available data support, in general, the Fried hypothesis. It is of some importance, however, to bear in mind that (1) current methods of erythropoietin assay do not permit a clear distinction between normal and low levels of activity; (2) there tends to be an inverse relation between the erythroid marrow cellularity and the level of plasma erythropoietin, suggesting that marrow utilization of the hormone is a kinetic factor in special need of measurement [9]; and (3) it is sometimes difficult to distinguish between a primary physiologic defect and secondary compensatory deviations.

The anemia of renal failure merits separate consideration. Evidence from several laboratories now indicates that at least two factors

TABLE 1. Classification of Pathophysiologic Mechanisms of Anemia

Mechanism	Erythropoietin Activity	Clinical Example
I. Underproduction		
A. Intrinsic marrow failure	↑	Toxic marrow aplasia
B. Decreased marrow stimulation	?↓	Myxedema
II. Hemolysis		
A. Intra-erythrocytic defect	↑	Sickle cell anemia
B. Extra-erythrocytic defect	↑	Coombs-positive hemolytic anemia
III. Hemorrhage		
A. Acute (with or without iron-store depletion)	↑	Acute hemorrhagic shock
B. Chronic (with eventual iron-store depletion)	?↑	Iron deficiency anemia
IV. Ineffective erythropoiesis*	↑	Addisonian pernicious anemia

* See text and figure 1B.
↑ = increased; ↓ = decreased.

are involved in this form of anemia: diminished elaboration of erythropoietin by the diseased kidney, and impaired marrow red cell production due to the suppressive effect of the uremic state.[10] In addition, shortened red cell survival and bleeding may also contribute to the anemia. Since studies of anephric man reveal some capacity for red cell production, other sites besides the kidney may be of some importance for erythropoietin production, and/or erythropoietin may not have the dominant role that has heretofore been attributed to it.[11]

Ineffective erythropoiesis[12] refers to a mechanism that also involves multiple factors (fig. 1B). The essential defect is destruction of marrow erythroid precursors, and thus, inadequate delivery of cells into the blood. Some degree of ineffective erythropoiesis is believed to occur normally, early lysis presumably involving only the small fraction of poorly-formed cells.[9, 13] With full expression, however, such as in the megaloblastic states and in thalassemia, there are, in addition, shortened survival of circulating red cells, and severe anemia, in spite of increased "compensatory" erythropoietic activity and augmented total red cell production.

Mechanisms of Erythrocythemia

Expansion of the red cell compartment, erythrocythemia, also may be classified in terms of some of the pathophysiologic principles just described, as shown in table 2 and in figure 2E and F.

Polycythemia vera appears to result from a hemopoietic proliferative lesion presumably centering on the primitive stem cell, since all of the major marrow cell lineages may be involved, and the disorder displays certain autonomous features.[14] Thus, accompanying the erythrocythemia, are usually granulocytosis and thrombocytosis, and in the marrow, panhyperplasia, with progression to fibrosis and osteosclerosis, and myeloid metaplasia. According to the scheme in figure 2F, red cell plethora suppresses elaboration of erythropoietin. While there is supporting evidence for this hypothesis in the hypertransfused rodent, reports on patients with polycythemia vera have been conflicting.

Secondary erythrocythemia may take any of three forms (table 2). With (1) hypoxemia (fig. 2E) or (2) inappropriate secretion of erythropoietin, by lesions such as renal carcinoma and cysts, Wilms tumor, cerebellar hemangioma, pheochromocytoma and hepatoma, hypererythropoietinemia may be demonstrable. A third subclass (3) excess oxyhemoglobin, may result from transfusion plethora. The iatrogenetic condition may occur during certain surgical procedures in which large volumes of blood are administered for prolonged periods. Such situations may arise during operations requiring cardiopulmonary bypass. In these instances, reduced plasma erythropoietin activity would be expected.

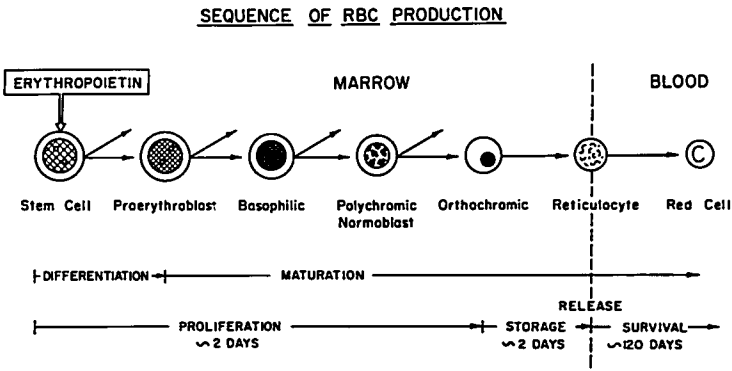

FIG. 3. Erythropoiesis is initiated by the hormone erythropoietin when it induces the conversion of the undifferentiated stem cell into the blast of the erythroid lineage. Proliferation and maturation follow and overlap. Total intramedullary time from the initial step is normally 4 to 5 days, but this period may be shortened under stress.

Cellular and Molecular Aspects of Erythropoiesis

Although the mature red cell is comparatively simple in structure and composition, the processes by which it arrives at that end stage of development are complex. Nevertheless, because of the relative simplicity and ready availability of separate red cells and their precursors, more is now known about the intricacies of red cell synthesis than of any other somatic cell in the adult mammalian organism.[15]

Morphologically, normal marrow red cell formation follows the familiar sequence shown in figure 3. Differentiation in this scheme refers to the commitment of the primitive stem cell to the erythroid lineage by conversion to the proerythroblast, the earliest cell recognizable as being capable of giving rise to the mature red cell. Proliferation is the process of cell multiplication by mitotic division, and this extends from the stem cell through the early polychromic normoblast. Maturation is the term covering detectable changes with time, but without necessary reference to proliferation. Normally, most of the maturing red cells pass through all of the stages depicted. As discussed above, evidence suggests that in ineffective erythropoiesis a small proportion of marrow erythroid precursors is normally destroyed before reaching maturity. Under conditions of enhanced erythropoiesis, skipping of some cell stages in the sequence, as well as shortening of storage time and early release of red cells, may occur.[9, 13, 15]

Cytochemical accompaniments[16] of special importance in the maturation scheme are: a progressive decline in ribonucleic acids (RNA) manifested by disappearance of nucleoli and decrease in cytoplasmic basophilia; and a reciprocal rise in hemoglobin, reflected in the progressive acidophilia of the cytoplasm.

Into this fabric of morphologic and cytochemical observations, may now be woven recent biochemical evidence interrelating these phenomena. The exciting researches of Goldwasser and his co-workers[17-19] using *in vitro* rodent marrow preparations, suggest that erythropoietin induces differentiation of the stem cell into the erythroid lineage by acting as a chemical derepressor. This is to say that, under the influence of this hormone, previously repressed genes of the stem cell are permitted to function as DNA templates for the synthesis of messenger RNAs. Messenger RNAs, in turn, program the manufac-

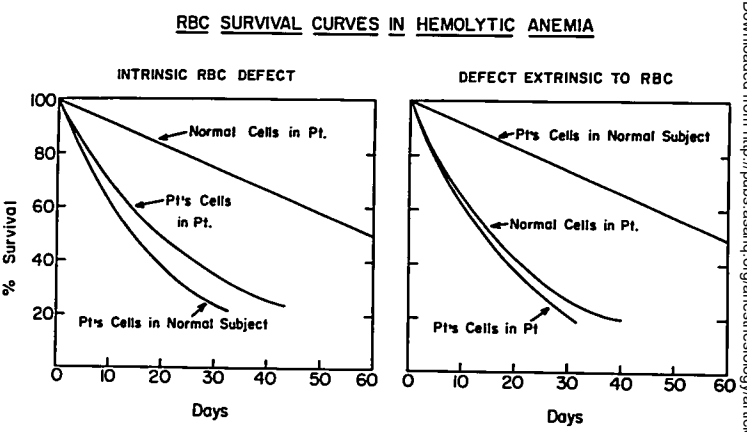

FIG. 4. Differentiation of intrinsic as opposed to extrinsic hemolytic defects by the cross-transfusion technique using labeled cells. In (A), an intra-erythrocytic abnormality is indicated by rapid destruction of the patient's cells even in a normal milieu; while in (B), even normal red cells are swiftly eliminated in the affected patient.

ture of new cellular proteins, such as hemoglobin, which can be readily measured in this experimental system. These studies represent the first demonstration of the biochemical basis of cytodifferentiation in a mammalian cell preparation.

Important advances in our knowledge of the metabolism of erythropoietin can be expected, when more is known of its physical and chemical properties. Currently,[12] the hormone appears to be a nondialyzable glycoprotein of relatively low antigenicity, and with a molecular weight of about 60,000.

When one considers the numerous constituents of the red cell, it is evident that many other substances are essential to red cell production. There may be other "erythropoietins" [9, 13] acting at other sites along the sequence depicted in figure 3. There is also some evidence to suggest that contact inhibition [15] or mitosis inhibition [20] may constitute feed-back mechanisms for control of erythropoiesis in the marrow.

It is of some importance to distinguish between two additional classes of substances that may influence, but do not regulate, erythropoiesis: (1) essential building units, such as iron, amino acids, cofactors (vitamin B_{12} and folate), and lipids; and (2) a variety of other materials that can affect erythropoiesis under special conditions. Pituitary and thyroid hormones appear to act through general tissue metabolic effects, probably on the postulated oxygen supply-demand monitor; while estrogens in large doses inhibit the action of erythropoietin on the marrow [21]; and androgens in pharmacologic amounts have been shown to exert a synergistic effect with erythropoietin.[22] The augmentation of erythropoiesis by cobalt is also, at least in part, mediated through erythropoietin.[15]

Having surveyed current concepts of red cell production, we may now consider in more detail the fate of this important structure.

Red Cell Survival

Survival of the red cell may be considered to be the reciprocal of its destruction. After the normal cell's 120-day sojourn in the blood stream, it undergoes lysis. In spite of its lack of a nucleus, the cell is endowed with unique metabolic machinery maintaining its size, biconcave disc-shape and plasticity, and thus, accounting for its viability. With exhaustion

TABLE 2. Classification of Pathophysiologic Mechanisms of Erythrocythemia

Mechanism	Plasma Erythropoietin	Clinical Example
I. Primary		
Intrinsic hemopoietic proliferative defect	? Normal or ↓	Polycythemia vera
II. Secondary		
A. Hypoxemia from cardiac, vascular, pulmonary lesions, or high altitude	↑	Tetralogy of Fallot
B. Inappropriate elaboration of erythropoietin	↑	Renal carcinoma (in relatively rare instances)
C. Excess oxyhemoglobin	↓	Transfusion plethora

↑ = increased; ↓ = decreased.

TABLE 3. Classification of Some Intrinsic Red Cell Defects

Erythrocyte Component	Defect	Clinical Example
I. Hemoglobin	Faulty amino acid sequence in globin moiety	Sickle cell anemia
	Faulty amino acid sequence in globin, with instability of heme iron	Hb M methemoglobinemia*
	Impaired rate of globin chain synthesis	Thalassemia
	Abnormal globin chain combination	Hb H disease
II. Membrane	Increased permeability to cations	Hereditary spherocytosis
	Abnormal affinity for subcomponent of third complement component	Paroxysmal nocturnal hemoglobinuria
III. Soluble substances	Deficiency of:	
	Glucose-6-phosphate dehydrogenase	Hereditary non-spherocytic hemolytic anemia
	Glutathione	
	Glutathione reductase	
	Pyruvate kinase	
	6-Phosphogluconic dehydrogenase	
	2,3-Diphosphoglycerate mutase	
	Adenosine triphosphatase	
	Triosephosphate isomerase	
	Diphosphopyridine nucleotide diaphorase	Hereditary methemoglobinemia*

* May not be associated with anemia in all cases.

of this energy-producing apparatus, the cell becomes prey for the physico-chemical destructive effects of the circulation and the reticuloendothelial tissues.

Measurement of Survival. Red cell life span in the circulation is readily determined by isotopic labeling. If a sample of normal blood is tagged *in vitro*, and then is injected into the circulation of a compatible normal subject, the corrected decline of labeled cells in the blood of the recipient will be linear (fig. 4). This is consistent with a progressive removal of the older cells with time rather than with a random destructive process.[2, 3]

Any more rapid descent of such curves represents shortened red cell survival and thus a hemolytic state. The major hemolytic factor may be intrinsic to the red cell or reside in the cell's environment. Definitive determination of one or the other may be obtained by cross-transfusion observations,[2, 3] such as are illustrated in figure 4.

Other Evidences of a Hemolytic State. Before considering specific hemolytic disorders, comments concerning other general manifestations of hemolytic disorders are in order. Hemoglobin released from the early lysis of red cells may be detectable in the plasma and

urine. Levels of haptoglobin, the hemoglobin-binding plasma protein, characteristically fall. The hemoglobin that is not immediately excreted by way of the kidneys is taken up by the reticuloendothelial system. In these tissues, the iron is removed from the heme, and the resulting porphyrin is catabolized and excreted as the pyrrole pigment bilirubin, chiefly by way of the bile, and then into the intestine where the pigment is converted to urobilinogen. Bilirubin may rise above normal levels in the serum if its production exceeds the ability of the liver to conjugate and excrete it. The globin moiety is dismembered into amino acids, which are then available for reutilization in protein synthesis. The iron is also available for reuse, and rapid iron turnover is characteristic of the hemolytic process (fig. 1B).

As described in the earlier section, the resulting anemia triggers the release of more plasma erythropoietin, which induces marrow hyperplasia, so that reticulocytosis becomes a valuable diagnostic sign.

Intrinsic Red Cell Defects. Although in some cases, both intrinsic and extrinsic factors account for the hemolytic state, it is helpful, at least initially, to consider these two classes separately.

Intra-erythrocytic lesions that usually predispose the cell to early death, may be considered in relation to three components: the hemoglobin molecule, the cell membrane and soluble substances, such as enzymes, coenzymes, and substrates of glucose metabolism.[22] Selected instances of each, with the basic defect, insofar as it can be presently defined, and clinical examples, are given in table 3.

The precise sequence of biochemical steps, with their anatomical correlations, leading to complete dissolution of the red cell is not completely known. Nevertheless, the following salient points provide a broad outline for an understanding of these complex mechanisms.[23, 24] The energy for preservation of the optimal oxidation-reduction state of hemoglobin and the generation of reduced triphosphopyridine nucleotide (TPNH) and adenosine triphosphate (ATP), is derived only from the breakdown of glucose to lactic acid, for the mature red cell has no high-energy-yielding Krebs cycle. With aging of the cell *in vivo*, there is decline in activity of the two key enzymes, hexokinase and glucose-6-phosphate dehydrogenase of the energy-producing glycolytic and pentose-phosphate pathways. Membrane permeability and active transport are closely related and are also dependent upon the energy derived from glucose metabolism. Red cells may be conditioned by a variety of mechanisms and substances that render the cells, particularly the older ones, susceptible to lysis when there is stasis in reticuloendothelial tissues, such as the spleen and liver.

TABLE 4. Drugs and Other Chemical Agents that May Induce Hemolysis *In Vivo*

Analgesics	Other antibacterials
*Acetanilid	*Nitrofurantoin (Furadantin)
*Acetylsalicylic acid (Aspirin)	†Chloramphenicol (Chloromycetin)
*Acetophenetidin (Phenacetin)	*Paraaminosalicylic acid
	‡Penicillin
Sulfonamides and sulfones	Miscellaneous
*Sulfanilamide	*Naphthalene (moth balls)
*Sulfisoxazole (Gantrisin)	*Vitamin K (water-soluble forms)
*Salicylazosulfapyridine (Azulfadine)	*Methylene blue
*Sulfamethoxypyridazine (Kynex)	*Dimercaprol (BAL)
*Sulfoxone (Diasone)	†Quinidine
*Thiazolsulfone (Promizole)	†Fava bean
	Lead
Antimalarials	Nitrobenzene
†‡Quinine	
*Primaquine	
*Quinacrine (Atrabrine)	

* Particularly toxic in subjects with glucose-6-phosphate dehydrogenase deficiency.
† Hemolytic in Caucasians, but not in Negroes with glucose-6-phosphate dehydrogenase deficiency.
‡ Immune hemolytic mechanism demonstrated in a few instances.

An assortment of drugs and other chemicals, when given to some subjects, may induce hemolysis.[25] Examples of these agents are listed in table 4. Some are commonly used compounds, such as acetylsalicylic acid (Aspirin) and sulfisoxazole (Gantrisin). A major advance in our understanding of these drug-induced hemolytic states, with important implications in the fields of biochemistry, genetics and anthropology, as well as in clinical medicine, came with the discovery by Alving and his associates,[23, 25] beginning in 1952, that a large proportion of individuals susceptible to drug-induced hemolysis, have an inherited red cell defect. This latent disorder occurs in highest frequency among Negroes (males, 13 per cent; females, about 20 per cent) and Sephardic Jews (2 to 36 per cent). The basic abnormality is an X-linked deficiency of glu-

TABLE 5. Classification of Some Extra-Erythrocytic Hemolytic Disorders

General Class	Nature of Abnormality	Clinical Example
I. Immune disturbance	Exogenous iso-antibody	Incompatible blood transfusion
	Transplacental isoantibody to infant's red cells	Erythroblastosis fetalis
	Warm autoantibody to red cell	Coombs-positive hemolytic anemia
	Cold autoagglutinin	Cold agglutinin disease
	Cold autohemolysin	Paroxysmal cold hemoglobinuria
	Antibody to drug-red cell complex	Quinidine-induced hemolytic anemia
II. Secondary or symptomatic	Parasitization of red cell	Malaria
	Unwholesome milieu: precise causal factors not known	Neoplasia
	Trauma	Vascular or valvular prosthesis (angiopathic hemolytic anemia)
III. Hypersplenism	Hypersequestration, conditioning, and destruction of red cells in spleen	Cirrhosis with congestive splenomegaly

cose-6-phosphate dehydrogenase, which appears to render the cell incapable of regenerating reduced triphosphopyridine nucleotide (TPNH) with sufficient rapidity to overcome the oxidant effect of the partially-degraded drug. A number of simple methods may be used to detect this biochemical lesion, such as the methemoglobin reduction test. Such a defect, however, is frequently unsuspected and may become apparent only after a catastrophic hemolytic event. Infections, metabolic derangements and extensive surgical procedures may enhance the severity of the hemolysis. Even patients without a biochemical lesion of the red cell, if severely stressed by concurrent systemic illness, and given high doses of challenging compounds, may exhibit at least mild to moderate degrees of hemolysis. This latter group of subjects, without intra-erythrocytic abnormalities, properly belongs in the class of patients with extra-erythrocytic disorders, which may now be considered.

Extrinsic Hemolytic Factors. Hemolytic lesions extrinsic to the red cell are numerous, diverse, and occur most often in patients with underlying disorders, hematologic and otherwise. A simple and useful classification is given in table 5, although distinctions between these classes are not always sharp. For example, the hemolytic anemia of chronic lymphocytic leukemia may be Coombs-positive; by definition it has to be considered secondary or symptomatic; and it may also be in part hypersplenic.

Immune hemolysis may take several forms, varying from well-known incompatible blood transfusion reactions to rarer instances in which a drug, such as quinidine, may sensitize the individual to early red cell destruction when the antiarrhythmic agent is readministered.[24]

Another form of hemolytic anemia, of special significance to members of surgical teams, is that due to the "Waring Blendor effect" on the circulating red cells of vascular or valvular prostheses.[29]

Hypersplenism refers to any variety or combination of blood cytopenia somehow attributable to the spleen, so that splenectomy may be expected to correct the disturbance.[26, 27] The spleen is usually, although not necessarily, palpably enlarged, and the marrow is characteristically hyperplastic in the formed element deficient in the blood. Hypersplenic anemia usually implies a hemolytic state due to hypersequestration and early destruction of red cells by the spleen. In most instances, the enlarged spleen is "congested," or is the site of involvement with lymphoma, chronic leukemia, or related disturbances, granuloma or storage disease. In such cases, hypersequestration in the spleen may be demonstrated by the rapid appearance of relatively high radioactivity over this organ, with reciprocal decline of counts in blood samples when isotopi-

cally-labeled red cells are injected into the patient's circulation.[27] The precise mechanisms whereby the spleen accelerates red cell destruction is not clear, but splenomegaly in itself, with associated erythrostasis, may condition the trapped pool of cells for early destruction.[24, 26, 27] Hypersplenism may also be superimposed upon intra-erythrocytic hemolytic states, so that occasionally children with thalassemia major and sickle cell disease may derive some benefit from splenectomy.[26] The disorder classically and most regularly "cured" by splenectomy is hereditary spherocytosis (HS). Whether one considers this disturbance "hypersplenic" is a moot point. Although the essential criteria for hypersplenism, as given above, are satisfied in HS, cross-transfusion experiments indicate that the disease is a consequence of *normal* splenic reaction to abnormal red cells. Thus, most current writers do not consider HS to be an example of the hypersplenic syndrome. This is another illustration of the sometimes arbitrary distinction between intrinsic and extrinsic hemolytic disorders.

This survey of the principal factors which account for survival of the erythrocyte, and the multiple lesions which may cause its early elimination has also made apparent the gaps in our knowledge of this important cell. Nevertheless, the growing interest and pace of research of investigators in many disciplines are rapidly supplying needed information, while raising new and important questions which remain to be answered.

Summary and Conclusions

Red cell production and destruction are normally delicately balanced, maintaining a remarkably constant circulating erythron, in spite of continuous moment-to-moment shifts and rapid turnover of some of the red cell constituents. Regulation of this homeostatic mechanism is mediated, at least in large part, by erythropoietin, a hormone whose major site of origin is the kidney. Erythropoietin functions by committing the marrow stem cell to erythroid differentiation. Recent evidence suggests that this important step is achieved, at the molecular level, by derepression of stem cell genes governing messenger RNA synthesis.

Application of basic concepts of the dynamic relationships of red cell production and removal, and correlations with erythropoietin assays, permit an understanding of the pathophysiology of the anemias and the erythrocythemias.

Erythrocyte survival is largely a testimony to the unique metabolic machinery of this non-nucleated cell, which survives four months of continuous abuse in the circulation. Death of the cell is normally the result of a wearing-down of its energy-generating apparatus, with concomitant aberrations in membrane permeability and ion transport.

The hemolytic states are conveniently understood in terms of intra-erythrocytic defects of hemoglobin, stroma, and glucose metabolism as contrasted with extra-erythrocytic disturbances. In some instances, however, the distinctions are relative.

Drug-induced hemolytic anemias are worthy of special emphasis because they are frequent, are often unsuspected and unrecognized, may be precipitated or aggravated by infectious, systemic illnesses, and associated surgical procedures, are dose-dependent and thus, may be avoided or more promptly treated if the physician is alerted to them.

It has been said that two fundamental problems in biology warrant special attention: cytodifferentiation and membrane transport. The red cell shows promise of being an ideal model for elucidation of the intricate mechanisms of these two essential processes of multicellular organisms.

Grateful acknowledgment is made to Doctors Alvin R. Tarlov, Sanford B. Krantz, Richard L. DeGowin and Margot M. Doyle for their helpful criticisms of this manuscript.

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PLATELET TRANSFUSIONS The safety and value of platelet transfusions have been demonstrated in plastic anemia, thrombocytopenias, massive transfusion syndrome, Thai hemorrhagic fever, leukemias and generalized neoplasias with thrombocytopenia. The routine use of platelet transfusions will benefit greatly from the availability of stored platelets. A method for freezing of human platelets without loss of viability was developed in Boston based on the simultaneous use of two preservative agents—dimethylsulfoxide and dextrose. Bleeding time, clot retraction and hemostasis were improved with platelet transfusions. (*Djerassi, I.: The Role of Platelet Administration in a Blood Transfusion Service, Transfusion 6: 55 (Jan.) 1966.*)