THE REMARKABLE ADVANCE which has taken place during the last ten years in our understanding of the hemolytic anemias is due to two lines of inquiry having been pursued simultaneously although more or less independently. On the one hand, hematologists have become more and more aware of the frequency with which hemolytic phenomena occur in a variety of diseases, and on the other, physiologists and physical chemists have become more and more interested in the fundamental nature of hemolytic processes. It is the purpose of this review to try to bring these two lines of inquiry together.

One must admit at the outset that the attempt can be only partially successful because so many important points are still undecided, but if we try to answer a number of rather general questions we shall summarize much of our knowledge and at the same time shall call attention to much that remains to be discovered. These questions are: What is the normal life span of the red cells, and what normally limits its life? If its life is limited by hemolytic processes, either under normal conditions or in disease, what kind of substances injure it and what are their general properties? What happens when normally occurring lytic mechanisms, which must be of a low order of activity (some investigators, indeed, believe that these lytic mechanisms are of only subsidiary importance) become the very active hemolytic mechanisms which are involved in the hemolytic episode of disease? How shall we classify these lysins in terms of how they produce their effects? And finally, to what special hemolytic mechanisms are such abnormally constituted cells as the sickle cell or the leptocyte of Mediterranean disease, a prey?

1. The Normal Life Span and the Aging Process

It is now recognized that the life of the red cell in man, as well as in several other mammals in which it has been measured, is about one hundred to one hundred and twenty days. This duration of life has been found by several methods which are in substantial agreement. One of the most versatile of these methods is the Ashby technic of transfusing serologically compatible and serologically recognizable blood, e.g., blood of group O, into a recipient of another group, e.g., of group A. At intervals after the transfusion, a sample of the blood of the recipient is withdrawn; his own cells of group A are agglutinated with an anti-A serum, and the number of surviving donor cells are counted. A fairly good straight line ("linear Ashby curve") is obtained when the number of surviving cells are plotted against time; the slope of this line gives the rate of red cell

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destruction (about 1 per cent per day) and the point at which the line cuts the
time-axis of the graph gives the average duration of the life of the cell (about
one hundred and ten days). Although the mathematical analysis of Ashby curves
has not been too successful, the approximately straight line relation would be
expected in a situation in which each red cell lives for about one hundred days
but no more; at the end of this time, some terminal event removes the aged cell
from the circulation.

The process which ends in the destruction of the cell is presumably an intrinsic
one, i.e., one which takes place within the red cell itself. It is often described
as one of "wear and tear" brought about by the cell's having been buffeted
about in the circulation for such a long time. This way of describing it is some-
what vague and unsatisfactory; in its place, let us think of the cell as aging
continuously, and of the aging process as involving a using-up of enzymes (and
perhaps other material) essential to the cell's integrity. Among the large number
of enzyme systems which the red cell contains, there is one which has highly
suggestive properties.2

A reversil)le oxidation of oxyhemoglobin to methemoglobin is always going on
in the red cell, the reaction velocities being such that the rate of methemoglobin
accumulation is very slow. The velocity of the oxidative reaction can be in-
creased by hydrogen donors, such as ascorbic acid or reduced glutathione, both
of which are present in the cell with the result that a peroxide intermediary,
Hb-H2O2, is formed. This then becomes methemoglobin with its ferric iron
replacing the ferrous iron of hemoglobin. When the hydrogen donors are present
in sufficient quantity to make the reaction really rapid, another pigment, chole-
globin, may be formed from the peroxide intermediary and may reach a concen-
tration in the cell of more than 20 per cent. Such choleglobin-containing cells
have a dusky color which becomes greenish as the concentration of choleglobin
increases; in some systems, indeed, such as those containing mouse red cells and
liver slices, the cells become as green as if they contained chlorophyl.3

The appearance of this green pigment is a step in the direction of red cell
disintegration. At least when the cells hemolyze, and under many circumstances
(e.g., in systems containing phenylhydrazine) even before hemolysis, choleglobin
becomes denatured and splits off its ferric iron. The other products are denatured
globin (globan) and biliverdin; the globan can be recognized as Heinz bodies in
intact cells or as a precipitate in hemolysates, and the iron may stain as granules
in siderocytes. Ultimately the ferric iron becomes incorporated in ferritin, in
which form it is transported and stored. No doubt the breakdown of choleglobin
occurs to a more limited extent even before Heinz bodies and iron granules
appear. Since red cells containing even relatively small amounts of choleglobin
are abnormally fragile, both osmotically and mechanically, in vitro, and are un-
usually easily phagocytosed in vivo, the appearance of choleglobin is a step in the
direction of red cell destruction. This is so whether the terminal event which
removes the red cell from the circulation is hemolysis on the one hand or phago-
cytosis on the other.

The idea that the preferential destruction of old red cells is related to their
increased content of choleglobin and to the instability which results gains support
from another observation. Red cells contain catalase, an enzyme which prevents
the formation and accumulation of the peroxide intermediary and which therefore slows the rate of formation of methemoglobin and of choleglobin. On the hypothesis that the aging process involves the accumulation of choleglobin and an increased susceptibility towards terminal events, one would expect aging to be slow in red cells which contain a large amount of catalase and rapid in cells in which the amount of catalase is small. The life of the catalase-rich mammalian red cell is much longer than that of the catalase-poor avian red cell, but whether the generalization will prove to be good when the lives and catalase contents of the erythrocytes of other animals are measured remains to be seen.

While there is still much room for discussion about details, the idea that the life of the red cell is limited by the degradation of its metabolic processes is certainly an improvement over the idea of wear and tear due to its continual movement around the circulation. The point which is most debatable is the nature of the terminal event which removes the aged cell. Twenty-five years ago, the position was stated by Rous: “The thesis that hemolysis is concerned with red cell destruction must be looked upon as unproven. . . . Only two methods have so far been described whereby worn out cells leave the circulation, namely fragmentation and phagocytosis.” Even now, the evidence for red cell destruction by hemolysis is none too impressive as long as one confines oneself to normal material; if evidence from pathologic material is considered in addition, there has never been much doubt about it (see Rous’s review). The difference between the point of view which we hold now and that which was held twenty-five years ago is that we have come to recognize a number of normally occurring substances which are at least potential lysins; among these are the fatty acids and soaps discharged into the blood stream from the chyle, the bile salts and the fatty acids associated with them, and a variety of lytic substances, soap-like or lysolecithin-like, which have been extracted from plasma and from tissues. Perhaps these substances exist in such a low effective concentration that their lytic action is slower than that of a simultaneously occurring fragmentation process; in this case the fragmentation process, accompanied by phagocytosis, will be the normal mechanism for removing old cells from the circulation. By an increase in the effective concentration of the lysins, however, hemolysis could become the faster of the two processes, and destruction by lysis would then occur before destruction by fragmentation. The idea of potential lysins, normally operating at a low level of activity, is at least a simplifying hypothesis which leads to inquiries as to the usual state in which such lysins are found. This turns out to be a state in which the lyasin is associated, in complexes, with other substances which act as inhibitors or accelerators of its action.

II. Lysin-Inhibitor-Accelerator Complexes

There is no question but that plasma contains potentially lytic substances such as the fatty acids and their soaps, nor that various ether soluble and ether insoluble substances can be extracted from it with alcohol. At the same time, plasma itself is not lytic, although it can be rendered capable of turning red cells into the prolytic spherical form if it is heated to 37 C. for a few hours. Plasma also contains proteins and lipids which, singly and collectively, act as inhibitors of the lytic activity of substances such as the bile salts, fatty
acids and soaps. There is considerable evidence that the lysins in plasma are bound, in a somewhat loose and partially reversible manner, to inhibitory material, and that the non-lytic complex formed is in a state of equilibrium with a much smaller concentration of free lysin.\textsuperscript{9} The same is true of tissues; lytic components can be extracted, sometimes even with saline, but in their natural state these seem to be bound to inhibitory material to form relatively non-lytic complexes, the components of which are separable by alcohol extraction, and, in some cases at least, by ultracentrifugation.\textsuperscript{10} The inhibitory material of these complexes is probably partly protein and partly lipid; since so much of the lysin is bound, the only measurement which is possible is one of the effective concentration of a lysin in plasma or in a tissue extract, and the only possible way of judging it is by the effect on added red cells. This effective concentration will be expected to increase either if the quantity of lysin increases, or if the quantity of associated inhibitor falls.

A somewhat analogous situation exists with respect to the lipids of plasma. We know that the cholesterol, cholesterol ester, lecithin and other lipids which have for decades been extracted from plasma and estimated as isolated substances, are really bound in complexes with one another and with plasma proteins, oriented water molecules being an integral part of the complexes.\textsuperscript{11} As a result we have come to be suspicious of the actual lytic nature of substances which must be extracted from plasma with alcohol, ether and similar solvents. As often as not, they may be said to be laboratory creations, and products of the breakdown of the naturally occurring unit, the lysin-inhibitor-accelerator complex, into fractions which are often determined to a large extent by the method of extraction used. Lysins obtainable only by extraction with organic solvents have the same suspicion attached to them, and often represent, as in the case of some of the tissue lysins (see below), the lytic part of a much less lytic lysin-inhibitor complex.

The tendency of lysins such as the soaps and fatty acids to form naturally occurring lysin-inhibitor complexes has been deduced from a variety of in vitro experiments, although in a few cases the existence of the complexes has been demonstrated directly.\textsuperscript{10, 12} There is equally good evidence, again resting largely on deductions from in vitro experiments, that the activity of lysins and of lysin-inhibitor complexes can be enhanced by a variety of accelerators, so that the unit, for practical purposes, is the lysin-inhibitor-accelerator complex. In the case of a lysin acting in vivo, the only meaningful measurement which we can make is one of its activity as influenced both by the substances which are associated with it and which tend to render it inactive, and by the substances associated with it which tend to enhance its action. This will be better understood if it is illustrated by a few examples.

1. Plasma normally contains soaps derived from the lacteals and the thoracic duct, and largely combined with plasma inhibitors such as protein and lipid. After a meal rich in fat, the soap concentration in the plasma rises from about 1 mg. per cent to as much as 6 mg. per cent, an increase in lysin concentration which is sufficient to increase the rate of red cell destruction by about one-third. The animal (dog) would presumably develop an anemia were it not that the rate of red cell production is increased proportionately.\textsuperscript{13} This is an example of in-
creased hemolysis occurring because of an increase in the concentration of a circulating hemolysin; in this case the lysin is a simple and naturally occurring substance.

2. Dogs fed on a special vitamin deficient diet develop a severe anemia if indol is administered simultaneously. Indol itself is not a lysin in the low concentrations in which it occurs in the blood stream, but it is an accelerator of both bile salt hemolysis and of hemolysis by lysolecithin-like substances. The anemia which results is attributable to indol acting as an accelerator of these naturally occurring hemolysins.

3. Horses and dogs develop an anemia when fed phenothiazine, used as a worm remedy. Phenothiazine itself is not lytic, but is an accelerator for several naturally occurring lysins, particularly lysolecithin. It has been suggested that it produces a hemolytic anemia in this way.

4. Children who chew moth balls develop a hemolytic anemia. The active material (naphthalene and substances related to it) is not hemolytic in the concentrations in which it occurs in the blood stream, but is an accelerator for a number of lysins of the soap and fatty acid class.

Doubtless, many drugs have the property of being accelerators of hemolysins rather than of being lysins themselves. One would expect that there would also be instances in which increased hemolysis develops because of a reduction in the concentration of plasma inhibitors. Brinkman was unable to produce a hemolytic anemia in rabbits by the injection of lysolecithin-like material except when the animals were deprived of cholesterol, one of the more important plasma inhibitors, and it has been observed that the lytic action of plasma in cases of Mediterranean anemia can be demonstrated only if the plasma cholesterol is very low. These may be two instances in which the effectiveness of a hemolytic system depends on associated inhibitors being reduced in concentration.

III. The Hemolytic Episode

A hemolytic episode can occur when the concentration of an existing lysin rises, when an accelerator enhances its effect sufficiently, or when there is sufficient decrease in the concentration of the inhibitors which hold its effects in check. It can also occur when a new or foreign hemolytic mechanism is established, and this is what probably happens in the majority of cases of hemolytic disease. Under all of these circumstances, a hemolytic anemia results if the rate of red cell destruction is greater than the rate of red cell production. This may develop suddenly to produce a more or less severe hemolytic crisis; it may be transient if the rate of red cell production rises so as to keep pace with the increased rate of red cell destruction, or it may develop slowly and be persistent. Almost any combination of the possibilities can occur, and the effects which follow may vary greatly in degree. In addition to the anemia, the abnormal hemolytic process, whether abnormal in nature or merely abnormal in intensity, results in a change in the type of curve obtained by the Ashby technic, in the appearance of spherical forms in the blood stream, and in an increase in the products of red cell breakdown.

If the red cells in the normal individual can be likened to a population which is growing old and dying of old age, the cells during a hemolytic episode can be
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likened to a population exposed to an epidemic which kills young and old alike. The fall in the red cell count during an acute hemolytic episode is curvilinear, and since the lysins in the circulation are often capable of destroying not only the individual's own red cells but also the red cells of a donor, the Ashby curve is curvilinear also ("exponential Ashby curve"). This form of the Ashby curve indicates that the cause of the red cell destruction is an extrinsic catastrophe, and not the gradual aging and death from a terminal event which gives rise to the normal linear Ashby curve. If the individual's cells are removed during the hemolytic episode and transferred to a normal recipient's circulation, their death rate again may become one of the normal linear type because they have been removed from the destructive environment.

By variations in the Ashby technic it can be shown, for example, that the destruction of Rh positive red cells in the infant of an immunized mother is an extrinsic process due to the presence of anti-Rh antibodies in the infant's plasma, that an extrinsic process is involved in the destruction of normal red cells transfused into some recipients with acquired hemolytic anemia, that the hemolysis of the red cells in congenital hemolytic icterus is intrinsic, proceeding as readily when the cells are transfused into a normal recipient as it does in the individual affected with the disease, and so on.18 It is only when one tries to extract quantitative information from Ashby curves that they are disappointing; this is partly because they are rarely as smooth as one gathers from the ones used as illustrations, and partly because the mathematical treatment of even the simplest of them is formidable.19, 20 Even after the expenditure of much labor on the problem, the present position is that it is possible to construct Ashby curves on the basis of certain assumptions about the mechanisms involved, but not possible to derive much information about the mechanisms from a given Ashby curve, except perhaps in a few simple cases.

The small spherical cells which appear in the blood stream during a severe hemolytic episode21 are similar to the spherical forms found in vitro when red cells are exposed to almost any hemolysin. These spherical forms are apparently due to effects of the lysin on the components of the cell's surface ultrastructure, which becomes so disorganized that the maintenance of the special discoidal shape is impossible. With the passage of time or in higher concentrations of the lysin, the disorganization progresses and the cell finally hemolyzes.

It is unfortunate that the term "spherocyte" should have been used to describe both these spherical forms and the abnormally thick red cells of congenital hemolytic icterus. The latter appear to be congenitally misshapen cells which are abnormally thick even in an isotonic medium such as their own plasma, and which have an increased osmotic fragility because the thick red cell is able to undergo less than the normal increase in volume before it becomes a sphere with a surface equal to that of the original disk.22, 23 It is true that the spherical forms found during hemolytic episodes also have an increased osmotic fragility, but the underlying cause of the increased fragility is not so much the cell shape as that circulating lysins have disorganized their structure. It does not help matters to find that there is still a third form of "spherocyte" found when red cells of either normal or abnormal shape swell in hypotonic media. The swelling is accompanied by shape changes, usually in the direction of cup-shaped forms,
but at a certain stage some restraining structure seems to give way, the cell then becomes a sphere, and further swelling leads to hemolysis. This type of “spherocyte” is observed in blood kept under conditions of stasis; here the principal feature is the volume increase, which may depend on the accumulation of metabolites. By contrast, the volumes of the spherical forms which result from the action of lysins and the volumes of the spherocytes of congenital hemolytic icterus are substantially the same as those of typical disks. The general adoption of Crosby’s terms “hereditary spherocyte,” “acquired spherocyte” and “osmotic spherocyte” would be a step in the direction of clearing up the confusion.

The acute hemolytic episode may be accompanied by hemoglobinemia. Hemoglobinuria occurs if the level of plasma hemoglobin rises to about 130 mg./100 ml.; once started, it may continue until the plasma hemoglobin falls to 50 mg./100 ml. Increased red cell destruction, as shown by anemia and an increased output of urobilinogen, etc., in the absence of hemoglobinemia and hemoglobinuria points to the removal of red cells by sequestration and phagocytosis rather than by frank intravascular hemolysis, but does so somewhat uncertainly since changes in plasma hemoglobin level are determined by the difference between the rate of addition of hemoglobin to, and the rate of removal of hemoglobin from, the plasma. This difference may be quite small if the hemolytic process is slow.

The other accompaniments of the hemolytic process are indications of increased bone marrow activity, notably reticulocytosis and an enlargement of the liver, spleen and the reticulo-endothelial system in general, which becomes laden with the products of red cell breakdown.

Both of these manifestations are responses on the part of the body to the hemolysis and, at best, measure a combination of the intensity of the hemolytic process and its duration. They are apt to be late manifestations. In hemolytic anemia of the newborn, for example, an anemia is an earlier sign than a reticulocytosis; a reticulocytosis is an earlier sign than an erythroblastosis; all three occur before the enlargement of the liver and spleen.

IV. A Classification of Lysins Which Act in Vivo

The lysins which produce hemolysis in vivo can be divided into a group of naturally occurring hemolytic substances such as the bile salts, the fatty acids, lyssolecithin and other lytic materials obtainable from tissues and plasma (the “tissue lysins”), and a group of hemolytic substances which are essentially foreign to the individual, such as the bacterial lysins, many drugs and the many agglutinins and lysins of the immune body type. The list of these substances is enormous, but what is known about the mechanism of their action can be summarized comparatively briefly.

1. Naturally occurring lysins, including the “tissue lysins.” Little need be added to what has already been said about the conditions under which the bile salts, the fatty acids and the soaps can become hemolytic in vivo; what is required is a suitably high effective concentration of the lysin, this depending not on the concentration of lysin alone, but on the concentrations and potencies of its associated inhibitors and accelerators as well. It seems that the soaps derived from the diet can bring about a certain amount of hemolysis in vivo, and it is
easy to bring about intravascular hemolysis in animals by injecting a variety of
soaps, e.g., oleate, bile salts and lecithins or allied substances. Quite apart
from the question as to whether pure taurocholates and glycocholates are lytic,
the evidence is rather against any appreciable amount of in vivo hemolysis
being produced by the normally circulating bile salts. In view of what is known
about systems containing the same lysins in vitro, there is nothing unexpected
about any of these results.

The situation is very different when we turn to the subject of the lytic sub-
stances which can be obtained from tissues, to the question of what they are,
and of whether they play any part in physiology or pathology. The “tissue lysins”
have been known since the time of Metchnikoff, who discovered them when he
tried to obtain phagocytosis of red cells by pieces of chopped-up lymph glands,
etc., in vitro. Hemolysis occurred instead. Metchnikoff showed that the activity
of the lysin involved is lessened by heating, and after the Ehrlich school had
satisfied itself that a property of the then recently discovered complement was
not involved, the subject was almost completely dropped.

Two ideas about the tissue lysins, however, have been associated with them
from the beginning, and the persistence of these ideas has brought about a
revival of interest in tissue lysins from time to time. One is that they have some
relation to the normal destruction of red cells and to hemolytic episodes; the
other is that the lysins sometimes found in tumors are responsible for the anemia
often seen in patients with cancer, and that the hemolysins may be associated
with cytolytins which may explain the invasiveness of malignant tumors. The
methods used for extracting the lysins from tissues have largely depended on
whether the investigator was influenced by the first of these ideas or by the
second. Those interested in the cancer problem have naturally started with
tumors and normal controls which they extract with saline (as Metchnikoff did),
whereas those interested in the life of the red cell have naturally started with
plasma and, to obtain lytic substances at all from this normally nonlytic ma-
terial, extract it with organic solvents. This apparently trivial difference in
procedure accounts for much of the complexity and confusion which the literature
on the subject contains.

In 1907, Weiß made a detailed study of the tissue lysins obtained from dog
liver and kidney, as well as from several kinds of tumor, by extraction with saline;
the weak lysins which he found in normal tissue and in non-necrotic tumors
seemed to be similar, but other lysins, characterized by greater diffusibility,
were found in necrotic tumors. His work was forgotten. In 1943, Macgregor,
Findlay and Martin demonstrated a lytic agent obtained by incubating small
pieces of normal tissue in saline; they thought the lysin to be a species-specific
enzyme, but this part of their observation has not been confirmed. Gross later
found an even more active lysin in saline extracts of mouse carcinoma. It should
be emphasized, however, that none of these saline-extracted lysins is really very
lytic, the most active of them (Gross’s lysin) taking from thirty minutes to an
hour to hemolyze the usual test suspension of washed red cells. It should also be
noticed that, in order to obtain even weak lysins, it is necessary to incubate the
red cells with the pieces of tissue for a period of hours, or alternatively to pre-
incubate the pieces of tissue before removing the supernatant saline and adding it to the test suspension of red cells.

Much more actively lytic substances can be obtained by extracting tissues and plasma with organic solvents. By extracting blood with alcohol, petroleum ether and chloroform, Brinkman obtained a hemolytic substance, not very clearly identified but apparently a mixture of phospholipids and the soaps of fatty acids, the hemolytic effect of which is inhibited by cholesterol. It is worth remarking that these lytic substances and also their inhibitor, cholesterol, can be extracted from the red cells themselves, and so one can think of the red cell as containing all the essentials of a lysin-inhibitor system within its own structure.

It therefore contains, conceivably, a mechanism for its own destruction. This possibility is emphasized by Laser, whose ether soluble lysin, like Brinkman’s, is obtained from the red cells themselves. Bergenhem and Fahraeus also obtained lytic substances from plasma by alcohol extraction, but these are distinguished from Brinkman’s and from Laser’s by being relatively insoluble in cold ether. They are usually referred to as lysolecithin or lysolecithin-like substances. Singer has devised a method for estimating them, although there is a question as to its quantitative reliability.31 Lysolecithin-like substances are found in particularly high concentration in the blood of the splenic pulp, and may be responsible for hemolysis there. More recently, Laser has extracted an alcohol and ether soluble lysin, identified as \( \text{cis} \) 11–12 octadecenoic acid (a relative of stearic acid), from various tissues and from red cells themselves.

As the situation stands at the present time, it seems that the weakly lytic substances which can be extracted with saline from preincubated homogenates of tissues and from plasma exist as lysin-inhibitor complexes. These complexes are produced enzymatically, during preincubation, from less lytic or nonlytic precursors. Heating the system destroys the necessary enzymes. Highly lytic components of these complexes can be extracted with various solvents; these highly lytic substances, however, are laboratory creations in the sense that they result from the breaking-up of naturally occurring complexes. Tyler has used the ultracentrifuge to separate a preponderantly lytic fraction of the lysin-inhibitor complex of guinea pig liver from a preponderantly inhibitory fraction; the former is in the microsome fraction, and the latter in the mitochondrial fraction. There does not seem to be much doubt as to the existence of the complexes as such.

Very little can be said about the nature of the components of these complexes, particularly as there is probably more than one identifiable complex in both plasma and in tissue homogenates. At least one of the lysins is alcohol and ether soluble, and may be identical with Laser’s lysin, i.e., a soap of a C-18 fatty acid. The inhibitor may be a protein, a lipid, or a lipoprotein complex. The lytic substance is probably firmly bound in the tissues, the enzymatic process producing the weakly lytic lysin-inhibitory complexes which appear in saline extracts, accompanied by small quantities of free lysin. At any stage, however, large amounts of highly lytic free lysin can be extracted with alcohol from the complexes in which it is bound. There is further evidence that lysins relatively insoluble in cold ether (lysolecithin-like substances), as well as ether soluble lysins, are bound
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in similar complexes, and that they can be obtained from incubated blood, plasma and tissue homogenates. Smaller quantities can also be obtained from fresh blood and tissues, i.e., some of the lytic material is preformed.\(^3\)

The question remains as to whether the tissue lysins are responsible for in vivo hemolysis either under normal or under pathologic conditions. A high concentration of lysolecithin-like substances in the blood of the splenic pulp would certainly provide a mechanism by means of which the spleen could dispose of red cells sequestered there. Other mechanisms, such as the effect of stasis in producing the “osmotic spherocyte,” may be involved at the same time, and, apart from the spleen’s peculiar property of being able to sequester unusually thick red cells,\(^33\) what goes on in the spleen may also go on in any sluggish region of the circulation. Castle and his collaborators suggest that the tissue lysins, together with hypoxia, provide a mechanism for the lysis of agglutinated red cells in the capillaries of organs such as the liver, and that this mechanism provides the much sought-for link between the process of in vivo agglutination and in vivo hemolysis (see below). Other investigators, such as Brückmann and Wertheimer,\(^34\) look upon the tissue lysins as products of autolysis and as having no connection with normal or pathologic blood destruction. The only possible conclusion is that the question is undecided. Nor is it decided that the lysin of mouse carcinoma is different from that of normal tissues, for its greater activity may be due to its being present in greater concentration; it is not even decided, indeed, that the anemia in the tumor-bearing mouse is a hemolytic anemia.

2. Lysins which do not occur naturally. Since this review is concerned with the mechanisms which are involved in hemolysis in vivo, no attempt will be made to catalogue the great number of drugs and toxins which have been described as increasing the rate of red cell destruction, particularly as it is not clear, in many instances, whether the increase in red cell destruction is due to hemolysis or to phagocytosis. It will be sufficient to say that the changes which one large group of substances produce are primarily changes in the red cell interior, in red cell metabolism and particularly in the hemoglobin-methemoglobin-choleglobin system, while the changes produced by another large group of substances are primarily surface changes which involve hemolysis or agglutination followed by hemolysis.

Intracellular hemoglobin breakdown is brought about by phenylhydrazine and by aromatic amino and nitro compounds, the effect of these drugs being essentially to accelerate the rate of formation of methemoglobin and choleglobin. The latter breaks down to form bile pigment precursors, globin and the ferric iron seen in siderocytes and the H\(_2\)S cells described by Granick. Similar effects are produced by silver and lead salts, by carbon tetrachloride and bisulfide, and even by sulfonamides. Since red cells containing choleglobin have increased osmotic and mechanical fragilities,\(^2\) and since red cells containing methemoglobin are quickly phagocyted by the reticulo-endothelial system,\(^35\) there is no difficulty in seeing how drugs of this class can lead to increased red cell destruction and anemia.

There is a remarkably close relation between the surface injury which results in agglutination and the surface injury which results in hemolysis, so much, indeed, that it is almost a rule that conditions can be found under which lytic
substances become agglutinating, and vice versa. The problem is to find the conditions: when does an agglutinin become a lysin? Sometimes the answer is straight forward, but more often it is not.

In the case of some of the simplest substances, such as the inorganic salts, agglutination occurs in some concentrations and lysis in others, and in the case of many other agglutinating systems the factor necessary for lysis is complement. This statement covers the agglutinins tannin and colloidal silicic acid, the agglutinins of the ABO system, the heterophile antibody agglutinin of mononucleosis and many of the atypical antibodies now being detected by the anti-human globulin test and by the use of trypsinized red cells. It does not cover the vegetable agglutinins concavalin A and ricin, nor does it cover the agglutinins of the Rh system, for these are not lytic in vitro even if complement is present. They nevertheless produce a hemolytic anemia in vivo.

This hemolytic anemia has two additional features. While in vitro agglutination is rapid, the anemia takes some hours to be produced in vivo, i.e., there is a delay. Much smaller quantities of agglutinin (at least if the agglutinin is an anti-serum) are required for agglutination and lysis in vivo than are required in vitro, i.e., there is an enhancement of effect. Presumably there is some process, present in vivo but absent in vitro, which takes time and which brings about enhancement.

Castle and his collaborators, after having shown that the mechanical and osmotic fragilities of red cells increase only some hours after the injection of an agglutinating antibody, infer that the initial effect of the agglutination is to produce stasis and sequestration of the red cells in the capillaries of various organs, where substances liberated by the tissues (the “tissue lysins”) bring about the changes in fragility. The increased fragility, in turn, determines that the cells shall be readily destroyed, either while still trapped in the capillaries (because of their increased osmotic fragility) or when liberated into the circulation (because of their increased mechanical fragility).

The idea of this train of events is at variance for the moment with the earlier idea that the red cell, after having its surface injured by the agglutinin, is hemolyzed by the stresses it experiences in the circulation (mechanical fragility) or by its inability to swell without bursting (osmotic fragility, rendering the cell vulnerable to stasis), or by other mechanisms. It may, of course, be true that the mechanism involved in the destruction of agglutinated red cells is not hemolysis per se but phagocytosis, although in the particular case in which the agglutination-hemolysis takes place in the presence of complement, there is no problem at all in understanding the mechanism involved.

It will probably occur to the reader that what is lacking, both as regards the differences between the in vitro and in vivo effects of immune bodies, as regards the “tissue lysins,” and even as regards the basic idea of simple lysins existing in lysin-inhibitor-accelerator complexes, is extensive and suitably planned experiments on animals.

Excluding the cases in which hemolysis is caused by heat, drugs or the lysins associated with bacteria, most cases of acquired hemolytic anemia in man can be attributed to the presence of antibodies, either lytic in themselves, or agglutinating primarily and lytic secondarily because of the obscure enhancing
mechanisms which occur in vivo. A statement of this kind, which is a return to the point of view of Widal, Chauffard and other workers of the beginning of this century, would certainly not have been made fourteen years ago, when Schwartz and Dameshek's description of lysins of the immune body type in 3 consecutive cases of acquired hemolytic anemia was something of a novelty. Now the situation is entirely reversed. An embarrassingly large variety of antibodies is appearing as a result of newer methods of testing (which employ trypsinized red cells, paroxysmal nocturnal hemoglobinuria red cells, direct and indirect anti-human globulin tests [Coombs], etc.). The problem now is to decide when an antibody is nonspecific and when it can reasonably be held responsible for a hemolytic anemia.

V. Hemolysis of Red Cells with Intrinsic Defects

The abnormally shaped red cells of congenital hemolytic icterus, of sickle cell anemia, of Mediterranean anemia and of pernicious anemia are all peculiarly vulnerable to hemolytic processes. These cells have two defects in their architecture, a defect in shape and an abnormality in the nature of their hemoglobin. In each case, the Ashby technic shows that the cells are hemolyzed because of intrinsic defects, for the abnormal cells are destroyed unusually rapidly when they are transferred into the circulation of normal individuals. The hemolytic mechanism involved is different, however, for each abnormal type of cell.

The red cell of congenital hemolytic icterus (the “hereditary spherocyte”) is unusually thick in relation to its diameter, and is preferentially sequestered in the spleen of the individual affected with the disease and in the spleen of the normal individual as well. Because of its small surface to volume ratio, the “hereditary spherocyte” readily becomes an “osmotic spherocyte.” In the splenic pulp, hemolysis, accompanied or followed by phagocytosis, results from the effects of stasis perhaps aided by the action of the lysolecithin-like substances and other “tissue lysins” which are found in relatively high concentration in pulp and pulp plasma. The red cells in congenital hemolytic icterus also show increased mechanical fragility, perhaps another consequence of their shape and of their small surface to volume ratio. Abnormal antibodies may be found in the hemolytic crises of the disease, and during the crisis the splenic destructive mechanisms become more active.

The abnormal red cell in sickle cell anemia undergoes its curious and usually reversible shape change when exposed to low O₂ tensions, and contains para-crystalline and birefringent hemoglobin when it is in its sickled form. Pauling and his collaborators have shown that this hemoglobin is abnormal electrophoretically. Part of the explanation for the sickling may be that the hemoglobin has an unusually small solubility, although the cell architecture may be abnormal in other ways as well. The sickle cells themselves are poor osmometers and have an increased mechanical fragility. It has been suggested that hemolysis occurs when masses of sickle cells are impacted in the capillaries of various organs; under these conditions, which are much the same as those which occur in hemorrhagic infarcts, hemolysis is brought about by the stasis, anoxia and accumulation of metabolites, possibly assisted by the lytic action of locally produced lysins. Even the transition from the biconcave disk to the sickle, and
from the sickle to the disk, is often accompanied by the loss of filaments, resembling myelin forms, from the red cell surface; if frequently repeated, such transitions are likely to contribute to red cell breakdown.

The red cells of Mediterranean anemia in its major form (Cooley's anemia) are unusually flat (leptocytes) and abnormally resistant osmotically. Very little is known about the process which destroys these cells, but Ashby curves show that an intrinsic defect is present, at least in some cases, which renders them shortlived. Just as lysis is closely related to the spherical form, so fragmentation is related to the discoidal form. Fragmentation of the very flat disks of Mediterranean disease is a conspicuous phenomenon, and this may be the process which destroys them.

The Ashby curves obtained when red cells from cases of pernicious anemia in relapse are transfused into normal recipients show that the cells are shortlived. Johnson and his collaborators have also shown that the red cells in pernicious anemia are specially susceptible to lysis by lipemic serum.

Finally, it seems more than a coincidence that in each of these diseases the abnormality of red cell shape is accompanied by an abnormality in the contained hemoglobin. This has been most completely studied in the sickle cell but the investigations are only beginning. The hemoglobin of the red cell of Mediterranean disease shows a peculiarity in the rate at which it is denatured by alkali. There is also some evidence that the hemoglobin of the cell of congenital hemolytic icterus is abnormally soluble, and that the hemoglobin in the misshapen red cells of pernicious anemia becomes readily birefringent. There have been many speculations as to the possible relation between red cell shape and the molecular arrangement of intracorpuscular hemoglobin. Convincing evidence of an arrangement of hemoglobin, intermediate between that of the highly ordered crystal lattice and that of the disordered dilute solution, has now been obtained in normal red cells, and the possible relation between abnormal hemoglobin, abnormal red cell shapes, and abnormal rates or even forms of red cell destruction will be scrutinized most carefully from now on.

**Summary**

The factors which normally limit the life of the red cell are described as being a continuous metabolic process involving the enzymatic oxidation of Hb and a terminal event which may be hemolysis, fragmentation or phagocytosis.

A number of lytic substances, such as soaps, lipids and lyssolecithin-like substances can be extracted from plasma and from tissues. These substances are associated with inhibitors and accelerators to form complexes. The activity of these naturally occurring hemolytic complexes tends to be small, although it can increase to such an extent that appreciable red cell destruction results. Instances are given in which hemolysis in vivo results from the concentration of one of these lysins increasing (as when fat is fed), from an accelerator of one of the lysins being introduced (usually as a drug), and from the concentration of inhibitory material being reduced (as by a low cholesterol diet).

Most hemolytic episodes are due to the establishment of a new hemolytic mechanism, which may appear after the introduction of a drug, of an agglutinin such as silicic acid or ricin, of an immune agglutinin which is not itself a hemolysin.
CERTAIN HEMOLYTIC MECHANISMS IN HEMOLYTIC ANEMIA

or of an agglutinin which is a lysin in the presence of complement. The various mechanisms which may result in hemolysis are discussed. The case in which the agglutinin becomes a lysin in the presence of complement presents no difficulty; in other cases the mechanism of hemolysis is not so clear, nor is it clear whether red cell destruction depends primarily on hemolysis or primarily on phagocytosis.

Special processes are involved in the destruction of red cells which have intrinsic defects of structure. The abnormally thick red cells of congenital icterus are selectively sequestered in the spleen, where there are a number of hemolytic mechanisms which can destroy them the more readily because of their abnormal shape. The sickle cell, with its poor osmotic properties, its reduced mechanical fragility, and its tendency to lose part of its structure as filaments at each disk-sickle transformation, is destroyed by processes which are probably hemolytic but less easy to specify. The flat red cells of Mediterranean disease are abnormally prone to fragmentation. In all these diseases the abnormal shape of the red cell seems to be accompanied by peculiarities in its contained hemoglobin, an observation which requires further study before its significance is clear.

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