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203

The Mode of Action and Biologic Importance of the Plasma Factor Stimulating Erythroblastic Mitoses

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Carnot's observation [4] according to which the serum of bled animals stimulates erythropoiesis when injected to a normal animal, and the consequent implication that an erythropoietic factor (erythropoietin) appears in plasma of normal animals after bleeding, were the starting points for the study of humoral factors controlling erythropoiesis.

For a long time *Carnot*'s discovery was underestimated, having been submitted to a too precocious clinical application; but, later on, the physiological, chemical and clinical problems on *erythropoietin* became the object of the most careful and extensive research.

From a physiological standpoint, a most important fact was pointed out by demonstrating that not only the bleeding but all conditions lowering blood pO_2 too, were effective stimuli for erythropoiesis (table I). The pO_2 decrease must not

Table I

Blood p(O_2) Reduction

– anemia	– hypoxia
– bleeding	– low external p (O_2)
– myeloid aplasia	– $CoCl_2$
	– high tissue O_2 utilization (thyroxine)

high erythropoietic plasma activity

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necessarily be severe: any lowering of blood pO_2 beneath the individual's need seems to stimulate physiologically the production of erythropoietic factors[8].

In all likelihood, the plasma erythropoietic factor is the humoral agent normally devoted to the stimulation of medullary erythropoiesis.

The statement that the erythropoietic factor is a distinct chemical substance lies both on the demonstration that this factor passes into the milk and urine of bled animals[10,11,24,26], and on *Reissmann*'s finding according to which the barometric depression in an animal being in a parabiotic state causes an erythropoietic marrow stimulation also in the un-decompressed partner[22].

A great deal of speculation has been made about the question whether there are many erythropoietic factors or a single one. The bulk of evidence seems to support the former hypothesis; each erythropoietic factor appearing in plasma after bleeding would be then characterized by a specific mode of action. We refer to:

a) a factor devoted to the stimulation of erythroblastic reproduction (erythroblastic mitosis stimulating factor)[14,15];

b) a factor stimulating hemo-synthesis[2,3,19];

c) a factor stimulating iron uptake by the erythroblasts. Definite evidence about the reliability of such a statement is still lacking, notwithstanding *Austoni*'s observation[1] of a more active Fe^{59} incorporation into the erythroblasts of bled animals, in comparison with the normal ones;

d) a reticulocyte ripening factor[20], causing an earlier disappearance of the brilliant cresyl blue reaction in the erythrocytes.

In this paper, only the plasma factor stimulating erythroblastic mitosis will be considered.

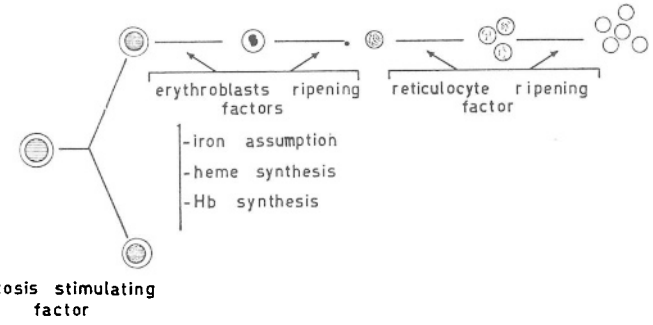


Fig. 1. Possible type of action of various factors influencing erythropoiesis.

The methods devised to assay the plasma-erythropoietic factor may be classified in two main groups:

Indirect methods: reticulocyte and red blood cell counts; hematocrit appraisal; evaluation of Fe^{59} incorporation rate into red cell and of the dilution of Cr^{51}-tagged erythrocytes.

Direct methods: histologic bone marrow examination; absolute count of erythro-

blasts in the bone marrow; bone marrow hematocrit; erythroblastic mitosis arrest at metaphase caused by colchicine.

Nearly no information can be afforded by indirect methods about the mode of action of the plasma erythropoietic factors. Such indirect methods provide many valuable data about peripheral erythrocyte changes, without specifying, however, through which mechanism these changes took place.

Among the direct methods, a simple one is, in our opinion, the method proposed by us after *Dustin*, *Astaldi* and coll., based on the property of colchicine to arrest mitoses at metaphase without inhibiting their onset. In this way, we were enabled to estimate the reproductive activity of any tissue, through a counting of the percentage of mitoses arrested by the alkaloid.

Since hypophysectomy brings about a striking decrease in the erythroblastic mitotic rate in bone marrow[17], the hypophysectomized animal, treated with colchicine, has been chosen as the test animal to assay the erythropoietic activity of various substances.

According to this test, the erythropoietic activity of a substance is expressed by the increase of erythroblastic mitosis percentage in the bone marrow of a rat after intraperitoneal injection of 2 ml of the material in assay. The animal must be hypophysectomized since ten days, and 1 mg/kg of colchicine must be injected intraperitoneally 25 hours after the injection of the substance. The injected rats are killed 9 hours after administration of colchicine (fig. 2).

By this method, not only the approximate measurement of the erythropoietic activity of various sera could be obtained, but it could also be shown that a marked stimulation of erythroblastic reproductive activity is one of the paramount actions

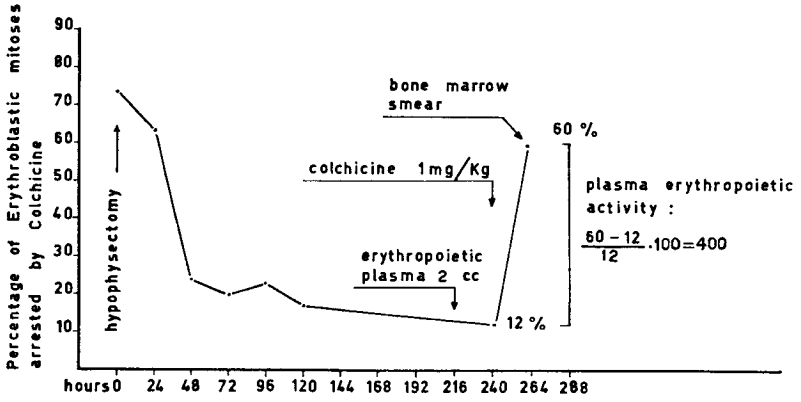


Fig. 2. Quantitative direct evaluation of plasma erythropoietic activity. Ten days after hypophysectomy 2 cc of erythropoietically active plasma are injected intraperitoneally in a rat. A day after the injection, 1 mg/kg of colchicine is introduced in the peritoneum. Nine hours after the colchicine injection, the animal is sacrificed and its femoral bone marrow examined. The percentage of erythroblastic mitosis is referred to the percentage of mitoses in hypophysectomized control rats as in the formula.

of anemic sera. This is the first time, as far as we know, that the existence of a factor acting as a stimulus on erythroblastic mitosis is demonstrated.

The problem of the influence of endocrine glands upon the formation of the plasma factor stimulating erythroblastic mitosis is a difficult and important one. No endocrine gland seems to be really essential for the increase of erythroblastic reproductive activity in bled animals, but the hypophysis, more than the other glands, seems to be involved in this process.

These facts have been variously interpreted. A group of investigators from Berkeley, Calif. has suggested that the hypophysis secretes directly a powerful hormone able to stimulate normal erythropoiesis, *the pituitary erythropoietic hormone*[6, 25]. This assumption is not supported by the finding that even in hypophysectomized animals a slight reticulocyte reaction may arise, provided severe hypo-oxemia be induced[7].

Some other workers, therefore, state that the pituitary gland does not produce any erythropoietic hormone. The hypophysis should merely exert a control on the oxygen needs of the body; the changes in red blood cell mass should be closely related to these needs[8].

We have found that the hypophysectomized animals react to the bleeding with a later and lower erythropoietic factor production than the normal ones (fig. 3).

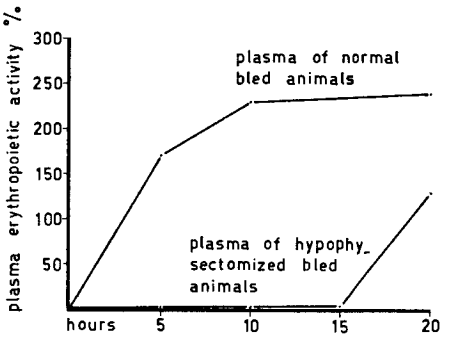


Fig. 3. The injection of plasma of repeatedly bled rats showing a very active bone marrow and a high reticulocytosis in the blood, promotes erythroblastic mitosis in the recipient 5–10 hours after the injection. Plasma of hypophysectomized rats repeatedly bled as the normals acts much more feebly and later.

Perhaps the hypophysis does not produce by itself the erythropoietic plasma factor; it is possible, however, that the pituitary affects its formation, influencing in a hitherto unknown way its synthesis.

Little information is available about chemical and physicochemical properties of the plasma erythropoietic factor. According to *Slaunwhite*[23], such a factor could be a low molecular weight polypeptide; it has also been suggested that it could be a low molecular weight acidic glycoprotein[21]: its metachromatic properties are supposed to lie on such a structure. The assumption that the erythropoietic factor is a fatty

substance is not accepted, although the hypothesis that it may be a low molecular weight lipo-protein, cannot be rejected. *Gley*'s hypothesis on its steroid nature[9] seems to be less acceptable to-day.

Particular interest has been directed towards study of the erythropoietic plasma factor in blood-donors. Our findings[16] can be summarized as follows:

a) A weak erythropoiesis stimulating activity arises in the plasma of a normal individual, 48 hours after a blood-letting (300–400 ml); such activity will gradually disappear within one week. Ten days after the blood-letting, no erythropoiesis stimulating activity can be detected (fig. 4).

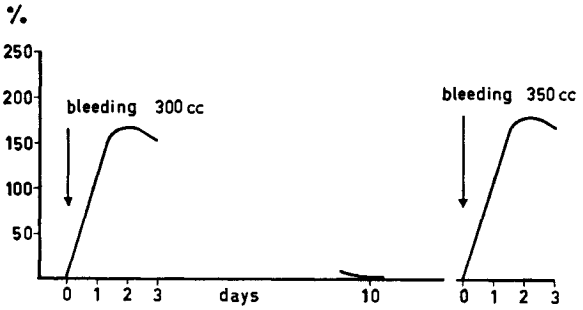


Fig. 4. Serum erythropoietic activity of a blood donor after two successive blood donations.

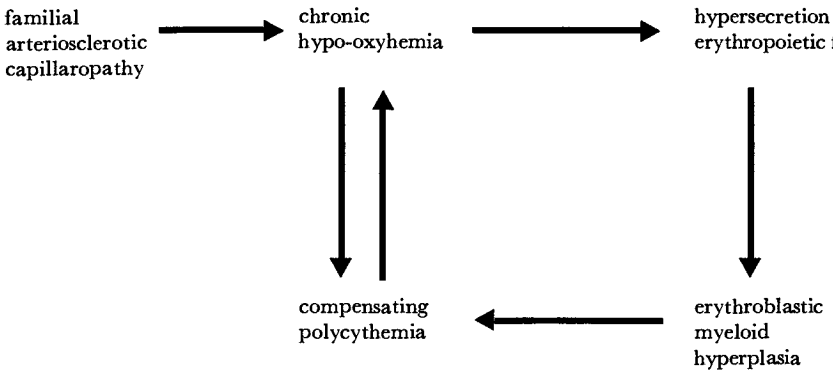
b) It has been possible to concentrate by lyophilization the donor's erythropoietic active plasma. In this way, we have obtained a donor's plasma with an activity slightly lower than that induced in an animal after a blood letting equivalent to 2 % of its body weight.

From a clinical standpoint, the assay of the plasma erythropoiesis stimulating activity may provide a new approach to the pathogenetic appraisal of some blood disorders. In the plasma of many polycythemic individuals a very high erythropoiesis stimulating activity is occasionally detectable; it has been suggested the clinical features of polycythemia be related to such a biological condition[5, 12]. We have had the opportunity of studying a family of polycythemic individuals whose plasma showed high amounts of erythroblastic mitosis stimulating factor. In this family the disease, as we have suggested[15], could be related to a widespread atherosclerotic constitutional capillary damage with hypo-oxemia resulting in a rise of plasma erythropoietic factor level (table II).

In individuals with thalassemia the plasma erythropoiesis stimulating activity is highly increased in comparison with the other types of anaemia as suggested by *Medici* and co-workers[18] and by us. In the plasma of both heterozygous and homozygous subjects (rsp. Cooley's trait carriers and Cooley's anaemia patients), the erythropoiesis stimulating activity is markedly increased. The biological condition of the "trait" carriers is, however, different from that of the Cooley's anaemia patients. *Marinone*[13] has shown that the rise of the erythropoiesis stimulating activity in the plasma of a "trait" carrier is chiefly related to the slight concen-

Table II

Possible Physiopathological Mechanism of Polyglobulia in a Case of Familial Generalized Angiopathy with Polycythemia



tration of hemoglobin in his red cells. The blood pO_2 in these patients is very low, indeed, owing to a congenital defect in the synthesis of hemoglobin; the erythrocyte hypochromia is severe and cannot be corrected. This condition leads to an increase of erythropoietic plasma factor and, consequently, to a stimulation of marrow reproductive activity. Consecutive polyglobulia counterbalance the congenital decrease in hemoglobin content of red blood cells (fig. 5).

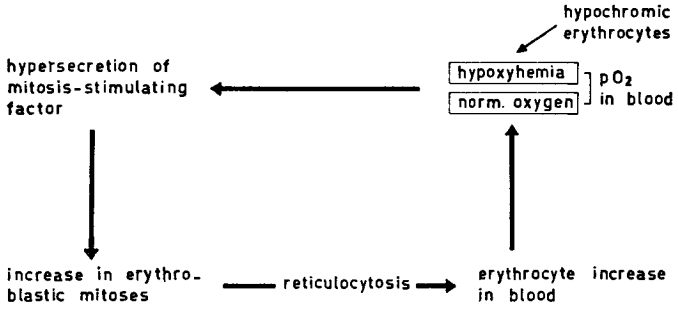


Fig. 5. Possible physiopathological explication of the bone marrow erythroblastic hyperplasia and of the slight peripheral polyglobulia in some cases of thalassemia minima.

On the contrary, the erythropoiesis stimulating activity in the Cooley's patient's plasma is enhanced, above all, by the severe red cells destruction. When Cooley's anaemia is completely corrected by repeated blood transfusions, the erythropoietic plasma factor gets a lower level and the erythroblastic quota in bone marrow is markedly depressed[18] (fig. 6).

The assay of the erythropoietic plasma factor is also very profitable in the study of aplastic anaemias. These diseases may be grouped into two classes, according to the erythropoietic factor content in plasma:

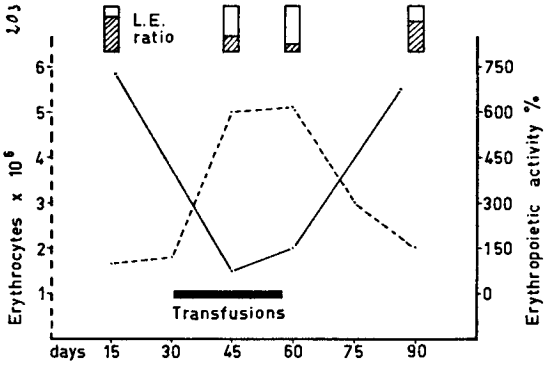


Fig. 6. Erythrocytes level and plasma erythropoietic activity in Cooley's disease during transfusion therapy, as determined with the author's personal test.

in the first group, the erythroblastic medullary quota is low, while the erythropoiesis stimulating activity in plasma is increased (these are the *true myelopathic aplasias* related to a direct peripheral bone marrow impairment of myeloid reproductive activity);

in the second group, there is no erythropoiesis stimulating activity in the plasma: the erythroblastic aplasia seems to follow an inadequate stimulation of the erythron (erythroblastic aplasias due to central premedullary asthenia or *asthenic aplasias*).

Some cases of acute leukaemia seem to belong to the latter group, as if the marrow aplasia were not secondary to the massive hemocytoblastic infiltration, but were closely related to the absence of the plasma erythropoietic factor. If the myeloasthenic nature of anemia in some cases of acute leukaemia could be confirmed, the pathogenetic and perhaps the therapeutic problems of some among these malignant hemopathies could probably be considered in quite a different way.

It appears undoubtedly difficult to draw any conclusions from these data. The following data seem to be ascertained:

1. It is advisable to get plasma with a high erythropoietic factor concentration, in order to arrive at the isolation of this factor.

2. Such an "active" plasma should be utilized for therapeutic trials in such anaemic individuals whose plasma is *lacking* the erythropoietic factor.

3. It is not adoisable to transfuse large amounts of erythrocytes to anaemic individuals with confirmed erythroblastic hypoplasia. If the plasma of these patients shows an increase of the erythropoietic factor (myelopathic aplasias), its formation could be dangerously hindered.

4. A patient with a marrow hypoplasia and no erythropoietic factor in his plasma (myeloasthenic aplasia) should be given, besides a proper amount of blood, also large quantities of "active" plasma.

Studies are in progress, in order to obtain further information about the plasma erythropoietic factors.

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217

Natrium Alginatum von niedriger Polymerisation als ein neues Blutersatzmittel

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Mit Rücksicht auf die geographische Eigentümlichkeit unseres Landes als Inselreich haben wir ein neues Blutersatzmittel aus Natriumsalz der Alginsäure herstellen können, die ein Bestandteil der Zellmembrane der Braunalge ist.

Das Blutersatzmittel ist aus Natriumsalz der Alginsäure als ein Hochpolymer der Mannuronsäure hergestellt worden. Die Polymerisation dieser Säure als Blutersatzmittel muß bis zum geeigneten Grade herabgesetzt werden. Die zweckmäßige Herabsetzung dieser Polymerisation steht im Mittelpunkt dieses Studiums, wobei es keine Nebenerscheinungen hervorruft und genügende Stabilisierungswirkung auf das Zirkulationssystem bewahren kann. Der Durchschnittswert dieses optimalen Polymerisationsgrades ist ca. 100 (Molekulargewicht ca. 20000) nach der Messung mittels Ultrazentrifuge oder Lichtzerstreuungsapparates, wobei die Viskositätszahl (intrinsic viscosity) ca. 0,072 (l/g) ist.

Das Rezept unseres neu bedingten Blutersatzmittels lautet folgendermaßen:

Natrium Alginatum (Viskositätszahl 0,072 l/g)	0,4 g
Traubenzucker	5,0 g
NaCl	0,3 g
Wasser	100 cc

(Der kolloid-osmotische Druck dieser Lösung: ca. 9,8 cm H_2O in 25° C.)

Um dieses Präparat zu gewinnen, muß das von Braunalge extrahierte und gereinigte Alginatum bis 0,072 (l/g) von der Viskositätszahl depolymerisiert werden.

Nach den mehrfachen tierexperimentellen sowie klinischen Untersuchungen