Studies of Plasma Erythropoietic Factor in Anemic Human Patients

By T. C. PRENTICE AND E. A. MIRAND

The existence of a humoral erythropoietic stimulating factor which controls red cell production has been suspected for many years. Carrel and Deflandre postulated in 1906 that such might be true. In 1950 Reissmann, using parabiotic rats, published convincing experimental evidence of a circulating erythropoietic factor induced by placing one member of the pair in a hypoxic atmosphere. In 1952 an excellent review concerning the fundamental stimulus for erythropoiesis was written by Grant and Root. A blood-borne substance capable of stimulating erythropoiesis was strongly suggested. Subsequently many laboratories have taken up and expanded this concept, using several different methods of study. Thus plasma, or a "deproteinized" extract of plasma, obtained from animals rendered anemic by bleeding was shown to have the ability to stimulate increased erythropoiesis in recipient animals. Similar material from animals with phenylhydrazine-induced hemolytic anemia possessed the same properties, though somewhat more potent. Plasma from animals kept in a low oxygen atmosphere has exerted significant erythropoietic activity. Also there is evidence that a substance may be present in the milk of anoxic rats and mice which produces an increased hemoglobin content of the nursing young.

As studies with experimental animals have progressed, increasing interest has been centered on the significance of these findings in patients with various types of anemia and polycythemia. This phase, the study of human patients, has been aided tremendously by the demonstration that boiled extracts of human plasma retain their erythropoietic stimulating properties. This discovery enabled investigators to test the erythropoietic stimulating properties of extracts of human plasma in other animal species without detectable reactions due to foreign protein. Also smaller amounts of plasma were then adequate, since the extract could be assayed in small animals. Reports of such studies, using plasma extracts from human patients with varied forms of anemia and polycythemia, are now beginning to appear. They have indicated, in general, marked increases of erythropoietin in plasma from patients with Cooley's anemia, less consistent increases in Sickle cell anemia, no increase in one case of hypoplastic anemia and increased amounts in patients with polycythemia vera and secondary polycythemia. The present study is a report of 9 further patients with anemia of varied etiology in whom plasma erythropoietin has been assayed. Ancillary studies have been done in some of these cases in an effort to further clarify the significance of this factor in the particular individual.

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PLASMA ERYTHROPOIETIC FACTOR IN ANEMIC HUMAN PATIENTS

MATERIALS AND METHODS

The patients were selected at random, anyone with significant anemia being used as a subject. One hundred cc. of blood was drawn from each patient. .2 cc. of Heparin (20 mg.) was used as the anticoagulant. The blood was then centrifuged and the plasma removed. This plasma was then "deproteinized" using the method of Borsook,12 and concentrated to one third of its original volume. It was then frozen until used. This amount of plasma extract enabled the use of 3–4 test animals for each patient. The bio-assay technique was carried out using hypophysectomized rats as previously described21. Two cc of extract was given subcutaneously daily to each hypophysectomized rat for 3 days. On day 4, 1 μc Fe59 was given I.V. and on day 5 a 24-hour uptake was done. Controls were hypophysectomized rats given normal human plasma extract concentrated to one third its original volume.

In addition a control group of saline injected or untreated hypophysectomized rats was used in each instance. The experimental and control results were expressed as the average of the 24-hour Fe59 uptakes in the test animals used for each individual.

RESULTS

Table 1 summarizes the results in the 9 patients. It can be seen that, using the "t" test for significance, 4 of the 9 patients possessed significantly increased amounts of erythropoietic factor in their plasma. These 4 patients with positive results did not have a greater degree of anemia as a group than the other 5. There was considerable spread of Fe59 uptake values within the various test groups. As a result the mean uptake values for a given test group had to be more than double the control group before the <1 per cent level of significance was reached. It is probable that with further improvement of assay methods better differentiation between experimental and control groups will be possible. No significant difference was noted between those control groups receiving normal human plasma and those receiving either saline or nothing. Again better assay methods may eventually enable detection of small amounts of erythropoietin which may be present in normal plasma.

DISCUSSION

The significance of the plasma erythropoietic stimulating factor (EPF) in the various clinical forms of anemia and polycythemia remains to be elucidated. There does not appear to be a consistent relationship between the demonstrable level of EPF in the plasma and the level of erythropoietic activity in an individual. One sees very high levels of plasma EPF in some patients with no evidence in the blood or marrow of increased erythropoiesis (see patient J. P.). Others show marked marrow erythroid hyperplasia and reticulocytosis without detectable elevation of plasma EPF (see patient C. K.). Likewise, in normal rats subjected to hypoxia7 there is invariably a marked elevation of plasma EPF at 24 hours, with relatively little evidence of erythropoietic response in marrow or blood. At 48 hours, when marrow erythroid hyperplasia and reticulocytosis is slightly more pronounced, the plasma EPF values have in every instance fallen back to normal. After 5 days in hypoxic atmosphere the EPF values remain down while evidence of increased erythropoiesis is now quite striking. Perhaps some of these discrepancies could be explained by deficiencies in the assay method. However, in the instances cited above, where such a marked contrast
Table 1. Showing degree of anemia and plasma titer of erythropoietic factor in each patient

<table>
<thead>
<tr>
<th>Pt.</th>
<th>Blood</th>
<th>Diagnosis</th>
<th>Remarks</th>
<th>Fe(^{68}) Uptake</th>
<th>Retics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>VN</td>
<td>Hb. 5.6</td>
<td>Ca of colon with retroperitoneal metastases.</td>
<td>Depressed marrow erythropoiesis. Retics. 5%. Short RBC survival time.</td>
<td>2 13.0</td>
<td>9.0-17.0</td>
</tr>
<tr>
<td>JB</td>
<td>Hb. 7.7</td>
<td>Chronic lymphatic leukemia.</td>
<td></td>
<td>3 18.4</td>
<td>11.5-21.7</td>
</tr>
<tr>
<td>AC</td>
<td>Hb. 9.1</td>
<td>Ca of ascending colon</td>
<td></td>
<td>3 9.6</td>
<td>5.7-12.0</td>
</tr>
<tr>
<td>AB</td>
<td>Hb. 8.5</td>
<td>Ca of cervix—receiving radiation</td>
<td></td>
<td>3 26.1</td>
<td>15.3-36.8</td>
</tr>
<tr>
<td>MY</td>
<td>Hb. 9.0</td>
<td>Ca of cervix—receiving radiation</td>
<td></td>
<td>3 19.6</td>
<td>10.5-26.9</td>
</tr>
</tbody>
</table>

A Normal Human Control
B Saline Control

A Normal Human Control
B Untreated Control

FC Hb. 8.5 | Chronic lymphatic leukemia. | Normal marrow erythropoiesis. Retics. 1.6% Sl. reduced Cr\. RBC survival. Evidence of increased hemolysis. Retics 5.5%. Reduced Cr\. RBC survival. | 3 13.7 | 5.9-17.2 | ±5.0 | Insig. | 0.3 0.1-0.4±0.22 |

GR Hb. 10.4 | Chronic lymphatic leukemia. | | 4 23.6 | 8.5-45.9 | ±17.0 | Insig. | 1.4 0.4-2.3±1.35 |

A Normal Human Control
B Untreated Control

* The P. values opposite each patient indicate a comparison between the patient and the normal human control group. Those opposite the control groups indicate a comparison between the controls themselves.

is seen, this seems unlikely. In other situations, such as anemia due to hemorrhage or hemolytic anemia due to phenylhydrazine, high levels of plasma EPF are consistently associated with evidence in blood and marrow of increased erythropoiesis. The primary difficulty in the interpretation of the significances of the plasma EPF level in a given patient may well be our ignorance concerning the relative rates of production and utilization or destruction of this material. High plasma levels could be due to increased production, decreased utilization or decreased destruction. Similarly decreased levels could be attributed to variation in any
one or all of these parameters. It would appear therefore that until means are devised to evaluate rates of production and utilization or destruction of this material the interpretation of the meaning of a given level of plasma EPF will be extremely difficult. Much work on the source of EPF is now being done.22-25 As further progress in this phase of the problem is made, perhaps more quantitative data as to rates of production under various conditions will become available. Little has been done with the problem of utilization or destruction of EPF. Why, for instance, is the plasma of some anemic animals capable of stimulating recipient test animals and yet incapable of stimulating themselves?

Further clarification of the reliability and validity of the presently used test for erythropoietic activity in plasma is also needed. Different laboratories are using different methods. Do the assay methods always mean what they are interpreted to mean?

Summary

Nine patients with anemia of varied origin have been studied. The relationship between erythron response and the content of erythropoietic factor in the plasma has been observed in these patients. Wide discrepancies between these variables were seen to exist. Further investigation of this aspect of the problem is needed.

Summary in Interlingua

Nove patientes con anemia de varie origines esseva studiate. Le relation inter le responsa erythronic e le contento de factor erythropoietic in le plasma esseva observate in iste patientes. Esseva notate que il existe forte discrepantias inter iste variabiles. Iste aspecto del problema require investigationes additional.

References

11 Mirand, E. A., and Prentice, T. C.: Fe59 Assessment of erythropoiesis in mice follow-


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