Evidence for a Humoral Regulation of Erythropoiesis

Studies on a Patient with Polycythemia Secondary to Regional Hypoxia

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THE REGULAR OCCURRENCE of polycythemia in chronic hypoxic states\(^1\)\(^2\) and the suppression of erythropoiesis by increases in barometric pressure\(^3\) or the oxygen concentration of inspired air\(^4\)\(^5\) indicate that the state of blood oxygenation is a major factor in regulating the rate of erythrocytogenesis. In the past, most investigators have considered blood or tissue oxygen tension as the critical factor.\(^7\)\(^8\) Recent evidence, however, suggests that the oxygen content is equally as important as the \(pO_2\) in the regulation of erythropoiesis. For example, Grant and Root\(^9\) found a reduction of \(O_2\) content in arterial blood and the bone marrow of subjects with acute or chronic anemia. Also, in transfusion polycythemia there is an increased \(O_2\) content and suppression of erythropoiesis even though the \(pO_2\) of peripheral blood, and presumably tissue, is unchanged.\(^1\)\(^2\)

The mechanisms by which alteration in \(pO_2\) or \(O_2\) content control the rate of red cell production are the subject of speculation. It has been suggested that either the partial pressure of oxygen (\(pO_2\)) in the bone marrow regulates the rate of erythropoiesis\(^8\)\(^11\) or a decrease in arterial oxygen tension results in the formation of an unidentified humoral (plasma) factor, "hemopoietine," capable of stimulating erythropoiesis.\(^12\)\(^13\) Some clinical and experimental observations might suggest that the latter is elaborated by the pituitary\(^14\)\(^18\) or thyroid\(^19\)\(^20\) glands. A third explanation is that the erythropoietic response of hypoxia is mediated through nervous pathways.\(^21\)\(^25\)

In the present report, data will be presented which are compatible with a humoral regulation of erythrocytogenesis. The studies were conducted on a patient with polycythemia, secondary to a patent ductus arteriosus with a reverse shunt.\(^24\)\(^35\) Characteristic of the syndrome are primary pulmonary hypertension, erythrocytosis, and an oxygen differential between brachial and femoral arterial blood. In the absence of clinical evidence of cardiac or pulmonary decompensation, as was the case in this patient, the oxygen saturation of brachial artery blood was normal and hypoxia was confined to the lower half of the body.\(^33\)\(^34\) The resultant difference in bone marrow oxygenation presented an opportunity to evaluate the nature of the influence of hypoxia on red cell formation.
HUMORAL REGULATION OF ERYTHROPOIESIS

CASE REPORT

R. S.—an 18 year old colored woman—was first seen in the out-patient clinic January 13, 1953, for a urinary tract infection and hypertension (160/130). Erythromycin sensitive hemolytic Staphylococcus aureus was cultured from the urine and intravenous pyelography revealed poor filling of the left kidney. Erythromycin therapy was instituted and hospitalization advised for evaluation of her hypertension. She was admitted February 6, 1953.

Her health in the past had been excellent except for one episode of hemoptysis, the previous year, for which she did not seek medical attention. Her activities never had been limited by easy fatigability, weakness, or dyspnea, and she could ascend three flights of stairs without difficulty. Her menstrual history was normal and she had no complaints of a hematologic, cardiovascular, or gastrointestinal nature.

Physical Examination

T 99, R 20, P 76, BP 130/100. The patient was a slender, well nourished, apprehensive female, who appeared neither acutely nor chronically ill. The conjunctiva, fingernails of both upper extremities, and nasal and buccal mucosa were pink, but there was definite cyanosis of the toenails and vaginal mucosa. Racial melanin pigmentation precluded evaluation of skin cyanosis. She had minimal but definite clubbing of the toes but none of the fingers. Several observers failed to detect cardiac murmurs. The lungs were clear. A standard bicycle exercise test was tolerated normally without a change in the degree or extent of cyanosis. Otherwise, the examination was within normal limits.

Hospital Course

The renal infection cleared on erythromycin therapy, following which her blood pressure returned to normal, and a repeat intravenous pyelogram on February 14 was normal. Thereafter, her course was uneventful until February 28 when she suddenly became apprehensive, developed dyspnea, orthopnea, and generalized cyanosis, and lapsed into a semicoma state. At this time a grade 3 systolic murmur was heard over the entire precordium but her lungs were clear. An EKG showed sinoauricular tachycardia followed by complete heart block, ventricular tachycardia, ventricular fibrillation, and death within three hours of the onset of the acute episode.

Laboratory Data

Hematology: Red cell count 7.33 million per cu. mm.; hemoglobin 20.5 Gm. per cent; hematocrit 62 per cent; mean corpuscular volume 85 cu. µ; mean corpuscular hemoglobin 28.77; mean corpuscular hemoglobin concentration 33 per cent; reticulocyte count 1.5 to 2 per cent; red cell mass (Cr¹) 43.5 cc./Kg. (normal 27.0 cc./Kg.); plasma volume (T-1824) 46 cc./Kg. (normal 42 cc./Kg.); total blood volume 80.5 cc./Kg. The metabisulfite test for sickle cells was negative; red cell fragility in hypotonic saline was normal; Coombs' test negative; white blood cell count 7,000 per cu. mm., with a normal differential; platelet count (modified Rees-Ecker technic) 154,000 per cu. mm.; bleeding time (Duke) 3 min.; clotting time (glass tube) 7 min.; one stage prothrombin time 14.2 sec. (control 14.0); serum prothrombin time 35 sec. (control 52 sec.); recalcification time 100 sec. (control 90 sec.); capillary fragility normal; fibrinogen 260 mg. per cent.

Chemistry: Blood nonprotein nitrogen 44, 50, 29 mg. per cent.; CO₂ combining power 22.5, 23.2 mEq./L.; chlorides 95 mEq./L.; sodium 140 mEq./L.; glucose tolerance test normal; serum bilirubin .84 mg. per cent.; 5 per cent bromsulfalein retention in 45 min.; thymol turbidity 4.2 MacLaglen units; cephalin cholesterol flocculation negative; alkaline phosphatase 3.8 and 9.0 B.U.; total protein 8.6 Gm. per cent.; albumin 6 Gm. per cent.; globulin 2.6 Gm. per cent.

Other laboratory data: The urine concentrated to 1.020 (Fishberg) and at the time of hospital admission showed only an occasional WBC and O -- + + albuminuria. Urine urobilinogen 0.15 Ehrlich units in two hours. 15 min. P.S.P. excretion: (2/7) 5 per cent; (2/11) 5 per cent; (2/21) 20 per cent. The stool was negative for occult blood. Circulation time:
arm to lung (ether) 8 sec.; arm to tongue (Decholin) 18 sec.; EKG: right ventricular hypertrophy. Chest x-ray and fluoroscopy: there was an accentuation of vascular markings with bilateral enlargement of the pulmonary artery suggestive of congenital heart disease.

Postmortem Findings

A patent ductus arteriosus 1.7 cm. in diameter entered the aorta 1 cm. distal to the subclavian artery. The heart showed marked right ventricular hypertrophy 1.2 to 1.7 cm. in thickness, but otherwise was normal. There was marked arteriosclerosis of pulmonary vessels. There was no evidence of chronic congestive failure.

Special Studies

Methods

Direct intravascular pressures were recorded at the time of cardiac catheterization using a Statham strain-gage and Sanborn strain-gage amplifier and recorder. Mean pressures in pulmonary and systemic arteries were obtained by planimetric integration of the pulse wave. Samples for oxygen determinations were obtained at the time of catheterization (table 1).

Fasting peripheral blood samples were collected without stasis with the patient in the recumbent position under moderate sedation. The first 2 to 3 cc. collected were discarded. In the second syringe, 1.0 cc. was collected without suction for a direct pH determination using the glass electrode technic. Six cc. were then withdrawn for O₂ analysis into a chilled, oiled syringe, the dead space of which previously had been filled with heparin. This was immediately placed in an ice bath. Bone marrow samples were obtained from the sternum at the level of the first rib and from both iliac crests. The marrow was entered in the usual fashion with a 16 gage needle and 0.2 cc. withdrawn for cytology. Following the aspiration for cytology, samples were collected for pH and O₂ determinations as previously described. Bone marrow sections for evaluation of cellularity and morphology were obtained at post-mortem examination from the sternum, the ribs at the costochondral junction, lumbar vertebrae, and iliac crests.

Oxygen content and capacity were done by the method of Van Slyke and Neill. Oxygen tensions were derived by interpolation of pH and O₂ saturation on a standard oxygen dissociation curve calculated for constant pH, which has been found more reliable than those constructed under conditions of constant pCO₂. Data for the average pO₂ in the normal female (table 3) were obtained from the dissociation curve using an oxygen capacity of 18.8 to 19.4 vol. per cent (14.0 ± 0.2 X 1.36); oxygen content of 17.9 vol. per cent and pH of 7.41 for arterial blood and 13.7 vol. per cent and 7.37 for venous blood. Oxygen tensions of normal human bone marrow were calculated on the dissociation curve from the data of Schwartz and Stats and Berk, et al. using a value of 7.37 for pH when the latter was not determined.
Results

Hemodynamic and oxygen studies

The diagnosis of patent ductus arteriosus with reverse flow was substantiated by cardiac catheterization (table 1), which revealed a mean pulmonary artery pressure of 102 and a mean systemic arterial pressure of 96 mm Hg. The resultant pressure gradient favored a right to left flow. The marked reduction in oxygenation of femoral arterial blood (tables 1 and 2) furnished conclusive evidence that venous blood actually was passing from the pulmonary artery into the aorta. Moreover, the normal oxygen values for brachial arterial blood (tables 1 and 2) indicated that an insignificant amount of unoxygenated blood was being channeled into the proximal portion of the aorta. Finally, it was concluded that a unidirectional type of flow existed at the time of catheterization, since the \( O_2 \) contents of the right auricle and ventricle, in the tricuspid area as well as the out-flow tract, were almost identical to those of the pulmonary artery.

It will be noted that the \( pO_2 \) values for brachial arterial blood (88 and 71.1 mm Hg) determined indirectly by interpolation on a standard dissociation curve, were lower than the average normal of 97.1 mm Hg reported with direct measurements of arterial \( pO_2 \) by Comroe and Dripps. The \( O_2 \) saturation was also less than the normal of 98 to 99.2 per cent obtained by Drabkin, et al., with a direct spectrophotometric technic. However, the brachial arterial \( O_2 \) values in this patient were within the normal limits reported for the methods employed (Bock, et al., 82.2 to 96 per cent saturation, \( pO_2 \) 67 to 79 mm Hg; Cullen and Cook, 80.5 to 96.5 per cent saturation, \( pO_2 \) 67 to 80 mm Hg; Wasserman, et al., 90 to 98 per cent saturation). Moreover, oxygenation of brachial arterial blood was significantly greater than that reported in secondary polycythemia (Berk, et al., \( O_2 \) saturation 66.3 to 87.8 per cent, \( pO_2 \) 25 to 37 mm Hg; Wasserman, et al., \( O_2 \) saturation 75 to 88 per cent, \( pO_2 \) 41 to 57.2 mm Hg (\( pO_2 \) calculated, assigning a pH of 7.4); Hecht and Samuels, \( pO_2 \) 35 mm Hg).

The discrepancy between the direct and indirect measurements of \( pO_2 \) apparently arises from errors inherent in the determination of \( O_2 \) saturation by the

![Figure 1](www.bloodjournal.org)
Van Slyke-Neill technique. Roughton, et al.,\textsuperscript{51} in analyzing these errors, demonstrated that due to the gradual reversion of inactive hemoglobin to the gas-combining form, dissociation of traces of COHb, and other minor factors, the O\textsubscript{2} capacity by this method is 2 per cent higher, and consequently the saturation lower, than at the moment of withdrawal of blood from the body. A 2 per cent error in saturation on the flat portion of the dissociation curve produces an error of 10 to 20 mm. Hg in deriving pO\textsubscript{2} by interpolation. Consequently, if the arterial O\textsubscript{2} saturation exceeds 85 per cent, a 2 per cent error produces a significant variation in the value of pO\textsubscript{2}. On the other hand, if O\textsubscript{2} saturation is less than 85 per cent, as in venous blood or the femoral arterial samples in this patient, the variation between direct and indirect determination of pO\textsubscript{2} is negligible.

While these studies suggested that tissues deriving their blood supply from the aorta proximal to the entanglement of the ductus were normally oxygenated and those distal to the ductus were hypoxic, the critical determinations were the pO\textsubscript{2} and oxygen contents in the marrow. These values along with similar data from the peripheral blood are presented in table 2 and graphically represented in figure 1.

Oxygen values for sternal marrow blood were within the normal range reported by Schwartz and Stats\textsuperscript{43} and Berk, et al.\textsuperscript{44} (table 2), and those observed in five hematologically normal hospitalized patients in our laboratory, saturation 65 to 87 per cent, pO\textsubscript{2} 36 to 56 mm. Hg. In marked contrast was the iliac marrow saturation of 42 and 34 per cent and pO\textsubscript{2} of 21.9 and 26.6 mm. Hg. These values are similar to those observed in polycythemia secondary to chronic lung disease by Hecht and Samuels,\textsuperscript{50} pO\textsubscript{2} 25 mm. Hg, but lower than in a patient with polycythemia secondary to a tetralogy of Fallot studied in our laboratory, 53.5 per cent saturation, pO\textsubscript{2} 29 mm. Hg, and those in secondary polycythemia observed by Berk,\textsuperscript{44} 61.8 to 72.5 per cent saturation with a pO\textsubscript{2} of 31 to 37 mm. Hg.

The objection might be raised that in withdrawing 5 to 7 cc. of blood from the marrow cavity, one obtains a mixture of peripheral blood and marrow. However, table 2.

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>O\textsubscript{2} content (Vol. %)</th>
<th>O\textsubscript{2} saturation (%)</th>
<th>pO\textsubscript{2} (mm. Hg)</th>
</tr>
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<tbody>
<tr>
<td>Femoral vein</td>
<td>2/23/53</td>
<td>4.3</td>
<td>18.6</td>
<td>13.8</td>
</tr>
<tr>
<td>Antecubital vein</td>
<td>2/23/53</td>
<td>17.06</td>
<td>73.8</td>
<td>43.8</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>2/17/53</td>
<td>14.34</td>
<td>62.0</td>
<td>35.8</td>
</tr>
<tr>
<td></td>
<td>2/23/53</td>
<td>10.64</td>
<td>46.15</td>
<td>28.4</td>
</tr>
<tr>
<td>Brachial artery</td>
<td>2/17/53</td>
<td>22.08</td>
<td>95.7</td>
<td>88.0</td>
</tr>
<tr>
<td></td>
<td>2/23/53</td>
<td>21.18</td>
<td>92.1</td>
<td>71.1</td>
</tr>
<tr>
<td>Iliac bone marrow</td>
<td>2/19/53</td>
<td>7.93</td>
<td>34.4</td>
<td>21.9</td>
</tr>
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<td></td>
<td>2/23/53</td>
<td>9.9</td>
<td>42.8</td>
<td>26.6</td>
</tr>
<tr>
<td>Sternal bone marrow</td>
<td>2/17/53</td>
<td>17.2</td>
<td>74.5</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td>2/23/53</td>
<td>15.96</td>
<td>69.0</td>
<td>41.1</td>
</tr>
</tbody>
</table>

\* Derivation of these values is given under "Methods."
\dagger O\textsubscript{2} saturation = O\textsubscript{2} content/O\textsubscript{2} capacity.
\ddagger After Schwarz and Stats.\textsuperscript{43}
\S After Berk, et al.\textsuperscript{44}
Humoral Regulation of Erythropoiesis

Grant and Root, using the micro method of Roughton and Scholander, were unable to detect in dogs a significant variation between the O\textsubscript{2} content or saturation of the first drop of blood obtained on entering the marrow and a similar sample obtained after the removal of 3 to 10 cc. Excluding the theoretic possibility of a localized vascular abnormality, as has been postulated in polycythemia vera\textsuperscript{48} the oxygen content of the adjacent peripheral blood should reflect the state of oxygenation of the marrow itself. The O\textsubscript{2} values of the marrow, reported by Grant\textsuperscript{49} and Hecht,\textsuperscript{50} approximated those obtained simultaneously on jugular or right heart blood. Berk, et al.,\textsuperscript{44} however, observed marrow oxygen values intermediate between those of peripheral artery and vein. They suggested that the oxygenation of marrow samples reflects in part, at least, the rate of O\textsubscript{2} consumption. The data presented here is in keeping with the latter; the O\textsubscript{2} values of the hyperplastic sternal marrow being equivalent to those of the antecubital vein, while the O\textsubscript{2} determinations of the less cellular iliac crest were intermediate between those of the femoral artery and vein.

Morphology

Iliac crest: Marrow aspirations on three occasions were relatively and absolutely hypocellular. Sections obtained at postmortem examination, while less cellular than similar preparations obtained from normally oxygenated areas, were within the range of normal cellularity (fig. 2). Red cell hyperplasia was not observed in these sections.

A 2000 cell differential was done on Wright stained preparations of marrow obtained by aspiration (table 3). The myeloid elements, megakaryocytes, monocytes, plasma, and reticulum cells were normal. Maturation and hemoglobin formation of the erythroid series appeared normal. The myeloid:erythroid ratio of 2.3:1 is probably within normal limits and is greater than the M:E ratio of 1.1:1 reported by Braun\textsuperscript{51} in secondary polycythemia and that of 1:1.4 reported by Merino\textsuperscript{54} in altitude polycythemia.

Fig. 2.—Postmortem section (6 µ) of iliac (hypoxic) marrow (X 300).
TABLE 3.—Relative Number of Red Cells in Hypoxic (Iliac) and Normally Oxygenated (Sternal) Marrow of Patient R. S. Compared with Normal* and Secondary Polycythemia†

<table>
<thead>
<tr>
<th>Cells</th>
<th>Normal</th>
<th>Patient R. S.</th>
<th>Secondary polycythemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Iliac 2:18</td>
<td>Sternal 2:18</td>
</tr>
<tr>
<td>Myeloblasts</td>
<td>0.3-5.0</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>1.0-8.0</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Neutrophiles:</td>
<td>5.0-19.0</td>
<td>3.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>0.5-3.0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Basophiles</td>
<td>0.0-0.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Metamyelocytes and bands</td>
<td>13.0-32.0</td>
<td>14.5</td>
<td>11.0</td>
</tr>
<tr>
<td>Neutrophiles</td>
<td>12.8</td>
<td>9.6</td>
<td>22.4</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>1.6</td>
<td>1.3</td>
<td>0.43</td>
</tr>
<tr>
<td>Basophiles</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Polymorphonuclear:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophiles</td>
<td>7.0-30.0</td>
<td>32.6</td>
<td>15.2</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>0.5-4.0</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Basophiles</td>
<td>0.0-0.7</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3.0-17.0</td>
<td>17.6</td>
<td>9.4</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.5-5.0</td>
<td>3.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0.0-2.0</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Reticulum cells</td>
<td>0.1-2.0</td>
<td>0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td>0.0-3.0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Pronormoblasts</td>
<td>1.0-8.0</td>
<td>1.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Normoblasts</td>
<td>7.0-32.0</td>
<td>22.2</td>
<td>52.3</td>
</tr>
<tr>
<td>Early</td>
<td>3.1</td>
<td>12.8</td>
<td>11.8</td>
</tr>
<tr>
<td>Late</td>
<td>19.1</td>
<td>30.5</td>
<td>32.4</td>
</tr>
<tr>
<td>M:E ratio</td>
<td>3:1</td>
<td>2.3:1</td>
<td>1:1.7</td>
</tr>
</tbody>
</table>

* From Wintrone.†
† From Braun.33

FIG. 3.—Postmortem section (6 μ) of second rib at the costochondral junction demonstrating red cell hyperplasia in normally oxygenated areas (X 300).
Sternal marrow: Aspirations from the sternum at the level of the first rib, obtained at the same time as those of the iliac crest, were uniformly hypercellular. At autopsy, sections were obtained from the sternum and the ribs at the costochondral junction. The internal mammary artery is the principal, if not the sole source of blood supply to the latter and oxygenation of this marrow was presumably normal. These sections (fig. 3) uniformly showed a marked hypercellularity and red cell hyperplasia.

In a differential count of 2000 nucleated elements (table 3), 55.6 per cent were of the erythrocytic series, late normoblasts predominating. This percentage is in the range of those observed in high altitude polycythemia by Merino (mean 55.6 per cent) and greater than that observed by Braun in polycythemia secondary to cor pulmonale (mean 45 per cent). Maturation and hemoglobin formation appeared normal. The remainder of the nucleated elements were normal. The reversal of the myeloid:erythroid ratio, 1:1.7, was compatible with the red cell hyperplasia.

Lumbar vertebrae: Postmortem sections of the body of the first lumbar vertebrae showed a degree of cellularity intermediate between that of the ribs and the iliac crest with a moderate hyperplasia of red cell elements.

Discussion

From the physiologic and pathologic data presented it was concluded that this patient developed polycythemia secondary to hypoxia arising from the flow of unoxgenated blood into the systemic circulation by way of a patent ductus arteriosus. The major portion of the flow was channeled distally producing a decreased O2 content and pO2 in femoral arterial blood and presumably that of the descending aorta distal to the ductus. The increased red cell mass together with normal O2 saturation resulted in an increased O2 content but normal pO2 in the blood of the brachial artery. Consequently, arterial blood to the neural centers, pituitary, and those areas of the bone marrow deriving their blood supply from the aorta proximal to the entrance of the ductus, had a normal O2 saturation and pO2 with an increased O2 content. Other areas of the marrow, receiving blood from the distal aorta, were hypoxic. The altered hemodynamics and blood oxygenation provided a unique opportunity to evaluate in a single patient the effect of blood oxygenation on erythropoiesis.

The findings in our patient of normoblastic hyperplasia in normally oxygenated areas of the marrow (figure 3) are consistent with the hypothesis that under conditions of altered blood oxygenation red cell production is regulated by a humoral factor(s). The mechanism of development of the humoral stimulus in the hypoxic state might be attributed to: (1) an increased production of an erythropoietic stimulating factor; (2) a decreased catabolism or inactivation of a normally occurring stimulator; (3) a decreased production or increased breakdown of a normally occurring inhibitor.

Carnot and DeFlandre, in 1906, suggested that there was elaborated, in response to hypoxia, a plasma factor, "hemopoietine," capable of stimulating erythropoiesis. These and subsequent studies on the effect of hypoxic plasma, which have been reviewed by Westphal and recently by
Grant and Root,\textsuperscript{48} were inconclusive. Moreover, Gordin and Dubin,\textsuperscript{59} using the techniques originally described, were unable to demonstrate an effect on erythropoiesis from the injection of plasma from hypoxic animals into normal animals.

Reissmann, in 1949,\textsuperscript{60} presented the first convincing evidence for the existence of a humoral mechanism. He found that parabiotic rats developed a similar degree of polycythemia and normoblastic hyperplasia of the bone marrow, when one of the pair was exposed to an atmosphere of low oxygen tension, while the other breathed room air. It was demonstrated that the exchange of formed elements between parabionts was insufficient to lower the arterial oxygen saturation in the animal exposed to normal oxygen tension. Presumably, the interchange was sufficient to permit the passage of a humoral factor from the hypoxemic to the normal rat.

Grant\textsuperscript{61} arrived at a similar conclusion, using the anoxic lactating rat. The hemoglobin and red cell mass were greater in babies nursed by mothers exposed to intermittent anoxia than in litter mates nursed by normal mothers. Support for the existence of a similar mechanism in anemia following acute blood loss has been recently described by Erslev\textsuperscript{62} and Tohá.\textsuperscript{63}

The site of formation of the factor(s) controlling erythrocytogenesis is obscure. Various authors have suggested the spleen,\textsuperscript{64} reticuloendothelial system,\textsuperscript{65} pituitary,\textsuperscript{13, 16, 18} and the red cell itself\textsuperscript{66} as the site of formation of an erythropoietic hormone. Conclusive evidence for the existence of these hormones is lacking. Since in our patient the blood supplying the pituitary was normally oxygenated, it seems evident that an increased production of an erythropoietic hormone by the pituitary is not the primary lesion in hypoxic polycythemia. The findings in this patient do not permit comment on the possible role of the spleen or reticuloendothelial system in the regulation of erythropoiesis.

A commonly accepted theory has been that red cell production is regulated by the \( \text{pO}_2 \) or \( O_2 \) content in the erythropoietic centers of the bone marrow. If this was true, evidence of increased erythrocytogenesis in regional hypoxia should be confined to those areas where oxygenation is demonstrably diminished. In areas where \( O_2 \) saturation and \( \text{pO}_2 \) are normal and \( O_2 \) content increased, the picture should resemble that of transfusion polycythemia. Pace and his co-workers\textsuperscript{10} found a suppression of erythropoiesis, as measured by reticulocytosis, in four out of five men with transfusion polycythemia in whom the arterial \( O_2 \) values were equivalent to those observed in the brachial arterial blood of this patient. In two instances reticulocytes were absent. Birkhill and his colleagues\textsuperscript{67} produced a comparable degree of polycythemia in normal men following transfusion and noted a shift in the M:E ratio of marrow aspirates from pretransfusion values of 4:1 to 7:1 to figures of 20:1 to 37:1.

In this patient hypercellularity and normoblastic hyperplasia rather than suppression of erythropoiesis were present in both aspiration and postmortem sections of sternum and costal marrow where \( O_2 \) saturation and \( \text{pO}_2 \) were normal. These observations cannot be explained by a direct effect of hypoxia on the bone marrow. It may be argued that the \( O_2 \) content of sternal marrow samples, 15.96 and 17.2 vol. per cent, was within normal limits and suppression would not be expected. As mentioned previously, however, the blood obtained from bone
marrow aspirations is mixed blood that is more nearly venous than arterial. The O₂ content, then, should reflect in part at least the O₂ consumption of marrow elements. In the presence of red cell hyperplasia a high O₂ consumption⁴¹–⁴⁵ and a decrease in O₂ content would be expected. Actually, the sternal marrow O₂ content was within the upper limits observed in normal males⁴³–⁴⁴ and greater than in the few recorded determinations in normal females.⁴³

It has also been suggested that hypoxia produces a neural stimulation of erythropoiesis.²⁻²⁵ The evidence for this, which has been reviewed by Carpenter²² and Hortling,⁶⁹ is based on the occurrence of polycythemia in brain tumors and encephalitis²² and in experimental animals following destruction of vegetative centers,²¹ injection of air into the ventricular system,²³ and transection of the spinal cord.²¹, ²⁵ The interpretation of these data is difficult, since, in addition to destruction of neural centers there undoubtedly is alteration of endocrine, metabolic, or vascular function in many areas of the body. In the case presented, however, hypoxic polycythemia occurred without evidence of neurologic lesions and in the presence of normal oxygenation of vegetative centers. These findings seem to exclude a neural basis for hypoxic polycythemia.

The relative hypoplasia observed in the iliac marrow was an unexpected finding and requires explanation. Two interpretations are suggested: (1) The hypocellularity was a coincidental local phenomenon, to which little significance should be attached; this view might be strengthened by the moderate hypocellularity observed in postmortem sections of the first lumbar vertebrae. (2) On the other hand, it may signify that local anoxia was severe enough to prevent the response of the erythroid cells to the humoral stimulus. In support of such an explanation is the report of Talbott,⁷⁶ who found a maximum hematopoietic response at an altitude of 17,500 feet. On further ascent to 20,000 feet, where the arterial O₂ saturation was 65.6 per cent,⁷¹ a decrease in hemoglobin and erythrocytes was observed. Assuming that the blood obtained for O₂ analysis from the iliac marrow was pure venous blood and that 5 vol. per cent of O₂ had been consumed, then the calculated arterial O₂ saturation, 56 and 64 per cent, would be less than that which produced a decreased erythrocytogenesis in Talbott’s experience. Regardless of which interpretation one places on the findings in the iliac marrow, the basic conclusion that erythropoiesis in hypoxic states is regulated by a humoral factor is not altered.

SUMMARY

Data obtained from a patient with polycythemia secondary to a patent ductus arteriosus with reverse flow, have been presented. Oxygen saturation and tension of brachial arterial blood and sternal bone marrow were within normal limits, while those of the iliac marrow and femoral arterial blood were reduced.

The presence of polycythemia under such circumstances is thought to exclude a primary cerebral or pituitary mechanism. Similarly, the presence of hypercellularity and normoblastic hyperplasia in normally oxygenated areas of the bone marrow argue against a direct effect of lowered O₂ content or pO₂ on germinal erythroid cells.

These observations are compatible with a humoral regulation of erythropoiesis in the hypoxic state.
STOHLMAN, RATH, AND ROSE

SUMMARIO IN INTERLINGUA

Le concepto de un regulation humoral de erythropoiesis in stato hypoxic es compatibile con le datos hic presentate e obtenite ab un patiente con polycythemia (erythrocytosis anoxemic) resultante secundarimente de un patente ducto arterioso con fluxo revertite. Le saturation e le tension oxygenic del arteriosanguine brachial e del medulla ossee del steriso se manteneva intra limites normal durante que e le saturation e le tension oxygenic del arteriosanguine femoral e del medulla ili o eseva reducite.

Il pare que in iste conditiones le presentia de polycythemia exclude le possibilitate de un mechanismo primari o cerebral o pituitari. Similmente, le presentia de hypercellularitate e de hyperplasia normoblastic ins areas osseomedullari con oxygenation normal representa un argumento contra le supposition de un effecto directe de un reduce contenio de O₂ o un reduce pO₂ super le cellulas erythroide germinal.

REFERENCES

HUMORAL REGULATION OF ERYTHROPOIESIS


Evidence for a Humoral Regulation of Erythropoiesis: Studies on a Patient with Polycythemia Secondary to Regional Hypoxia

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