Polycythemia and Increased Erythropoietin Production in a Patient with Hypertrophy of the Juxta-Glomerular Apparatus

By J. JEPSON AND E. E. McGARRY

The specific renal cellular site responsible for the production of erythropoietin has been the object of much debate since Osnes' first implicated the juxtaglomerular apparatus (JGA). Several investigators, employing techniques in experimental animals which stimulate or suppress erythropoietin secretion, have reported increased and decreased granularity, respectively, of juxtaglomerular cells, suggesting the juxtaglomerular apparatus in experimental animals may play a direct or indirect role in either the production or release of erythropoietin.

The following case6 is presented in view of the association of polycythemia and increased erythropoietin secretion in a patient with histologically proven hypertrophy of the juxtaglomerular apparatus (Bartter's syndrome).

CASE HISTORY

J. H., a prepuberal 10 year-old normotensive Caucasian male with a known diagnosis of hypertrophy of the juxtaglomerular apparatus, was found to have an increased hemoglobin and hematocrit for his age. His hematologic status was investigated in detail.

The patient was born at 29 weeks of gestation, weighing 2 lb. 12 oz. He failed to attain normal growth, and at the age of 6 years, weighed 26 lb. and was 37 and 3/4 in. tall (height-age: 3 years, 2 months; bone age: 4 years). He was noted to have polyuria and polydipsia. Physical examination was normal except for growth retardation. Laboratory investigation demonstrated persistent refractory hypokalemia (1.0-2.9 mEq./l.) and hypochloremic alkalosis (chloride = 80-87 mEq./l., CO₂ of 26.9 mEq./l.). At the age of 8 years, the patient underwent intensive investigation on the metabolic ward of the Royal Victoria Hospital in Montreal, including renal and adrenal biopsies, which showed marked hypertrophy, hyperplasia, and hypergranularity of the juxtaglomerular apparatus, and hyperplasia of the zona glomerulosa of the adrenal.* The juxtaglomerular index7 of granularity

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This investigation was supported by a special U.S. Public Health Service Fellowship 2-F3-HE-18, 509-02 from the National Heart Institute, and by Grant MBT-1664 from the Medical Research Council of Canada.

First submitted November 4, 1967; accepted for publication December 15, 1967.

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*Detailed histologic analysis of the renal biopsy specimen was carried out by Dr. J. M. Rojo Ortega, Département de Recherches Cliniques, Hotel-Dieu de Montréal.
for 100 glomeruli was 168 (normal 4 ± 2), the juxtaglomerular cell count was 718/25 JGA (normal 190 ± 20), and the macula densa cell count was 229/25 macula densa (normal 7 ± 2). Hyperplasia and hypertrophy of the JGA was present in 95 per cent of the JGA. This was associated with increased aldosterone secretion rates, elevated blood renin, and angiotensin levels. Renal function, protein bound iodine, urinary 17-hydroxycorticoids and 17-keto steroids were normal. Detailed metabolic studies are to be reported elsewhere.

The hemoglobin was 16.4 Gm. per cent, hematocrit 50 per cent, reticulocytes 3.2 per cent, with normal platelet and white blood counts. Repeated hemoglobin determinations were between 15 and 17 Gm. per cent, the serum iron was 71 per cent, UIBC 156 sg per cent, TIBC 227 ηg per cent, with 30 per cent saturation of transferrin. Blood volume and iron kinetic studies are presented in Table 1.

MATERIALS AND METHODS

Whole plasma and 24-hour urines were collected and stored, and the urine extracted as described previously.10 The samples were assayed for erythropoietic activity in hypoxia-induced polycythemic mice.11 One-half ml. of whole plasma was injected subcutaneously on the fourth and fifth day following their removal from the hypoxic environment. To determine the dose-response curve of the urine extract which was found to have an erythropoietic stimulatory effect, 50-200 mg. of urine extract per 100 Gm. body weight was injected, subcutaneously, into polycythemic mice on the fifth day following removal from the hypoxic environment. Sixty hours after each mouse received the last injection of the material to be tested, 0.5 μc. 58FeCl3 was injected into the tail vein, and 48 hr. later, the mice were exsanguinated. The percent incorporation of 58Fe into erythrocytes was determined as a measure of the erythropoietic activity.11 Mice with hematocrits of less than 50 per cent were excluded.

The total blood volume of the patient was determined by the dilution technic, employing radio-iodinated serum albumin.12 Plasma-iron turnover rate and erythrocyte utilization of injected radio-iron were determined according to the method of Huff.13

RESULTS AND COMMENTS

The total blood volume, plasma volume, and red cell volume of the patient were increased over that of the normal predicted values for adults in our laboratory (Table 1).

Table 1.—Hematologic Values of a Patient with Hypertrophy of the Juxtaglomerular Apparatus Compared with the Predicted Values of His Weight

<table>
<thead>
<tr>
<th>Determination</th>
<th>Patient</th>
<th>Predicted for Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBV:</td>
<td>1362</td>
<td>1078</td>
</tr>
<tr>
<td>ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ml./kg.</td>
<td>89.5</td>
<td>71</td>
</tr>
<tr>
<td>RCV:</td>
<td>526</td>
<td>445</td>
</tr>
<tr>
<td>ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ml./kg.</td>
<td>35.0</td>
<td>27-29.0</td>
</tr>
<tr>
<td>PV:</td>
<td>836</td>
<td>626</td>
</tr>
<tr>
<td>ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ml./kg.</td>
<td>54.5</td>
<td>41.0</td>
</tr>
<tr>
<td>PITH:</td>
<td>13.3</td>
<td>38</td>
</tr>
<tr>
<td>iron mg./24 hr.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mg./kg./24 hr.</td>
<td>0.875</td>
<td>0.45-0.75</td>
</tr>
<tr>
<td>T ½ (min.)</td>
<td>44</td>
<td>60-120</td>
</tr>
<tr>
<td>10 days % 59Fe into RBC</td>
<td>65</td>
<td>60-100%</td>
</tr>
</tbody>
</table>
Fig. 1.—48 hour per cent incorporation of $^{59}$Fe into erythrocytes of polycythemic mice: dose response curve of urine extract and erythropoietic activity of plasma from a patient with hypertrophy of the JGA compared to dose response curve of erythropoietin. Minimum of five mice per group.

In prepuberal subjects, the red cell mass of males and females are similar, and fall within the adult female range (23.4 ml/Kg.)$^{14}$ According to the weight and height of the patient, the total blood volume should have been in the range of 900–1000 ml., the plasma volume 550–600 ml., and the red cell volume between 350–400 ml., or for his weight, 23–26 ml./Kg.$^{15,16}$ Despite the increased plasma volume, his hemoglobin ranged between 15–17 Gm. per cent, and he had a slight reticulocytosis.

The clearance time of 50 per cent of $^{59}$Fe-tagged plasma (T½) was decreased from that of the normal, and the 24 hour plasma-iron turnover rate per kilogram was increased. Utilization of radio-iron by erythrocytes was normal (Table 1).

All urine extracts assayed in polycythemic mice contained erythropoietic-stimulating activity. The dose-response curve paralleled that of a purified sheep erythropoietin. Plasma from this patient was also erythropoietically active, containing the equivalent of 0.55 u of erythropoietin per ml. of plasma (Fig. 1). Normal urine extracted by this method and normal human plasma$^{10}$ do not contain measurable erythropoietic activity.

**DISCUSSION**

Polycythemia and increased erythropoietin levels in the plasma and urine of a normotensive child with proven hypertrophy of the JGA associated with increased aldosterone secretion, blood renin, and angiotensin levels strongly suggest a correlation between the JGA and erythropoietin production since the
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aldosterone-renin-angiotensin system is known to be related to this group of
cells. Erythrocytosis, increased aldosterone, and erythropoietin levels have re-
cently been reported in a hypertensive patient with an adrenal adenoma who
had normal blood renin levels. Renin, aldosterone, and angiotensin II did not
stimulate erythropoiesis in starved rats, and maintenance of rats on low sodium
diets in order to activate the endogenous aldosterone-renin-angiotensin system
failed to increase the red cell mass, indicating that these hormones did not
directly stimulate erythropoiesis, and that endogenous activation of the aldos-
terone-renin-angiotensin system did not result in stimulation of erythropoie-
tin. Continuous intravenous drip of angiotensin II into rabbits resulted in
increased incorporation of $^{59}$Fe into erythrocytes. However, this was accom-
panied by reduced renal blood flow, and the increased erythropoietin produc-
tion was thought to be due to induction of renal ischemia.

Production of erythropoietin in the JGA has been difficult to demonstrate in
experimental animals. The experimental procedures employed in lower ani-
imals to induce erythropoietin production, such as renal artery constriction,
induction of hemolytic or hemorrhagic anemia, or those employed to suppress
endogenous erythropoietin such as hypertransfusion, also stimulate and sup-
press, respectively, the renin-angiotensin system. The changes in granularity
of the JGA in response to such stimuli to both systems then become difficult to
evaluate. A specific stimulus to erythropoietin, exposure to hypoxia, did not
alter the granularity of the JGA of rats, although erythropoietin levels were
increased. However, Mitus and Toyama made significant observations in
hydronephrotic rabbits, only some of which developed erythrocytosis. Those
rabbits which did develop erythrocytosis showed decreased granularity and
hyperplasia of the JGA cells, while hydronephrotic rabbits not demonstrating
erthrocytosis had normal granularity and hypoplasia of the JGA, suggesting
some functional relationship between these cells and erythropoietin production.

Attempts to demonstrate such a relationship between the juxtaglomerular
apparatus and erythropoietin immunologically are inconclusive. Application of
fluorescent antibody to sheep erythropoietin to renal tissue obtained from
sheep exposed to hypoxia failed to demonstrate fluorescence in the JGA, while
fluorescence was apparent in the capillary walls of the glomerular tufts. On
the other hand, if erythropoietin is produced at some site other than the kidney
and is activated by a renal factor described in lower animals by some
authors then antisera against erythropoietin would necessarily fail to show
fluorescence in renal tissue. Alternately, erythropoietin, if produced in the JGA,
may be present in a precursor form, and its antigen receptor sites may not be
available for combination with antisera.

Despite conflicting experimental evidence, the observed hypertrophy of the
JGA, increased erythropoietin levels, and activation of the aldosterone-renin-
angiotensin system in this patient suggest, but do not directly demonstrate, an
interdependence between the JGA and erythropoietin or a renal erythropoietic
factor in man. The hypertrophy of the JGA in this patient is undoubtedly also
related to the activation of the aldosterone-renin-angiotensin system with its
associated metabolic abnormalities. The interesting associated elevation of
erythropoietin and the polycythemia appear to indicate that overactivity of this system also resulted directly or indirectly in the observed effect on erythropoietin and secondarily on erythropoiesis. The persistent elevation of angiotensin in this patient could lead to renal ischemia secondary to reduced renal blood flow with subsequent increased production of erythropoietin or a renal erythropoietic factor, a situation similar to that produced in experimental animals. This was, however, not demonstrable in the patient since renal function and clearance appeared to be normal.

Both the presence of hypervolemia leading to overdistention of the renal intravascular bed and the presence of an increased red cell mass which would be expected to produce a hyperoxic, rather than a hypoxic, environment, suggest that another more direct mechanism of stimulation could be responsible for increased erythropoietin production. It is not clear whether this was a hormonal effect or due to undetectable renal ischemia. Perhaps further experiments in this direction will uncover a more direct relationship between these various hormones.

SUMMARY

A patient with proven hypertrophy of the juxtaglomerular apparatus was found to be polycythemic and hypervolemic. Increased levels of erythropoietin were present in the urine and plasma collected from the patient. The associated increase in aldosterone secretion rate, elevated blood renin and angiotensin levels suggest the juxtaglomerular apparatus may directly or indirectly play a role in the production of erythropoietin.

SUMMARIO IN INTERLINGUA

Un patiente con provate hypertrophia del apparato juxtaglomerular esseva recognoscite como polycythemic e hypervolemic. Augmentate nivellos de erythropoietina esseva presente in le urina e le plasma colligite ab le patiente. Le associate augmento in le intensitate del secretion de aldosterona e le elevate concentrationes de renina e angiotensina in le sanguine suggestiona que le apparato juxtaglomerular ha un rolo directe o indirecte in le production de erythropoietina.

ACKNOWLEDGMENTS

The authors are indebted to Dr. G. H. Nickerson for the opportunity to study this patient, and to Dr. N. K. M. de Leeuw for the use of laboratory facilities required in the investigation of iron-kinetic studies in this patient.

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