1958

Erythropoiesis: consideration of a humoral stimulatory substract

Nathan Richard Adkins
University of Nebraska Medical Center

ERYTHROPOIESIS: CONSIDERATION OF A HUMORAL STIMULATORY SUBSTANCE

Nathan Richard Adkins

Submitted in Partial Fulfillment for the Degree of Doctor of Medicine

College of Medicine, University of Nebraska

April 1, 1958

Omaha, Nebraska

TABLE OF CONTENTS

INTRODUCTION

The erythrocyte has long been a favorite object of study. Investigators, who have studied this unit, have noted a remarkable constancy with regard to its total mass and concentration in the blood. It was noted that any change in the red cell mass or concentration evoked a response on the part of the organism to restore the erythrocyte concentration to its original state. The carefully adjusted equilibrium, with regard to this unit, has stimulated investigators to search for the mechanism or mechanisms involved in red cell production and destruction. A prime object of study has been the physiologic basis for stimulation of red cell production.

Under ordinary circumstances, the red blood cell is known to be formed in the bone marrow. In certain disease states in which the bone marrow, for one reason or another, is unable to produce adequate numbers of red cells, the liver and spleen may become sites of extramedullary erythropoiesis. The erythrocyte precursors within the bone marrow combined with the circulating red cell mass are known as the erythron (1). Hyperplasia of this erythron is generally believed to be the result of anoxia, regardless of its

etiology. Thus, anoxia is thought to be the primary stimulus for erythropoiesis. The exact mechanism, which responds to the initial state of anoxia and causes the hyperplasia of the erythron, is not completely understood.

In any study of erythropoiesis, certain criteria have to be established as evidence of erythroid activity. Early studies in this field were based solely on the red cell concentration. In more recent years, factors of hemoconcentration, hemodilution, plasma loss, splenic release, and splenic withdrawal have been recognized and more accurate studies have resulted.

In the total examination of the erythron, a quantitative measure of both the circulating red cell mass and the active bone marrow is most desirable. At present, the former is possible, but the latter is only an approximation. Since the total erythron cannot be measured with any accuracy, the usual criterion for erythropoietic stimulation is the red cell concentration plus signs of erythrocyte precursor activity. These signs are usually a reticulocytosis or a hyperplasia of the erythroid elements of the bone marrow. A relative or absolute increase in the number of circulating reticulocytes,

preceding or occurring simultaneously with an increase in the red cell count, is the most widely used indirect criterion for stimulation of the erythroid bone marrow. More recent investigators have come to use radioactive iron (Fe^{59}) uptake studies with more accuracy. In addition to the more common methods listed above, the percentage of erythroid elements in the bone marrow, as well as the number of mitotic figures, have been used as criteria.

ERYTHROPOIESIS

It has been previously stated that, under normal conditions, the erythrocyte is formed in the bone marrow. From an original mother cell, the erythrocyte evolves by a number of mitotic divisions and structural changes. In this production of the mature cell, certain dietary and hormonal factors are necessary in the normal cellular metabolism. A deficiency in these components of the metabolic environment will impair the erythroblastic multiplication.

The passage of new cells into the circulation is manifested by the presence of reticulocytes in normal blood. When there is an increased need for

circulating red cells within an organism, the demand may be met by two means. The first and most rapid of the two is contraction of the spleen. Stimuli, such as severe exercise, sudden loss of blood, or diminished oxygen supply, may cause the spleen to contract and deliver corpuscles to the peripheral circulation. The second method, by which the body may respond to increased need for red cells, is increased activity of the hematopoietic system.

Jacobson and his group have reported extensive work with regard to erythropoiesis (36). This group of investigators believe that the dynamic equilibrium of the erythron is controlled by the relationship of the oxygen supply in the tissues, to the demand for oxygen, rather than by either one alone. Conditions that reduce the demand for oxygen while the supply remains normal, e.g., acute starvation and hypophysectomy, induce a decrease in the erythropoietic rate in rats. This decrease in the rate of erythropoiesis is also seen in conditions where there is an increase in the available oxygen, while the demand remains normal. Transfusion, polycythemia and states of hyperoxia are examples of this condition.

To expand this concept, Jacobson et al., have shown that measures which increase the demand for oxygen, while the supply remains normal, will increase the erythropoietic rate in rats. Examples of this condition are the administration of dinitrophenol or triiodothyronine. It has also been noted that decreasing the supply of exygen while the demand remains normal, as in bleeding or phenylhydrazine induced hemolysis, will also increase erythropoiesis in these animals (36).

Under normal conditions, the red cell disappears after a determinable and fairly constant period of time. There are a number of methods by which the life span of the red cell can be determined. In the majority of the procedures employed, the calculated life span of the erythrocyte in man is thought to be 110 to 130 days. If this be true, it appears that about fifty milliliters of blood is replaced each day (43).

The manner in which the destruction of the red cell takes place in the normal individual is not fully understood. It is known that certain large endothelial cells, found especially in the spleen, take up red corpuscles and destroy them. Robertson and Rous (42) searched the body, organ by organ, for

disintegrated red blood cells. They found little evidence of phagocytosis in man. They suggested that the chief means of erythrocyte destruction is fragmentation. In the circulation, the red blood cell is buffeted about and eventually becomes distorted and broken. This fragmentation progresses until a fine hemoglobin containing dust is removed by the spleen. This is but one hypothesis on the manner of destruction of the erythrocyte. In general, it is believed that the fragmented particles of the broken down erythrocytes are removed from the circulation by the reticulo-endothelial tissues of the body.

ACCELERATED ERYTHROPOIESIS

An increase in altitude is the most widely known condition which will cause an increased rate of erythropoiesis. It has been repeatedly shown that individuals living at higher altitudes have greater hemoglobin and red cell concentration than individuals living at sea level (3). The earlier studies, relating to altitude and its attending polycythemia, were clouded with doubt regarding the cause of the increased red count. Factors of hemoconcentration and unequal distribution of red blood cells, as well

as the splenic reservoir, had to be considered. It is now generally believed that residence at a sufficient altitude or an equivalent amount of anoxic anoxia will produce a slow increase in the total blood volume. This develops largely as an increase in the red cell mass.

A common form of anemic anoxia is that seen in carbon monoxide poisoning. In this condition, the hemoglobin of the red cell is partially saturated with carbon monoxide. This results in a proportionate decrease in the oxygen carrying capacity of the blood, without changing the red cell concentration. This has been an important method of study, since it enables one to evaluate the effect of a decrease in the oxygen carrying capacity of the blood alone. These studies have been carried out in animals (4) with a marked response in red counts, hemoglobin concentrations, and reticulocytosis. Although this has been a consistent response in animals, the results have not been conclusive in man (5). A possible explanation of this discrepancy lies in the fact that man has not been subjected to as high levels of carbon monoxide saturation. For obvious reasons, the duration of time of exposure maintained in man is not as great as that used in animals.

There are a number of pathological states in which man responds by a polycythemia. The majority of these conditions rely on a relative state of arterial anoxia, with varying degrees of arterial oxygen unsaturation. Congenital heart disease, in which there is active mixing of venous blood with arterial blood, is a prime example. Other states, such as chronic pulmonary diseases, Ayerza's disease, and pneumothorax often cause a polycythemia in man. This group is quite large and the literature is extensive on the subject.

Hemorrhage is a common cause of increased erythropoiesis. The regeneration of the circulating red cell mass, following loss by hemorrhage, involves an active erythropoietic stimulation. The red cell mass remains uniformly constant and is maintained by a balance between red cell production and destruction. A direct relationship exists between the severity of the anemia and the hemoglobin production, reticulocyte response, and the degree of marrow hyperplasia (6,7,8).

When anemic anoxia is added to or superimposed upon an anoxic anoxia, an additive effect is demonstrated. A standard red cell loss in dogs, produced either by hemorrhage or by the use of phenylhydrazine,

was recovered in 30-40% less time at 7,000 to 15,000 feet, than was required by anemia at sea level (9).

In the previous conditions, cited as examples in which an accelerated rate of erythropoiesis is demonstrable, a physiologic state of anoxia exists. This anoxia is either on an anemic or anoxic basis. Cobalt administration, either by an oral or parenteral route, over a period of several weeks, induces and maintains a polycythemia in man and in animals (9,10). The precise mechanism in this action of cobalt is not clearly understood. Early studies linked the administration of cobalt to a relative state of bone marrow anoxia. Other investigators have not been able to substantiate this conclusion. More recent articles relating to this subject attempt to correlate the administration of cobalt to the production of erythropoietin (12,13).

Erythropoietin is the name given to the substance, existing in anemic plasma of man and animals, that has a stimulatory effect on red cell production.

DECREASED ERYTHROPOIESIS

Since the loss of red blood cells from the

circulation or the reduction of the partial pressure of oxygen in the inspired air will stimulate erythropoiesis, it might be supposed that addition of extra red cells to the circulation or the breathing of oxygen rich air would show signs of decreased erythropoiesis.

When repeated transfusions of whole blood, or blood concentrated with respect to the erythrocytes, were given to rabbits or rats, a temporary polycythemia resulted. This polycythemia was associated with a reduction in the percentage of reticulocytes. Although there is a dilution factor involved, this reduction was considered as a sign of decreased erythropoiesis (14). A similar reduction in reticulocytes has been noted in patients with pernicious anemia, after they had received large transfusions of whole blood (15). A large decrease in the rate of red cell iron turnover also occurred after an artificial increase in the red cell volume of rats (14).

Studies in man, with regard to high oxygen partial pressure of the inspired air, have revealed minor decreases in the red cell concentrations, as well as other signs of decreased erythropoiesis (16). This result is not surprising, however, since the oxygen content of the arterial blood is little

increased by an elevation of the alveolar partial pressure of oxygen (9).

NON-HUMORAL THEORIES OF ERYTHROPOIESIS CONTROL

In the past sixty years, a number of theories have been advanced regarding the exact mechanism responsible for accelerated erythropoiesis. Anoxia, as previously stated, is generally considered to be the primary stimulus for erythropoiesis. The controversy exists, however, as to the mechanism or mechanisms involved from the primary state of anoxia to the final state of accelerated erythropoiesis.

Since the bone marrow is the site of erythropoiesis, a favorite assumption has been that bone marrow anoxia is the primary mechanism. This theory was first postulated by Mieschner in 1895 (17). He believed that the bone marrow was anoxic to a certain extent at all times, and anoxia provided a continuous stimulation of erythroid activity, counter-balancing the daily destruction of erythrocytes. This theory has been the most widely accepted. Grant and Root, however, contend that the evidence offered by other investigators has been indirect (18). In their studies, they determined the

oxygen content of the bone marrow of dogs. They were unable to demonstrate any decrease in the oxygen saturation of the bone marrow of dogs made anemic by bleeding. These dogs, at the same time, showed adequate evidence of accelerated erythropoiesis.

Carbon dioxide tension, acting on the bone marrow, has also been considered as the primary mechanism involved in erythropoiesis. This mechanism was first advanced by Jordan and Speidel in 1924 (19). Their initial work was based on an increased carbon dioxide production in tadpoles, correlated with an increased erythroid activity in the spleen. The evidence in general, however, does not establish carbon dioxide tension as the primary mechanism involved. Studies in carbon dioxide tensions at high altitudes show a decrease in arterial carbon dioxide tensions, while evidence of increased erythropoiesis may be observed.

The endocrine glands have been studied quite extensively, with the possibility that one or more of the hormones produced by these glands, is the primary agent involved in accelerated erythropoiesis.

The pituitary has been studied extensively with regard to any possible action on erythropoiesis.

There is no doubt that the hypophysectomized animal has a decreased number of red cells and a reduced hemoglobin concentration. It has also been demonstrated that the feeding of pituitary extracts has produced erythropoietic stimulation in the normal animal (9). One must remember, however, that a hypophysectomized animal is not a normal animal. A marked change in the normal physiology of the animal has occurred. The hypophysectomized animal is also capable of erythroid response to lowered barometric pressures and hemorrhage.

The gonads, the thyroid, and the adrenals have all been shown to exert some effect on the red counts of animals. However, the magnitude of these changes is not sufficient to consider them as the primary site of erythropoietic stimulation.

The nervous system has been considered mainly by European hematologists. They have considered the possibility of neural centers controlling erythropoiesis. This theory is quite plausible and some evidence has been presented which further strengthens this concept. However, it is difficult to devise controlled studies on the central nervous system. The studies of induced lesions in the vegetative center are also difficult to

evaluate, because of the proximity to the pituitary.

Other theories have been advanced, which deal with splenic hormones and destruction products of erythrocytes, as causative factors in accelerated erythropoiesis. These studies are quite controversial and not conclusive. Grant and Root consider the theories attractive, but lacking in evidence (9).

HUMORAL THEORY

The most widely accepted theory of the primary stimulus for erythropoiesis has been the concept of bone marrow anoxia. An opposing view favors the existence of an erythropoietic stimulating substance in the circulating blood. The first proponents of this theory were Carnot and De Flandre. In 1906, they suggested that the blood oxygenation regulates red cell production by means of an intermediary factor (20). According to this theory, arterial blood oxygen regulates the production of a factor capable of stimulating erythropoiesis. This factor is carried to the bone marrow by the blood. In testing this theory, Carnot and De Flandre injected 9 milliliters of plasma, obtained from slightly anemic rabbits, into normal rabbits. They noticed a small rise in the number of red blood cells in the

peripheral blood. However, they noted that plasma from severely anemic rabbits had no effect on the red count. Carnot introduced the term 'hemopoietine' to designate the unknown stimulating substance in the serum.

The original observation stimulated a long series of investigations (21,22) following this general pattern. An attempt was made to prove the existence of the factor. From 0.5 to 10 milliliters of plasma, obtained from anemic rabbits or rabbits maintained at low barometric pressure, were injected into test animals. The injections were followed by moderate or marked increases in the red cell counts. These studies, in general, were conflicting and unconvincing, but were interpreted as being in support of a humoral theory.

In the early 1930's, Gordon and Dubin (23) repeated the procedures, using the reticulocyte index. This method of interpretation was not available to the earlier investigators. They were unable to demonstrate any change in the number of erythrocytes or in the reticulocyte percentages.

In 1948, Bonsdorff and Jalavista (24) revived the humoral theory. They reported that 3 milliliters of plasma, from patients with congestive failure

or from rabbits exposed to low barometric pressure, produced a slight increase in red counts when injected into normal rabbits.

In 1950, Reissman (25) pointed out that the use of very small amounts of plasma to demonstrate the existence of a plasma factor, capable of stimulating red cell production, is almost certain to be unsuccessful. He used parabiotic rats as a more adequate approach. Parabiotic rats were conditioned for five weeks in a specially constructed breathing chamber. Defined gas mixtures were utilized in such a way that one rat was breathing an oxygen nitrogen mixture. The oxygen content of this mixture was 8 volumes per cent. The rat's partner was breathing normal air. Both partners developed normoblastic hyperplasia of the bone marrow. Blood gas studies were performed on several pairs. These studies revealed that the arterial oxygen tension corresponded to the air each partner was breathing. There was apparently no influence exerted by the blood mixing through the site of parabiosis.

Another approach was described in 1952 by Grant, who utilized the anoxic lactating rat and mouse (26). Mothers were placed in low pressure

chambers for 6 hours a day, while their litters remained at sea level pressure. After a week or two of this routine, it was noted that the circulating red cell concentration and hemoglobin levels were higher in the litters nursed by these mothers, than in a control group maintained at sea level.

In more recent years, there has been an increased number of articles in which the nature of this plasma substance has been described. Some studies regarding its location of formation are presently appearing in the literature.

Although the exact nature of the erythropoietic factor found in the plasma of anemic animals and man is not known, many investigators have attempted to determine its characteristics. Their reports, in general, are inconclusive and conflicting, but some light has been cast on the substance.

Erslev and Lavietes (27) have carried out experiments aimed at obtaining information concerning the biochemical and biologic nature of the erythropoietic factor. They used various fractionation procedures and also evaluated the effect of nitrogen mustard on its production. It is their conclusion that the factor is not produced in nitrogen

mustard sensitive tissues. They further maintain that the factor behaves like a serum albumin, alpha globulin, or beta globulin.

Borsook and his co-workers (28) have reported the presence of an erythrocyte stimulating factor in a non-protein plasma extract, obtained from anemic rabbits and assayed in normal rats. Gordon, et al., (29) have also demonstrated an erythropoietic factor in non-protein plasma extracts.

Hodgson and Toha (30) have stated that anemic rabbit plasma loses its erythropoietic effect after boiling at a pH of 5.5 for 15 minutes, but that the active factor can be extracted from the heat coagulated plasma proteins by either water or acetone (31). They have also reported that the active factor in anemic plasma is carried down with both the globulin and albumin fractions, after the addition of ammonium sulfate. Their preliminary studies suggest that it may be a fatty acid ester, and not an amino acid, purine or sugar.

Linman and Bethell (32) in their preliminary investigations, maintained that the factor was not destroyed by prolonged boiling and was not precipitated by perchloric acid. They suspected that the factor was a non-protein and suggested it

to be a lipid. In their subsequent follow-up studies (33), they contend that it is stable over wide ranges of temperature and is acid soluable. In their studies of the effect of irradiation on the production of the factor, they have found that plasma, obtained 24 hours post-total body irradiation, showed evidence of the factor. This was manifested by erythrocytosis and reticulocytosis. The plasma was obtained from rabbits, made anemic with phenylhydrazine, following the total body irradiation. There was evidence of a delayed response, as compared with a non-irradiated control group. It was also demonstrated that rabbits, rendered anemic by irradiation alone, were also able to produce an erythrocyte stimulating factor, but in decreased amounts.

Rambach and Alt (34) indicate that increased erythropoietic activity is associated with a protein having electrophoretic characteristics of an alpha-2-globulin. It is not precipitated by perchloric acid or by boiling. They maintain it to be heat stable and say that it stains for carbohydrate and nitrogen, but not for fat. It is not dialyzable and is precipitated by a 75% saturated solution of ammonium sulfate. They suggest

it may be a mucoprotein.

In an attempt to ascertain some of the properties of the factor, Slaunwhite and his co-workers (35) have treated plasma, obtained from anemic rabbits, in numerous ways. Assays were performed by determining the radioactive iron (Fe^{59}) uptake in hypophysectomized rats. They report that the factor was not destroyed by heating at a pH of 5.5 nor at a pH of 9, but was destroyed by heat at a pH of 1 and at a pH of 13. They further contend that it is non-dialyzable and is digested by pepsin, trypsin and chymotrypsin. It was also noted to be fairly stable in the presence of non-precipitable proteins of plasma to oxidation and reduction. Their conclusion is that the factor is probably a polypeptide. On the basis of their investigations, they state that the possibilities of the factor being a polysaccharide, glycoprotein, or lipoprotein cannot be ruled out.

It can be seen by the foregoing discussion, that the exact nature of the erythropoietic factor, is subject to debate. Some reference has already been made to the probable site of formation of the factor. Erslev and Lavietes (27) and Linman and Bethell (33) have shown the effect of irradiation

and nitrogen mustard on its production.

Previous attempts to determine the site of formation of the erythropoietic factor, by surgical removal of organs (38) and by organ extracts (39) have been unsuccessful. Jacobson and his group have been able to study the problem more thoroughly. They have had the advantage of more sensitive assay methods. The fact that a single dose of cobaltous chloride or an acute massive hemorrhage will elevate the plasma erythropoietin level in 10-12 hours (40), has also facilitated their investigations.

Rats, which have been subjected to hypophysectomy, thyroidectomy, splenectomy, adrenalectomy, and gonadectomy, retain the capacity to respond to repeated phlebotomy, with an increase in plasma content of erythropoietin, which is comparable to that observed in similarly bled normal animals (38). In addition, removal of seven-eighths of the liver, or removal of the adrenals, spleen, pancreas, stomach, or intestines, does not lessen the response to a single injection of cobalt (36).

After bilateral nephrectomy, neither rats nor rabbits have the capacity to respond to a single dose of cobaltous chloride or a single massive

hemorrhage by an elevation of erythropoietin. Plasma, obtained from nephrectomized rats withdrawn 10-12 hours after the injection of cobaltous chloride or bleeding, contains no erythropoietic activity as measured by the incorporation of radioactive iron (Fe^{59}) into the erythrocytes of starved or hypophysectomized rats.

In order to obtain a uremia at least equal to that which occurs 12 hours after nephrectomy, Jacobson and his group subjected animals to ligation of the ureters and waited 12 hours before giving the cobalt or bleeding the animals. In 12 hours, they collected the blood and assayed the plasma for the erythropoietin level. In this group, the plasma erythropoietin level was only slightly less than that obtained from similar assays from non-nephrectomized anemic plasma. The blood urea nitrogen of the nephrectomized rats reaches levels of 70-90 milligrams per cent, as compared to normal values of 21-22 milligrams per cent in controls. The rats, subjected to ureteral ligation, reached levels of 125-150 milligrams per cent in 24 hours.

Mirand and Prentice (41) have attempted to duplicate the results obtained by Jacobson and his group, by subjecting nephrectomized rats to hypoxic

hypoxia. Their studies indicate that erythropoietin production continues in the absence of the kidneys and-or the spleen.

The mode of action of this humoral substance, present in anemic plasma of animals and man, is not known. Whether the factor affects the erythropoietic system by increasing cell division, increasing the rate of maturation, or by increasing the rate of delivery from the hematopoietic centers, has not been defined. Some recent work (34) along this line has added some insight into its mode of action. By the combined use of plasma extracts from anemic rabbits and radioactive iron, an increase in the rate of delivery from the bone marrow is reflected by more rapid disappearance of radioactive iron, with increased levels noted in the peripheral red cells. The marrow life was shortened by about fifty per cent. This observation is interpreted as an indication of an increase in the rate of intra-marrow maturation.

One of the most commonly used methods of producing anemia in test animals is the administration of phenylhydrazine. Some investigators have noted that the plasma extracts obtained from these animals have a more potent erythrocytogenic property

than that obtained from bled animals (28,29). This finding has stimulated investigations aimed at determining the cause for the discrepancy. E. M. Jacobson and his collaborators (44) compared hemorrhage and phenylhydrazine with regard to the relative effectiveness of each in the production of the erythropoietic factor. They noted that, although the hematocrits in the two groups were equal, the oxygen carrying capacity of the blood of the phenylhydrazine group was significantly lower. They also observed that the phenylhydrazine group had methemoglobin concentrations of about 24%, while the corresponding level in the bled animals was essentially zero. They found that the most highly active plasma preparations were obtained from animals in the phenylhydrazine group, which had severely damaged livers. They postulate that the liver may act as a site of destruction of the erythropoietic factor.

Mirand and Prentice (45) have further expanded on this hypothesis. They noted that normal rats, placed in low oxygen atmospheres, did not elaborate in their plasma, a factor capable of stimulating increased incorporation of radioactive iron into red cells of normal or hypophysectomized rats.

However, when the livers of these test animals were first severely damaged by carbon tetrachloride, a significant level of the factor was demonstrable when the animals were maintained in an hypoxic atmosphere. This group maintains that the liver has a significant control of erythrocyte response under normal conditions.

In contrast to the results obtained by the preceding authors, Stohlman and Brecher (46), in their studies, could not establish a relationship between the activity of plasma extracts and coexistent liver disease. Their test animals were exposed to low oxygen tensions and phenylhydrazine.

Studies in man, with regard to the humoral theory of erythropoiesis control, are relatively few in number. Schmid and Gilbertsen (47) had the opportunity to study a case of patent ductus arteriosus with a reversed blood flow. This patient had a marked polycythemia. The shunt from the pulmonary system into the systemic circulation occurred distal to the origin of the left subclavian artery. The upper parts of the body were fully oxygenated, whereas the lower portions were only partially saturated. Bone marrow studies from the iliac and sternal areas were obtained, as well as

the bone marrow oxygen saturation. Both marrows showed a marked normoblastic hyperplasia. The oxygen saturations were markedly different. The iliac bone marrow was 53% saturated, while the sternum was 91% saturated. They feel that this evidence is contributory to the humoral theory of erythropoiesis.

A similar observation was made by Stohlman et al., on a patient with a polycythemia, secondary to a patent ductus arteriosus with reverse flow (48). They contend that the presence of polycythemia under such circumstances excludes a primary cerebral or pituitary mechanism. Similarly, the presence of hypercellularity and normoblastic hyperplasia in normal oxygenated areas of bone marrow, argue against marrow anoxia as a primary mechanism.

Other studies in man have dealt with the demonstration of the erythropoietic factor in anemic patients. Piliero and his group (49) have studied the filtrates of plasma, obtained from patients with Cooley's anemia and sickle cell anemia. These extracts stimulated erythropoiesis in rats, as evidenced by red blood counts, hemoglobin concentrations, hematocrits, reticulocyte percentage and

marrow nucleated red blood cells. A similarly prepared extract of urine from one patient with Cooley's anemia was also active. Plasma extracts, from normal subjects and extracts from one case of hypoplastic anemia, were inactive.

Prentice and Mirand (50) have duplicated these results with regard to Cooley's anemia and sickle cell anemia. They also noted that plasma extracts, from individuals with polycythemia vera and secondary polycythemia, were active. They have also failed to demonstrate significant levels of the factor in hypoplastic anemia.

SUMMARY

The erythrocyte, from its bone marrow origin, has a life cycle of 110-130 days, under normal conditions. Production and destruction of the erythrocyte is normally in a state of dynamic equilibrium, permitting very little alteration in the total red cell concentration or total mass. Any alteration in this total mass or concentration of the red cell evokes a response on the part of the organism to re-establish the equilibrium.

Jacobson and his group believe that this

dynamic equilibrium of the erythron is controlled by the relationship of the oxygen supply in the tissues, to the demand for oxygen, rather than by either one alone.

Certain changes in the environment of man and animals have long been known to produce an accelerated rate of erythropoiesis. The common denominator of this group is a relative state of anoxia. Conditions of increased altitude, carbon monoxide poisoning, pathological alterations in the circulatory or respiratory systems, and hemorrhage have been shown to increase the rate of erythropoiesis. The administration of cobalt also exerts an acceleratory effect on the production of red cells. The mechanism of action here is not clearly defined. Recent investigations link cobalt administration with erythropoietin production.

A decreased rate of erythropoiesis has been demonstrated after repeated transfusions of whole blood. The inspiration of oxygen-rich gases for variable times has also been shown to decrease red cell production. This decrease is not as marked, however, as the one seen in transfusion polycythemia.

A number of theories have been advanced as to the primary mechanism of control in erythropoiesis.

Marrow anoxia is the most widely accepted theory. Recent studies indicate that the evidence in support of this theory is primarily indirect in nature. Other theories advanced include: carbon dioxide tension, endocrine control, nervous system, splenic hormones and breakdown products of erythrocytes, as causative factors in erythropoiesis control. The majority of these theories have some evidence in their favor, but they remain controversial and inconclusive.

The humoral theory of erythropoiesis control was first advanced in 1906 by Carnot and De Flandre. They suggested that blood oxygenation regulated red cell production by means of an intermediary factor. This factor was thought to be carried to the bone marrow via the blood. They cited the erythropoietic effect of anemic plasma, injected into normal rabbits, as evidence for their theory.

In the succeeding years, a number of investigators have tried to demonstrate a factor in anemic plasma, capable of accelerating erythropoiesis. These studies have added some credence to the theory, and have demonstrated the existence of a substance in anemic plasma, capable of stimulating erythropoiesis.

There have been an increasing number of investigations in which an attempt is made to determine the characteristics of the factor. These reports are, in general, inconclusive and conflicting, but some information has been gained. The stimulatory substance apparently is heat stable, non-dialyzable, acid soluable, and not digested by pepsin, trypsin, or chymotrypsin. It has also been described as fairly stable, in the presence of non-precipitable plasma proteins, to oxidation and reduction. It must be remembered, however, that any one of the foregoing characteristics is subject to some debate.

Early attempts to localize the site of formation of the humoral factor by surgical removal of organs and organ extracts have been unsuccessful. More recent work by Jacobson and his group seems to implicate the kidney as the site of erythropoietin production. This work has not been reproduced by other investigators. The factor is apparently not produced in nitrogen mustard or radiation sensitive tissues.

The mode of action of erythropoietin has not been defined completely. Some preliminary investigations seem to indicate both an increase in intramarrow maturation, as well as an increased rate

of delivery from the marrow.

The liver has been postulated by several investigators as a possible site of erythropoietin destruction. More active plasma extracts have been obtained from animals in which liver destruction has occurred.

Studies in man, with regard to the factor, are limited. It has been demonstrated, however, that plasma extracts of patients suffering from Cooley's anemia, sickle cell anemia, polycythemia vera, and secondary polycythemia are more active than normal plasma. There is no apparent increase of the factor in the plasma of patients with hypoplastic anemia.

CONCLUSIONS

The humoral theory of erythropoiesis control is probably the best working hypothesis advanced at this time. The existence of a substance, in the plasma of anemic animals and man, capable of stimulating erythropoiesis, has been fairly well documented.

The possibility exists, however, that there is more than one mechanism involved in erythropoiesis.

It is also within the realm of possibility that there is more than one substance circulating in the blood, capable of stimulating red cell production. The present dilemma, with regard to the nature, action, site of formation, and manner of destruction, may be resolved by the demonstration of more than one stimulating substance.

The characteristics of this substance, the so-called erythropoietin, are subject to so many conflicting opinions that no definite conclusions can be drawn. Further biochemical and physiological investigations are indicated.

The usefulness of this substance as a clinically applicable drug is subject to much speculation. It may, in the future, be useful in states of impaired red cell formation. Such conditions, as hypoplastic and aplastic anemias, may be improved by its administration. It may also prove to be useful in the refractory anemias of chronic infection, chronic renal disease, and malignancy. Clinical application of the factor is merely speculation and only actual trial therapy will establish its usefulness.

ACKNOWLEDGMENT

I would like to extend my thanks to my advisor, E. Stanley Pederson, M.D., who gave so freely of his time and advice in the preparation of my thesis.

BIBLIOGRAPHY

1. Boycott, A. E.: The Blood as a Tissue: Hypertrophy and Atrophy of the Red Corpuscles. Proc. Roy. Soc. Med. 23:15, 1929.

2. Gordon, A. S.: Red Cell and Reticulocyte Counts in Guinea Pigs Following Exposure to Low Pressure. Proc. Soc. Exper. Biol. & Med. 37:507, 1937.

3. Hurtado, A. C., Merino, C., and Delgado, E.: Influence of Anoxemia on the Hemopoietic Activity. Arch. Int. Med. 75:284, 1945.

4. Williams, I. R., and Smith, E.: Blood Picture, Reproduction, and General Conditions During Daily Exposure to Illuminating Gas. Am. J. Physiol. 110:611, 1935.

5. Sievers, R. F., Edwards, T. I., Murray, A. L., and Schrenk, H. H.: Effect of Exposure to Known Concentrations of Carbon Monoxide. J. A. M. A. 118:585-588, 1942.

6. Robscheit-Robins, F. S., and Whipple, G. H.: Hemoglobin Production Increases with Severity of Anemia. Am. J. Physiol. 134:263, 1941.

7. Gordon, A. S.: Quantitative Nature of the Red Cell Response to a Single Bleeding. Proc. Soc. Exper. Biol. & Med. 31:563, 1934.

8. Steele, B. F.: The Effects of Blood Loss and Blood Destruction upon the Erythroid Cells in the Bone Marrow of Rabbits. J. Exper. Med. 57:881, 1933.

9. Grant, W. C., and Root, W. S.: Fundamental Stimulus for Erythropoiesis. Physiol. Rev. 32:449, 1952.

10. Schultze, M. O.: Metallic Elements and Blood Formation. Physiol. Rev. 20:37, 1940.

11. Barron, A. G., and Barron, E. S. G.: Mechanism of Cobalt Polycythemia, Effect of Ascorbic Acid. Proc. Soc. Exper. Biol. & Med. 35:407, 1936.

12. Goldwasser, C., Jacobson, L. O., Freid, W., and Plzak, L.: The Mechanism of the Erythropoietic Effect of Cobalt. Science 125:1085, 1957.

13. Goldwasser, C., Jacobson, L. O., Freid, W., and Plzak, L.: Studies on Erythropoiesis Part V. The Effect of Cobalt on the Production of Erythropoietin. Blood 13:55, 1958.

14. Elmlinger, P. J., Huff, R. L., and Oda, J. M.: Depression Red Cell Iron Turnover by Transfusion. Proc. Soc. Exper. Biol. & Med. 79: 16, 1952.

15. Vogel, K. M., and McCurdy, V. F.: Blood Transfusion and Regeneration in Pernicious Anemia. Arch. Int. Med. 12:707, 1913.

16. Barcroft, J., Hunt, G. H., and Dufton, D.: The Treatment of Chronic Cases of Gas Poisoning by Continuous Oxygen Administration in Chambers. Quart. J. Med. 13:179, 1920.

17. Miescher, F.: Ueber die Beziehungen zwischen Meereshoehe und Beschaffenheit des Blutes. Kor. Bl. f. schweiz. Aertze. 23:809, 1893.

18. Grant, W. C., and Root, W. S.: The Relation of O_2 in Bone Marrow Blood to Post-Hemorrhagic Erythropoiesis. Am. J. Physiol. 150:618, 1947.

19. Jordon, H. E., and Speidel, C. C.: The Fundamental Erythrocytopoietic Stimulus. Proc. Soc. Exper. Biol. & Med. 21:399, 1924.

20. Carnot, P., and De Flandre, C.: Sur l'activite' hemopoietique du serum au cours de le regeneration du sang. Compt. rend. Acad. d. sc. 143:384, 1906.

21. Gibelli, C.: Uber den Wert des Serum anamich gemachten Tiere bey der Regenerations des Blutes. Arch. f. Exper. Path. u. Pharmakol. 65:284, 1911.

22. Sandor, G.: Uber die Blutbildene Wirkung des Serum von Tieren, die in verdunnter Luft gehalten wurden. Ztschr. f. d. ges. exper. Med. 82:633, 1932.

23. Gordon, A. S., and Dubin, M.: On the Alleged Presence of "Hemopoietine" in the Blood Serum of Rabbits either Rendered Anemic or Subjected to Low Pressures. Am. J. Physiol. 107:704, 1934.

24. Bonsdorff, E., and Jalavisto, E.: A Humoral Mechanism in Anoxic Erythrocytosis. Acta. Physiol. Scandinav. 16:150, 1948.

25. Reissmann, K. R.: Studies on the Mechanism of Erythropoietic Stimulation in Parabiotic Rats During Hypoxia. Blood 5:372, 1950.

26. Grant, W. C.: Influence of Anoxia of a Lactating Rat on the Blood of Normal Baby Rats. Am. J. Physiol. 171:728, 1952.

27. Erslev, A. J., and Lavietes, P. H.: Observations on the Nature of the Erythropoietic Serum Factor. Blood 9:1055, 1954.

28. Borsook, H., Graybiel, A., Keighley, G., and Windsor, E.: Polycythemic Response in Normal Adult Rats to a Non-Protein Plasma Extract from Anemic Rabbits. Blood 9:734, 1954.

29. Gordon, A. S., Piliero, S. J., Kleinberg, W., and Friedman, H. H.: A Plasma Extract with Erythropoietic Activity. Proc. Soc. Exper. Biol. & Med. 86:255, 1954.

30. Hodgson, G. and Toha, J.: The Erythropoietic Effect of Urine and Plasma of Repeatedly Bled Rabbits. Blood 9:299, 1954.

31. Toha, J., Eskuche, I., Abarca, F., Salvatore, F., and Hodgson, G.: Chemical Properties of a Plasma Factor Accelerating Hemoglobin Recovery in Bled Rabbits. Nature 175:167, 1955.

32. Linman, J. W., and Bethell, F. H.: Observations on Circulating Erythrocytes and Bone Marrow of Rats Receiving Protein Free Extracts of Rabbit Plasma. Blood 11:310, 1956.

33. Linman, J. W. and Bethell, F. H.: The Effect of Irradiation on the Plasma Erythropoietic Stimulating Factor. Blood 12:123, 1957.

34. Rambach, W. A., Alt, H. L., and Cooper, J. D.: The Mode of Action and Nature of Heat Stable Plasma Erythropoietic Factor. Blood 12:1101, 1957.

35. Slaunwhite, W. R. Jr., Mirand, E. A. and Prentice, T. C.: Probable Polypeptide Nature of Erythropoietin. Proc. Soc. Exper. Biol. & Med. 96:616, 1957.

36. Jacobson, L. O., Goldwasser, E., Fried, W., and Plzak, L.: Role of the Kidney in Erythropoiesis. Nature 179:633, 1957.

37. Jacobson, L. O., Plzak, L., Fried, W., and Goldwasser, E.: Plasma Factor(s) Influencing Red Cell Production. Nature 177:1240, 1956.

38. Fried, W., Plzak, L., Jacobson, L. O., and Goldwasser, E.: Erythropoiesis II. Assay of Erythropoietin in Hypophysectomized Rats. Proc. Soc. Exper. Biol. & Med. 92:203, 1956.

39. Piliero, S. J., Medici, P. T., Pausky, B., Luhby, A. L., and Gordon, A. S.: Erythropoietic Stimulating Effects of Plasma Extracts from Anemic Human Subjects. Proc. Soc. Exper. Biol. & Med. 93:302, 1956.

40. Goldwasser, E., Jacobson, L. O., Plzak, L., and Fried, W.: Mechanism of the Erythropoietic Effect of Cobalt. Science 125:1085, 1957.

41. Mirand, E. A. and Prentice, T. C.: Presence of Plasma Erythropoietin in Hypoxic Rats with or without Kidney(s) and-or Spleen. Proc. Soc. Exper. Biol. & Med. 96:49, 1957.

42. Rous, P.: Destruction of the Red Blood Cells. Physiol. Rev. 3:75, 1923.

43. Wintrobe, M. M.: Clinical Hematology, Philadelphia, Lea & Febiger, 1956. p.155.

44. Jacobsen, E. M., Davis, A. K., and Alpen, E. L.: Relative Effectiveness of Phenylhydrazine Treatment and Hemorrhage in the Production of an Erythropoietic Factor. Blood 11:937, 1956.

45. Prentice, T. C. and Mirand, E. A.: Effect of Acute Liver Damage plus Hypoxia on Plasma Erythropoietin Content. Proc. Soc. Exper. Biol. & Med. 95:231, 1957.

46. Stohlman, F. Jr. and Brecher, G.: Humoral Regulation of Erythropoiesis IV. Relative Heat Stability of Erythropoietin. Proc. Soc. Exper. Biol. & Med. 95:797, 1957.

47. Schmid, R. and Gilbertsen, A. S.: Fundamental Observations on the Production of Compensatory Polycythemia in a Case of Patent Ductus Arteriosus with Reversed Blood Flow. Blood 10:247, 1955.

48. Stohlman, F. Jr., Rath, C. E. and Rose, J. C.: Evidence for a Humoral Regulation of Erythropoiesis, Studies on a Patient with Polycythemia, Secondary to Regional Hypoxia. Blood 9: 721, 1954.

49. Piliero, S. J., Medici, P. T., Pansky, B., Luhby, A. L. and Gordon, A. S.: Erythropoietic Stimulating Effects of Plasma Extracts from Anemic Patients. Proc. Soc. Exper. Biol. & Med. 93:302, 1956.

50. Prentice, T. C. and Mirand, E. A.: Studies of Plasma Erythropoietic Factor in Anemic Human Patients. Blood 12:993, 1957.