EMOLYTIC MECHANISMS

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ERYTHROBLASTOSIS foetalis is a violent acute hemolytic anemia occurring during the very first few days of life. It represents an example of acute hemolytic anemia occurring congenitally and due to the passive transfer from the mother to the fetus of antibodies which injure the fetal red cells. As an acute hemolytic anemia this disorder is subject to the same physiopathologic mechanisms as are found in other types of hemolytic anemia and I will discuss hemolytic mechanisms in general with the idea of presenting a background for the proper interpretation of hemolytic disease of the newborn.

Physiologic principles: The exact mechanisms involved in normal red blood cell destruction are quite obscure. It is known that the mature, non-nucleated erythrocyte has a life span of approximately 110-120 days from the time of its delivery from the bone marrow to the circulation. In this period of time, the cell passes through many miles of capillaries and is subjected to much squeezing and bumping, participates in many thousands of chemical exchanges in both the lungs and the tissue, and may remain stagnant for hours at a time in the spleen and in other sinusoidal organs. Despite the cell's plasticity and almost complete lack of its own metabolism (it is without a nucleus), it inevitably "wears out." Does it then simply become lysed by such normally present metabolites as "lysolecithin" or lecithin? Does it become fragmented or does it become swollen and disintegrate in the so-called graveyard of the red cell—the spleen? Answers to these questions are as yet not fully available. Peculiarly enough, more is known of abnormal blood destruction than of normal.

The course of the pigment hemoglobin is fairly well worked out, but the fate of the cell itself and of its many other constituents is rather obscure. The normal red cell, a biconcave disc with an average volume of 85-95 cu. micra and an average diameter of 7.5 micra, has an average thickness of 2.0 micra. This cell when placed in normal or isotonic salt solution (0.9 per cent NaCl) remains essentially unmodified. However, when placed in solutions of progressive hypotonicity—(0.8 per cent, 0.7 per cent, 0.65 per cent, etc.) the cell takes on more and more fluid. In a solution of 0.6 per cent NaCl, the red cell is thicker (with fluid) than in a solution of 0.8 per cent NaCl. Even though thicker, however, its total volume remains essentially unchanged. In other words, as the cell becomes thicker it becomes rounder, more spherical, and smaller in diameter. In 0.5 per cent NaCl solution, the red cell is almost completely spherical; although its volume is still 85-95 cu. micra, its diameter may now be only 4-5 micra and its thickness almost the same amount. In solutions of NaCl under 0.5 per cent the red cell finally bursts (hemolyzes). The differences in initial hemolysis indicate a difference in the thickness of the red cell population. The more resistant, i.e., the thinner red cells are probably for the most part the younger ones (reticulocytes) which have only recently been delivered from the

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The thicker cells are probably the older cells which have been buffeted about in the circulation and have remained stagnant in various sinusoidal areas. The thinnest cells do not become completely hemolyzed until concentrations of approximately 0.25 per cent NaCl are reached. Thus some normal red cells are hemolyzed at approximately 0.50 per cent NaCl solution and all are hemolyzed at approximately 2.5 per cent NaCl. The red cell, as Guest has pointed out, may be considered a perfect "osmometer," responding quickly to changes in hypotonicity and thus showing quick changes in thickness. These changes of the red cell with respect to hypotonicity are made use of in the fragility test. But they may have some physiologic importance as well. That is, the more effete red cells may be smaller and thicker than the relatively immature cells just released from the bone marrow. Although the question of hypotonicity does not enter into the ultimate hemolysis of the thickest red cells within the circulation, it is highly probable that these cells are more vulnerable to breakdown—say within the spleen—than their thinner fellows. The spleen may well be the graveyard of the thickest red cells.

Pathologic physiology of increased red cell destruction: The sequence of events which takes place during a bout of sudden blood destruction is best studied in the experimental animal. Guinea pigs, when injected with an anti-red cell hemolytic serum (see below), develop either fulminating, acute or subacute hemolytic processes depending upon the amount of hemolytic serum injected. In the fulminating type, there is hemoglobinuria, very rapidly developing anemia, extreme spherocytosis of the red cells. Evidences of regenerative activity on the part of the marrow are lacking. The hemoglobinuria and the violent reduction in red cell count (from about 5.0 M to 1.0 M or thereabouts in 24-36 hours) are indications of blood destruction which is so rapid that liberated hemoglobin cannot be modified to bilirubin but is excreted from the blood plasma into the urine.

Hemoglobin in the plasma is a threshold substance; it is present normally in a concentration of approximately 5 mg. per 100 cc. When the threshold of approximately 150 mg. is exceeded, hemoglobin is excreted into the urine. Hemoglobinuria thus indicates rapid and violent blood destruction which occurs usually within the circulation i.e., intravascularly. Hemoglobinuria is always accompanied by hemoglobinemia, but very definite hemoglobinemia (up to 150 mg. per 100 cc.) may be present without hemoglobinuria.

In fulminating hemolysis, the red cells in the circulation may become decreased by 60 per cent or more within a matter of a day or less. This may lead to a variety of shock, "hemolytic shock," due to the sudden loss of an osmotically important substance from the circulation. When one considers the great loss in total red cell volume, from approximately 2250 cc. in a normal human adult to (say) 450 cc. it is readily seen that the entire body must adjust itself quickly or death will supervene. The great strain on the circulation, more particularly on the heart, and on the tissue cells in a variety of organs can only be speculated upon.

The red cells which remain within the circulation after an "attack" by a hemolytic agent are presumably those which were originally the most resistant. Inspection of a blood smear at this point reveals that even these cells are now almost exclusively small, usually round, dense, brown-staining, and devoid of a central clear
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zone, i.e. spherocytes. These are unusually "fragile" in hypotonic solutions of sodium chloride. Extreme spherocytosis is the rule in violent hemolysis, whether in the experimental animal or in the hemolytic crisis clinically. The simultaneous occurrence of spherocytosis with anemia, and the coincidence of extreme degrees of spherocytosis with severe blood destruction suggest that the spherocyte in its various gradations, from a slightly thickened to an almost completely spherical cell, is the forerunner of complete hemolysis. The spherocyte may thus be considered as a red cell which has been injured by a hemolytic agent and as a stage between a normal mature circulating red cell and one which is completely hemolyzed. The greater the degree of spherocytosis which is present, the more fulminating is the hemolytic process.

In less fulminating hemolytic processes, in which the red cell count may drop from 5.0 M to 1.5 M in a matter of 3 to 10 days, hemoglobinemia and hemoglobinuria do not develop. The evidences of hemolytic shock are slight or completely lacking and there is far less strain on the circulation.

The anemia is variable and is normochromic, normocytic by cell volume, but microcytic by cell diameter, i.e. the red cells are unusually thick, and although their diameters are smaller than normal, their normal volumes are retained. The blood smears show 2 outstanding features which are of physiologic importance (1) a great diversity in red cell population, both in size and degree of maturity, and (2) evidence of regenerative activity on the part of red cells, white cells and platelets. The sudden, but not extremely violent hemolysis acts as a powerful stimulant to bone-marrow activity, which is reflected in the peripheral blood. Thus, together with partially hemolyzed cells, i.e. spherocytes, there are present relatively huge red cells newly arrived in the circulation; these are polychromatophilic reticulocytes. The disproportion between the small spherocyte, which is brown and "orthochromatic" and the large polychromatophilic red cell, which is bluish gray in color, is quite striking and is readily seen by inspection of a well-spread, well-stained blood smear. The cells represent 2 different physiologic processes; one destructive, the other regenerative. The diversity in size of these cells gives rise to 2 types of red cell population with respect to diameters and is well brought out in Price-Jones curves of red cell diameters, which show a "biphasic" character.

Other indications of increased hemolysis are to be found in the breakdown products of the hemoglobin. The plasma bilirubin becomes increased to variable levels depending in part upon the degree of blood destruction and in part upon the functional capacity of the liver to remove excess bilirubin from the circulation. Two individuals with the same degree of blood destruction may show bilirubin levels respectively of 2 and 4 mg. per 100 cc. of blood. The presumption is that in the latter case the hepatic cells remove bilirubin from the circulation more slowly than in the first instance. With rare exceptions, the bilirubinemia is always of the indirect variety and is thus associated with urine which is free of bilirubin, i.e. acholuric jaundice is present. Rarely is the amount of bilirubin presented to the hepatic cells so high, and the cells simultaneously inefficient (as in hemolytic anemia of the newborn) that a mixed type of bilirubinemia with bile appearing in the urine is present. The total amount of bilirubin in plasma rarely exceeds 10
mg. per 100 cc. and is usually between 2 and 5 mg. The biliary canaliculi are congested with bilirubin and the gall bladder is called upon to store unusually large quantities of bile laden with high concentrations of that pigment. As a result bilirubin may precipitate out and form stones with or without a calcium matrix.

The intestinal tract receives unusually large quantities of the pigment. In violent hemolysis, actual discharge of bile may occur; in less severe cases, the stools are highly colored, but otherwise normal in appearance. Except in certain rare instances of completely intravascular hemolysis, the urobilinogen in the feces is by far the surest index of increased blood destruction. It is conceivable in a given case that the liver may be functionally so adequate that it removes newly formed bilirubin almost as soon as it enters the bloodstream; the excess bilirubin is however passed into the intestine, where it is converted into urobilinogen. In acute hemolytic anemia, the output of urobilinogen may be increased 5 to 20 fold. In estimating the degree of hemolysis, one should always relate it to the hemoglobin content and even more accurately to the total intravascular hemoglobin. Thus, an output of 100 mg. of fecal urobilinogen may be normal for an adult weighing 150 pounds and with 15.0 Gm. of hemoglobin per 100 cc. For an adult with 5.0 Gm. of hemoglobin, however, 150 mg. of urobilinogen represents an approximately 3 times normal blood destruction; for a child weighing only 50 pounds, the same amount of urobilinogen at a similar hemoglobin level is about 9 times normal. The most accurate estimation of the degree of blood destruction is made by having recourse to the hemolytic index, which depends upon knowledge of the blood volume, the hemoglobin concentration, and the output of urobilinogen in the feces.

With an increase in urobilinogen in the intestines, the output in the urine usually becomes increased. The degree of urobilinogen is also conditioned to some extent by the hepatic function; thus, if the liver is normal, the urobilinogen may pass through quickly and re-enter the intestines. If on the other hand, the liver is damaged, there is a delay of urobilinogen excretion by this organ and a consequent increase in the general circulation and thus in the kidneys and urine. To rely solely on the content of urinary urobilinogen for the degree of blood destruction may lead to error.

Pathogenetic mechanisms of increased hemolysis: The various mechanisms responsible for increased blood destruction have recently been given some attention. They include the activity of hemolysins and agglutinins, the passive action of erythroblastosis, the role of the spleen, as well as of such physical factors as cold, heat, hydrogen ion concentration, etc. Attempts to incriminate a single mechanism as solely responsible for all hemolytic states are probably unwarranted.

Hemolysins: The older immunologists, including Bordet, Ehrlich, and others utilized the activity of hemolysins and their action upon red cells in their work on the theoretical aspects of immune processes. The Wassermann reaction is a direct outgrowth of this work. They demonstrated that if red cells of one species were repeatedly introduced into the circulation of another, the serum of the latter would eventually cause hemolysis of the red cells of the former. Thus, if guinea pig red cells were repeatedly injected in a rabbit, the rabbit's serum would eventually cause hemolysis of the guinea pig's red cells. Heating the serum to 56°C resulted in a
loss of this hemolytic ability, but when fresh guinea pig serum ("complement") was then added, hemolysis took place.

It was determined that hemolysis was a 2-step affair: (1) "sensitization" by antibody or "amboceptor," and (2) actual hemolysis by another substance "complement." These observations, although well-known for the most part by immunologists, were little if at all appreciated from their clinical standpoint. The first hemolysin which was related to clinical disease was that described by Donath and Landsteiner in cases of paroxysmal cold hemoglobinuria. These investigators demonstrated that certain, usually syphilitic, individuals had an autohemolysin in their sera which reacted with chilling and then reheating. The French observers Chauffard, Troisier, Widal, Abrami, and Brulé, and others demonstrated hemolysins in some of their cases of hemolytic anemia, some of which they called "hemolytic anemia." They believed that these hemolysins, which acted upon the patient's own red cells (auto-hemolysins) were causally related to the hemolytic process present.

Chauffard believed that a new speciality "immuno-hematology" might well be introduced. These observations, made between 1910-1915, were apparently disrupted by World War I and then frequently forgotten (or ignored) so that in publications during the next two decades or more, there was little mention of the possible role of hemolysins. Schwartz and I, in 1937 described 3 cases of acute hemolytic anemia, in 2 of which hemolysins of the immune body type were definitely present. The serum of these cases hemolyzed, not only the red cells of many prospective donors, but also the patient's own red cells. This hemolysis could be prevented by heating the serum to 56°C., but reappeared when guinea pig serum (complement) was added. The improvement of these patients following splenectomy, together with the cessation of spherocytosis and of increased hypotonic fragility, in association with the disappearance of hemolysin, led to the concept that the immune hemolysin might be responsible not only for the hemolytic state, but for the spherocytosis and increased hypotonic fragility as well. This was directly contrary to the concept of the numerous authorities that spherocytosis was due to a disorder of bone marrow erythrocyte production, and not to the activity of such extrinsic agents as hemolysins.

An immune hemolytic serum was produced by injecting guinea pig red cells repeatedly in rabbits. The rabbit serum was then injected in varying doses in normal guinea pigs and various types of hemolytic anemia were produced; fulminating, with extreme spherocytosis, hemoglobinuria, and death without regenerative activity on the part of the bone marrow; acute, with spherocytosis and marked changes in fragility, but without hemoglobinuria and with evidences of regeneration; subacute, with marked regeneration, many reticulocytes and polychromatophilic red cells giving a "pseudo-macrocytic" type of blood picture, etc. These observations demonstrated: (1) that a hemolytic serum could produce hemolytic syndromes in vivo comparable to clinical hemolytic syndromes, (2) that spherocytosis was a precursor of hemolysis and could be "acquired," and (3) that spherocytosis was produced outside the bone marrow, since the young red cells in the circulation were much larger than the spherocytes. From these and other observa-
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lations, we concluded that the hemolytic syndromes were in all probability more or less concerned with the activity of hemolysins of different types and acting in different concentrations. Thus a large dose of hemolysin might result in hemoglobinuria, and a smaller dose in subacute or chronic hemolytic anemia.

Further observations have borne out these original conceptions with the qualification that by a hemolysin is meant any substance, chemical, immune or other, which directly or indirectly tends to injure the red cell and thus cause its destruction. However, certain modifications and additions to the original concept must be made.

Hemolysins may be classified as simple and complex. A simple hemolysin, which may be of chemical nature (saponin, lecithin, lyssolecithin, arsine gas, some of the sulfonamides or their end products, benzol, etc.), acts directly on the red cell causing its immediate hemolysis without the intervention of any other substance. On the other hand, other hemolysins are "complex," requiring the preliminary action of "amboceptor" before hemolysis takes place by "complement." Of the complex hemolysins studied, including colloidal silicic acid, and immune hetero-hemolysin, the latter had all the properties of the iso-hemolysin which we had previously studied, and which had earlier been described by Chauffard and Troisier.

Complex hemolysin apparently acts first by injuring the red cell membrane as evidenced either by direct observation of the cells, which show irregular spinous processes or by the method of mechanical fragility. With the latter method, shaking of the red cells already acted upon by hemolysin in the absence of complement results in hemolysis within a certain period of time, control red cells showing no hemolysis. Prompt hemolysis of sensitized red cells takes place when complement is added, the latter agent apparently being the actual hemolysin. Complete hemolysis is probably antedated by the development of spherocytosis, although of this no complete evidence is available. Hemolysis in a complex system is probably facilitated by other factors such as that of pH, the amount of potassium or hydrogen ions, the temperature and perhaps the "vital" activities of certain cells, notably those in the spleen.

Agglutinins: Agglutinins are much more commonly found than hemolysins. What is more, the hemolytic activity of complex hemolysin is in part that of agglutinin (which is probably the same as amboceptor, or sensitizing agent). Agglutinins, when introduced into the circulation, result in hemolysis of red cells. They may be of different types, some acting at or above body temperature (warm agglutinin, e.g. the anti-Rh agglutinin), some acting well at any temperature, room, icebox, incubator (e.g. the iso-agglutinins, anti-A and anti-B), and some acting best at icebox temperature, i.e. cold agglutinin. Agglutinins may also be pure or simple in type, without any but an agglutinative action, or they may be more complex, i.e. act as agglutinins in one set of circumstances but as hemolysins under other conditions, e.g. when complement is added. This is the case with colloidal silicic acid and immune agglutinin. A further differentiation of agglutinins has recently been made depending upon whether they act well when diluted with normal salt solution or whether their activity is masked in such a solution to be brought out only if whole blood, plasma or albumin is used as a diluent. An at-
tempt has been made to divide agglutinins according to these differences into those which are "bivalent" and those which are "univalent." Agglutinins acting well in normal salt solution are said to be bivalent or complete antibodies, whereas those which require the use of a serum or plasma factor are said to be "univalent" or "incomplete" antibodies. The term "blocking antibody" has also been used for the incomplete anti-Rh agglutinin. Whether these concepts are correct, or whether the differences in antibody activity relative to salt solution or plasma rely solely on the type of diluent remain questions for further study.

Concanavalin-A. A protein derived from the jack-bean, causes intense agglutination of many species of red cells in very dilute concentrations. Actual injury to the red cell membrane occurs as evidenced by the behavior with mechanical trauma. Hemolysis in vivo is probably induced by the mechanical trauma of the active circulation upon the agglutinated corpuscles, although undoubtedly other factors such as temperature change, stasis, etc., have their effects.

Cold hemagglutinin, found in primary atypical pneumonia and at times in high concentration in other conditions, acts much like concanavalin. It is universal in its scope (panagglutinin) acting on all types of red cells, human and otherwise, but is limited in its activity with respect to temperature. It has a "'thermal amplitude'" of 0°C to 17–20°C. In this temperature range it causes intense agglutination of red cells. Agglutination, if continued, injures the red cells, as evidenced by mechanical hemolysis with trauma. The traumatizing effects of the circulatory pulsations upon agglutinated red cells are probably responsible for the in vivo hemolysis.

The iso-agglutinins anti-A and anti-B and the warm agglutinin anti-Rh cause intravascular hemolysis when introduced into the circulation in contact with susceptible red cells containing the appropriate agglutinogen. Again similar mechanisms are probably operative: injury to red cell membrane with the development of spherocytosis, the effects of trauma upon agglutinated red cells, complete hemolysis either intravascularly or in the spleen, with or without the added effects of stasis.

Erythrostasis: The effects of simple stasis in the development of hemolysis have been known for years. Ham and Castle chiefly on the basis of Knisely’s interesting histophysiologic studies of the spleen, concluded that the normal spleen has 2 main functions, both reproducible in the test-tube: erythrostasis and erythroconcentration. From further experimental data, they concluded that all hemolysis was a function of erythrostasis: either (1) of unusual degree, or (2) of normal degree in the presence of abnormal cells, i.e. spherocytes. Stasis of unusual degree was often the end result of agglutination. Stasis of normal degree with abnormal cells was present in various types of anemia with spherocytosis and occurred chiefly within the spleen.

Although there can be no doubt that erythrostasis, particularly within the spleen, may have some slight effect in the ultimate hemolysis of red cells, this theory does not explain the development of spherocytosis, nor does it indicate that the spherocyte is simply a red cell already in the process of hemolysis. Furthermore, as already indicated above, an agglutinating substance does more than simply remove a number of red cells from the active circulation to stagnate and to hemolyze; it actually injures the red cell envelope. Hemolysis is thus an active rather than a
passive mechanism, although it is possible that the passive factor may have some bearing. What is more, under conditions of extreme erythrostasis, we have found there is actually a decrease in hemolysis. Furthermore, the theory of erythrostasis has no bearing whatever in cases with actual hemolysinemia as in paroxysmal nocturnal hemoglobinuria; nor in March hemoglobinuria in which the reverse of stasis is present.

Splenic activity: The role of the spleen in normal and increased hemolysis is still obscure. Although it is attractive to consider the spleen as the "graveyard" of the red blood cell, this is by no means proved. In almost all conditions with increased hemolysis, the spleen is enlarged and in some of these splenectomy is followed by dramatic recovery. Does the spleen become enlarged because it must remove more abnormal red cells from the circulation than normally, or does it enlarge as a primary dysfunction and thus result in hemolysis? It would appear that both answers are probably correct, i.e. (1) the spleen often acts to remove red cells which have been partially hemolyzed elsewhere, and (2) in certain "hypersplenic" cases, the spleen appears to be primarily responsible for the increased hemolysis and its removal results in complete cessation of the hemolytic state. The "hypersplenic" cases are usually associated with leukopenia, granulocytopenia, and thrombocytopenia, i.e. pancytopenia, indicative according to our concepts, of an unusual degree of inhibitory effect of the hyperactive spleen upon bone marrow formation and delivery of the various cells. Definite proof other than splenectomy for a "hypersplenic" type of hemolysis has thus far been lacking, i.e. there is usually no histologic, or other direct evidence obtained through extracts, etc., that the spleen is the initiator of the entire hemolytic picture. Histologically, certain cases show intense erythrophagocytosis. It may be concluded that the spleen is certainly of aid in most hemolytic processes and that at times it initiates and carries through the entire reaction.

Certain chemical and physical factors: Certain chemicals, notably saponin, lecithin, arsine, phenylhydrazine, certain drugs containing the benzene ring and including the sulfonamide compounds, acetanilid, etc., have the property of injuring the red cell and causing its hemolysis. Inorganic acids, saturated fatty acids and their halogen derivatives, certain alcohols, also cause hemolysis. These probably act on the red cells in different ways. Physical factors, including extreme heat, cold (in the presence of agglutinin), certain radiations including the ultraviolet (especially in the presence of eosin or other like dyes) etc. may injure the red cell and result in its hemolysis. Contributing factors may be the pH, the concentration of potassium, sodium, sugar, etc. in the solution.

Summary of pathogenetic mechanisms: One may conclude that the red cell can be injured in a variety of ways, whether directly by a chemical factor or by hemolysis of simple variety, or by heat, or in a more complex fashion by an agglutination—hemolysin mechanism (immune hemolysin), or by the combination of agglutination and mechanical trauma. These methods of hemolysis may be aided by such factors as erythrostasis, an acid pH, etc. In any event, the red cell is actively injured and either partial hemolysis (spherocytosis) or complete hemolysis results. Erythrostasis, the spleen, and the pH are rarely the sole cause for the development of he-
niolysis, which is probably an "active" and not a "passive" process. The exact mechanisms which are operative in a given case of hemolytic anemia are quite obscure, but attempts to uncover them should always be made.

CLASSIFICATIONS OF HEMOLYTIC ANEMIA

I. Conventional or Nosographic Classification.

The classification which we have found most useful follows the conventional pattern:

Hemolytic Syndromes:

A. Hereditary
   1. Spherocytic (familial or congenital hemolytic jaundice or anemia)
   2. Mediterranean target-oval cell (including Cooley’s anemia in mild forms)
   3. Sickle cell (African target-sickle cell)

B. Acquired
   1. Chemical origin—phenylhydrazine, sulfonamides, etc.
   2. Bacterial origin—Streptococcus hemolyticus, B. coli, etc.
   3. Parasitic origin—malaria, Oroya fever
   4. "Symptomatic" origin—secondary or symptomatic of an underlying disease (Hodgkin’s disease, etc.)
   5. "Idiopathic" origin—with or without hemolysins or agglutinins, "hypersplenic" types.

C. Hemoglobinurias
   1. Paroxysmal cold. a. (Donath-Landsteiner) hemolysin; b. Cold hemagglutinin
   2. Paroxysmal march
   3. Paroxysmal nocturnal (Marchiafava-Micheli)
   4. Others

II. Types of Hemolysis: "Reticulo-endothelial" vs. Intravascular.

In general, 2 types of hemolysis can be discriminated. In one, there is a gradual disintegration of the red cell and its hemoglobin component. This evidently takes place outside the circulating blood and may be brought about in various sinusoidal areas with and without the activity of reticulo-endothelial cells. This type of hemolysis is unaccompanied by an increase in the plasma hemoglobin concentration but an increase in plasma bilirubin takes place, which is followed by an increase in the output of urobilinogen in the feces. The spleen becomes enlarged, at times excessively so, evidently because its blood destructive function is greatly increased.

In the second or intravascular type of hemolysis, a mass of red cells is suddenly or violently disrupted within the circulating blood itself. As a result, there is a quick liberation of hemoglobin and a consequent increase in plasma hemoglobin. If a sufficient amount of blood (roughly 30 cc. or over) is thus hemolyzed, the plasma hemoglobin concentration rises above the threshold level of 150 mg. per 100 cc. with the resultant passage of hemoglobinous urine. Hemoglobinuria is thus indicative of violent intravascular hemolysis of more than 30 cc. of blood. Hemoglobinuria must always be accompanied by hemoglobinemia, but the reverse is not
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necessarily true, since ordinarily plasma hemoglobin levels of less than 150 mg. are not accompanied by hemoglobinous urine. With the presence of an increased plasma hemoglobin level, an abnormal blood pigment called methemalbumin is formed. Small quantities of methemoglobin may also be produced. The spleen may not become enlarged even with successive bouts of violent hemolysis, apparently because red cell destruction is carried out chiefly within the circulating blood, and not in the reticulo-endothelial sinusoids. In paroxysmal nocturnal hemoglobinuria, both types of hemolysis seem to take place simultaneously. Intravascular hemolysis takes place with the patient in the supine position, generally at night, and normal or "reticulo-endothelial" hemolysis occurs during the rest of the day. In this condition, a mixed type of hemolysis is present with a resultant increase in both plasma hemoglobin and bilirubin levels, hemoglobinuria, a slight increase in fecal urobilinogen, and splenomegaly. There is in addition a very unusual type of iron removal from the hemoglobin molecule, with its deposition in renal tubules.

III. Types of Hemolysis as Determined from Red Blood Cell Survival Studies.

Numerous studies of the life span of the red cell by various methods indicate that it has a longevity of approximately 110 to 140 days. In our laboratory we have used a modified Ashby technic, utilizing high titre anti-A, anti-B, anti-M, anti-N, and anti-Rh testing sera for differential agglutination directly in the red blood cell counting pipet. By introducing normal red cells into the circulation of patients with hemolytic disease, it is possible to discriminate at least 2 types of hemolysis. In one, the introduced red cells are destroyed in a normal linear fashion, the life span being approximately normal. This occurs in familial spherocytosis and in the other hereditary hemolytic syndromes of Mediterranean target-oval cell disease and sickle cell disease. In certain cases of acquired hemolytic anemia, particularly in those associated with circulating iso-antibodies, the curve of red cell destruction is a much faster one. According to some investigators, this curve is of the exponential variety. In some of our cases, however, the curve has been linear in type, although a rapid loss of red cells took place. Thus, in certain cases of hemolytic anemia the disorder is evidently one which centers largely around the patient's own abnormal red cells, as a result of which the normal processes of hemolysis cause increased red cell destruction. According to a number of investigators this is the situation, for example, in familial spherocytosis, in which the abnormal red cells are destroyed at a rapid rate by a normal spleen. Normal red cells are destroyed at the normal slow rate. When the spleen is removed, increased red cell destruction ceases, even though spherocytosis persists. These observations apparently indicate that there is no abnormality in hemolysis per se but that the red cells themselves are abnormal. This explanation does not elucidate the fundamental cause of the spherocytosis. By analogy with our experiments concerning the production of spherocytosis and with the knowledge that the spherocyte represents a red cell which has been injured it would seem likely that the spherocyte of familial spherocytosis is a red cell which has been injured by "hemolysin" in the general sense. Since normal red cells are not destroyed at a more rapid rate in the circulation of a case of this disease,
it is possible that the patient's own tissues produce specific substances which act only upon the patient's own red cells.

Confirmatory of the presence of a substance causing spherocytosis of mature red cells is the extreme degree of this abnormality which develops during a hemolytic crisis. The hypotonic fragility increases during this time, and there is leukopenia, neutropenia, thrombocytopenia, and reticulocytopenia. These cytopenias suggest an active degree of hypersplenism. Recent studies in our laboratory indicate that the destruction of normal red cells may become increased at this time. In 2 cases an abnormal iso-antibody was demonstrable during crisis.

The rapid rate of hemolysis of introduced red cells in certain cases of acquired hemolytic anemia indicates a "hemolytic constitution," i.e. a mechanism by which introduced normal red cells as well as those of the patient are destroyed at an abnormally rapid rate. An exponential type of curve has been assumed by Brown et al. and has been confirmed in a few clinical observations. Our own studies indicate that the rapid hemolysis of normal red cells may proceed either exponentially or in a straight line fashion. With the possible exception of the crisis, therefore, the finding of a definitely decreased red cell life span indicates (1) acquired hemolytic anemia, and (2) a definite iso-antibody effect. Such cases as a rule are associated with the presence in the serum of abnormal agglutinins or hemolysins, which are usually of the immune body type (cf. below).

Still a third type of hemolysis, noncongenital, definitely acquired, but associated with a normal red cell life span, was recently observed in a case of acute hemolytic anemia associated with chemical poisoning (refrigerant). In this case, no abnormal iso-antibodies of any type were present.

IV. Types of Hemolysis as Determined from Study of Serum for Abnormal Iso-antibodies and from Study of Red Cells for Adsorbed Immune Antibody.

The cases of hereditary hemolytic disease (spherocytic, target-oval cell, target-sickle cell types) are not as a rule associated with the presence in the serum of demonstrable abnormal iso-antibodies, whether hemolysins or agglutinins. An exception, as noted above, may be during the hemolytic crisis of familial spherocytosis.

In cases of acquired hemolytic anemia, except those due to chemicals and those which are symptomatic or secondary to some other condition such as Hodgkin's disease, we have found an agglutinin which is apparently best brought out by the use of bovine albumin rather than normal salt solution as a serum diluent. This agglutinin is usually of the "cold" variety and may be of high titre. Its exact relationship to the hemolytic process is perhaps obscure since its reactions in vitro are best brought out at temperatures far below the normal body temperature. The potentiation of hemolytic activity of cold hemagglutinin has been pointed out recently by Boorman et al. Some cases with a warm agglutinin may show a greatly increased activity in albumin solutions as compared with saline. That this is due to the presence of a "univalent," "blocking," or "incomplete" antibody, as claimed by Wiener for anti-Rh agglutinins is debatable. We believe it more likely that the agglutinin activity is more readily brought out in solutions of albumin or in plasma
than in normal salt solution, not because the agglutinins are incomplete, but because these solutions are more physiologic diluting materials than normal salt solution. Erythroblastosis foetalis is an example of a congenital but acquired hemolytic disease due to the presence of abnormal agglutinins (usually of the anti-Rh type). These may be "complete," i.e. readily brought out in salt solution or "incomplete," i.e. not brought out in salt solution, but found only when albumin or plasma solutions are used for titration.

Some cases of acquired hemolytic anemia show an iso-hemolysin which is of the immune body variety and is active against red cells of all blood groups, as well as against the patient's own red cells.9 Cases of acquired hemolytic anemia which are due to chemical poisoning or are secondary to such conditions as Hodgkin's disease, lymphosarcoma, etc. do not show immune bodies of any type in the serum.

Recently Boorman, Dodd and Loutit17 described an interesting test by which they discriminated between familial and acquired cases of hemolytic disease. Beginning with the postulation previously advanced by Dameshek and Schwartz9 that in certain cases of acquired hemolytic anemia the serum might be devoid of demonstrable iso-antibody which was, however, adsorbed on the red cell, they prepared an anti-human serum rabbit serum. By using this serum against red cells of acquired and hereditary cases they found positive results in the acquired cases and negative results in the hereditary types. Negative results were also obtained in symptomatic and chemical cases. Although these results are in accord with our previous postulations and with the results of red cell longevity studies with studies of serum iso-antibodies, they require further confirmation before they can be completely accepted as to their reliability in differentiating between hereditary and acquired hemolytic disease.

V. Types of Hemolysis as Determined from "Differential Fragility Studies" of the Red Cells.

The hypotonic fragility test is an index simply of the degree of thickness of the red cell. The thicker the cell, the greater is its "fragility" to hypotonic solutions of sodium chloride. The red cell may be tested by other physical or chemical methods to determine possibly the type of substance which has reacted with it. Thus, by use of mechanical trauma with glass beads in a mechanical shaker (mechanical fragility) we can readily determine that red cells from one condition are hemolyzed at a far greater degree than normally.7 In our experience, this indicates the previous activity upon the red cell of agglutinin or the "sensitization" phenomena (first step) of hemolysis by a complex hemolysin. When a cold hemagglutinin is present, the mechanical fragility test must be performed with cold solutions or the tubes surrounded by ice. In certain hemoglobinurias, a cold hemagglutinin is present and therefore an abnormal mechanical fragility is present. In others, there is a complex autohemolysin which requires cold for sensitization and warmth and complement for hemolysis (Donath-Landsteiner hemolysin). In others, heat alone causes hemolysis (heat fragility increased); this occurs in paroxysmal nocturnal hemoglobin-
nuria. In this disease, there is also an increased acid hemolysis. One may tabulate these data as shown in table 1.

### Table 1.—Differential Fragility Tests

<table>
<thead>
<tr>
<th>Types of Hemolytic Disease</th>
<th>Hypotonic Fragility</th>
<th>Mechanical Fragility</th>
<th>Acid Fragility</th>
<th>Heat Fragility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hereditary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spherocytic</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Target cell</td>
<td>+ (resistant)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sickle cell</td>
<td>+ (resistant)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Acquired</strong></td>
<td>+ or -</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>With agglutinins</td>
<td>+ or -</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Without agglutinins</td>
<td>+ or -</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Hemoglobinurias</strong></td>
<td>-</td>
<td>-</td>
<td>+ (with cold)</td>
<td></td>
</tr>
<tr>
<td>Paroxysmal cold (syphilitic)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal cold (with agglutinin)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal nocturnal</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Paroxysmal march</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2.—Differentiation of Familial Spherocytosis from Acquired Spherocytosis

<table>
<thead>
<tr>
<th>Test used</th>
<th>Familial spherocytosis not in crisis</th>
<th>Familial spherocytosis in crisis</th>
<th>Acquired hemolytic anemia</th>
<th>Chemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell survival time</td>
<td>Normal</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Normal</td>
</tr>
<tr>
<td>Iso-antibodies</td>
<td>None</td>
<td>May be</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Anti-human-serum rabbit serum (Boorman, Dodd, Loutit)</td>
<td>Negative</td>
<td>?</td>
<td>+</td>
<td>Presumably negative</td>
</tr>
<tr>
<td>Mechanical fragility</td>
<td>Normal</td>
<td>?</td>
<td>+</td>
<td>Normal</td>
</tr>
</tbody>
</table>

A review in tabular form of some of the various tests which we have found useful in the differentiation of the familial spherocytic anemias from the acquired types is presented in table 2.

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**REFERENCES**

HEMOLYTIC MECHANISMS


HEMOLYTIC MECHANISMS
WILLIAM DAMESHEK