Effects of Partial Hepatectomy on Extrarenal Erythropoietin Production in Rats

By A. Anagnostou, S. Schade, J. Barone, and W. Fried

Studies were performed to determine the effects of partial hepatectomy on extrarenal erythropoietin production. Rats were either partially hepatectomized or sham operated. At intervals of from 5 min to 7 days afterward, both kidneys were removed from cohorts of the above two groups of rats and the animals were then exposed to hypoxia for 7.5 hr. Immediately afterward, their plasma was collected and its erythropoietin titer was assayed. Rats which were partially hepatectomized 2-4 days prior to nephrectomy and hypoxia had significantly higher plasma erythropoietin levels than did sham-operated controls, whereas rats hepatectomized 5 min, 1 day, or 7 days prior to nephrectomy and hypoxia did not. These data are consistent with the conclusion that extrarenal erythropoietin production is enhanced in association with rapid regeneration of hepatic cells.

ERYTHROPOIETIN (Ep), the hormone that regulates erythropoiesis, is produced mainly by the kidney. The liver has been suggested as a major site for deactivation of Ep, although several investigators have reported evidence to the contrary. Increased plasma Ep levels occur in some experimental and clinical conditions associated with liver damage. This phenomenon has generally been attributed to a decrease in the catabolic rate of the hormone. However, it is also possible that it results from increased Ep production by either damaged or regenerating liver cells. In recent years, the liver has been shown to produce a small, albeit significant, amount of Ep in nephrectomized, intensely hypoxic adult animals and to be the major site of Ep production in fetal and neonatal animals. The experiments to be reported here have been designed to show the effects of removing 50%-60% of the liver on extrarenal Ep production of rats. In these studies extrarenal Ep production has been determined at various times following partial hepatectomy.

MATERIALS AND METHODS

Female Sprague-Dawley rats weighing 200 g were used. They were maintained on regular rat chow (Purina Co., St. Louis, Mo.) and tap water ad libitum. Hypoxia was induced in a steel hypobaric chamber. The atmospheric pressure in the chamber was controlled by a vacuum pump and a spring valve. Removal of 50%-60% of the liver was carried out by excision of the median lobe and part of the left lateral lobe of the liver. Subtotal splenectomies were performed by amputating two-thirds of the spleen, leaving the hilum and adjacent tissue intact. Sham operations consisted of making an incision in the abdomen, identical to that made during partial hepatectomy, and then closing it with clips. Some groups had more traumatic sham operations, which consisted of making three large abdominal incisions, exteriorizing all of the bowel, the stomach, and the...
Table 1. Extrarenal Ep Production at Various Times After Partial Hepatectomy

<table>
<thead>
<tr>
<th>Time Interval Between Partial Hepatectomy or Sham Operation &amp; Bilateral Nephrectomy/Hypoxic Exposure</th>
<th>Partial Hepatectomy</th>
<th>Sham Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>59Fe Uptake Into RBC of Assay Mice*</td>
<td>Ep/ml Plasma</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(units)</td>
</tr>
<tr>
<td>5 min</td>
<td>0.9 ± 0.1 N.D.</td>
<td>3.0 ± 0.2 N.D.</td>
</tr>
<tr>
<td>Average</td>
<td>1.0 ± 0.1 N.D.</td>
<td>1.0 ± 0.1 N.D.</td>
</tr>
<tr>
<td>1 day</td>
<td>2.9 ± 2.0 N.D.</td>
<td>4.9 ± 0.4 0.03</td>
</tr>
<tr>
<td>Average</td>
<td>2.0 ± 0.2 N.D.</td>
<td>2.8 ± 0.2 N.D.</td>
</tr>
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<td></td>
<td>3.2 ± 0.3 N.D.</td>
<td>2.6 ± 0.3 N.D.</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>4.1 0.03</td>
</tr>
<tr>
<td>2 days</td>
<td>28.6 ± 3.10 0.72</td>
<td>5.0 ± 0.4 0.04</td>
</tr>
<tr>
<td>Average</td>
<td>14.0 ± 1.20 0.12</td>
<td>4.7 ± 0.5 0.04</td>
</tr>
<tr>
<td></td>
<td>21.3</td>
<td>4.8 0.04</td>
</tr>
<tr>
<td>3 days</td>
<td>15.6 ± 1.4 0.14</td>
<td>5.4 ± 0.4 0.04</td>
</tr>
<tr>
<td></td>
<td>12.4 ± 1.1 0.10</td>
<td>4.8 ± 0.5 0.04</td>
</tr>
<tr>
<td></td>
<td>18.6 ± 1.6 0.21</td>
<td>2.1 ± 0.2 N.D.</td>
</tr>
<tr>
<td></td>
<td>21.0 ± 2.2 0.28</td>
<td>3.2 ± 0.2 N.D.</td>
</tr>
<tr>
<td>Average</td>
<td>16.9</td>
<td>3.9 0.04</td>
</tr>
<tr>
<td>4 days</td>
<td>19.5 ± 2.0 0.24</td>
<td>8.1 ± 0.7 0.05</td>
</tr>
<tr>
<td></td>
<td>14.4 ± 1.2 0.12</td>
<td>4.8 ± 0.5 0.04</td>
</tr>
<tr>
<td></td>
<td>15.4 ± 4.1 0.14</td>
<td>3.2 ± 0.3 N.D.</td>
</tr>
<tr>
<td>Average</td>
<td>16.4</td>
<td>5.4 0.04</td>
</tr>
<tr>
<td>7 days</td>
<td>4.6 ± 0.5 0.03</td>
<td>2.3 ± 0.3 N.D.</td>
</tr>
<tr>
<td></td>
<td>2.2 ± 0.1 N.D.</td>
<td>4.4 ± 0.3 0.03</td>
</tr>
<tr>
<td>Average</td>
<td>3.4</td>
<td>3.3 0.03</td>
</tr>
</tbody>
</table>

*Each value represents the mean result from a single experiment ± 1 SEM.
†N.D. refers to values below the linear portion of the Ep dose–response curve, which begins at 3.5% 59Fe uptake or 0.03 Ep units.

spleen, and then replacing them and closing all incisions with clips. Bilateral nephrectomies were performed via a ventral incision, just prior to exposing the rats to 0.4 atmosphere of air for 7.5 hr. Immediately upon termination of hypoxia, the animals were bled by cardiocentesis and the plasma was collected and frozen at −20 °C until its Ep titer was determined.

Plasma Ep levels were assayed in posthypoxic polycythemic mice by the method of Gordon and Weintraub17 (6–8 mice received 0.8 ml of pooled plasma obtained from 4 rats per group; plasma from rats with hematocrits of less than 43% were not used). Units were expressed as International Reference Preparation (IRP) units by comparison to a dose–response curve produced using a standard Ep preparations with a specific activity of 76 U/mg protein. The Ep dose–response curve in our laboratory was linear between 0.025 and 1.0 unit when plotted on semi-log paper.

A group of 5 partially hepatectomized rats, and another group of 5 sham-operated rats were bilaterally nephrectomized 3 days postoperatively. They were then made hypoxic (0.4 atmosphere) for 7 hr, after which they were exsanguinated by cardiocentesis. The P502 of blood collected from each rat was determined using an IL PH-Blood Gas Analyzer, model 217.† The significance of differences between groups was tested by Student’s t test.

*Human urinary erythropoietin pool H-10-Talsl. Collected and concentrated by the Department of Physiology, University of the Northeast, Corrientes, Argentina, further processed and assayed by Hematology Research Laboratories, Children’s Hospital of Los Angeles, under Research Grant HL 10800 from the National Heart and Lung Institute.
†P502 determinations were performed in the laboratory of Dr. Helen Mauer at the Children’s Memorial Hospital, Chicago, Ill. with the technical assistance of Loyda Vida.
Table 2. Effect of Partial Hepatectomy and Partial Splenectomy on Extrarenal Ep

<table>
<thead>
<tr>
<th></th>
<th>3 Days*</th>
<th>4 Days*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{59}$Fe Uptake Into RBC of Assay Mice (%), Ep/ml Plasma (units)</td>
<td>$^{59}$Fe Uptake Into RBC of Assay Mice (%), Ep/ml Plasma (units)</td>
</tr>
<tr>
<td>Sham operation</td>
<td>3.1 ± 0.3†, N.D.</td>
<td>6.5 ± 0.5, 0.04</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>3.2 ± 0.3, N.D.</td>
<td>6.6 ± 0.5, 0.04</td>
</tr>
<tr>
<td>Partial hepatectomy</td>
<td>21.0 ± 2.0, 0.28</td>
<td>14.4 ± 1.6, 0.12</td>
</tr>
</tbody>
</table>

*Time interval between partial hepatectomy or sham operation and bilateral nephrectomy/hypoxic exposure.
†Each value represents the mean result from a single experiment ± 1 SEM.
N.D. refers to values below the linear portion of the Ep dose–response curve which begins at 3.5% $^{59}$Fe uptake or 0.03 Ep units.

RESULTS

Table 1 shows that the plasma Ep titer of rats that were nephrectomized and made hypoxic 5 min or 1 day following partial hepatectomy was slightly but not significantly lower than that of control animals which were sham-operated rather than partially hepatectomized. Groups of rats which were partially hepatectomized 2, 3, or 4 days prior to nephrectomy and exposure to hypoxia had significantly higher plasma Ep levels than did their corresponding sham-operated controls. There was no significant difference between the plasma Ep titer of control rats and that of rats which were partially hepatectomized 7 days prior to nephrectomy and exposure to hypoxia. The average wet liver weight of sham-operated rats was 5.9 g. One day posthepatectomy it had fallen to 3.1 g. Four days posthepatectomy it had regenerated to 6.0 g.

In order to determine if regeneration of other tissues also affected plasma Ep titers, rats had either two-thirds of the spleen removed, partial hepatectomy, or an especially traumatic laparotomy, as described in Materials and Methods. Three or four days later, the rats were bilaterally nephrectomized and exposed to 0.4 atmosphere of air for 7.5 hr. The plasma Ep titers of partially hepatectomized rats but not of partially splenectomized rats exceeded those of the sham-operated ones (Table 2).

To determine the effect of partial hepatectomy on Ep production by rats with intact kidneys, groups of 10 rats each were either partially hepatectomized or sham operated. Three and four days afterward, cohorts of 5 rats per group were

Table 3. Effect of Partial Hepatectomy or Sham Operation on Ep Production by Nonnephrectomized Rats

<table>
<thead>
<tr>
<th></th>
<th>3 Days*</th>
<th>4 Days*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$^{59}$Fe Uptake Into RBC of Assay Mice (%), Ep/ml Plasma (units)</td>
<td>$^{59}$Fe Uptake Into RBC of Assay Mice (%), Ep/ml Plasma (units)</td>
</tr>
<tr>
<td>Sham operation</td>
<td>24.4 ± 1.8, 0.83</td>
<td>14.1 ± 1.3, 0.12</td>
</tr>
<tr>
<td>Partial hepatectomy</td>
<td>23.2 ± 1.2, 1.05†</td>
<td>16.1 ± 1.8, 0.16†</td>
</tr>
</tbody>
</table>

*Time interval between partial hepatectomy or sham-operation and hypoxic exposure.
†Not significantly different from sham-operated mice, p > 0.1.
exposed to 0.5 atmosphere for 7 hr, and then were exsanguinated, and their plasma was collected and assayed for Ep. The results, shown in Table 3, indicate that the plasma Ep levels of partially hepatectomized, but nonnephrectomized, rats did not differ significantly from those of sham-operated ones. However, the results could not be considered definitive since the plasma Ep levels of sham-operated rats were already high and the sites of Ep production may therefore be relatively insensitive to conditions which would increase them even further.

$P_{50}O_2$ determinations were made on blood from partially hepatectomized and sham-operated rats that were nephrectomized 3 days postoperatively. These were $35.3 \pm 0.1$ and $29.8 \pm 0.1$ respectively (the difference was significant at $p < 0.01$).

**DISCUSSION**

Erythropoietin is a glycoprotein produced mainly by the kidneys.\(^1\) In adult nephrectomized animals and in fetal and neonatal animals, a small amount of Ep can also be generated by the liver,\(^14\, 16,18,19\) and possibly by the spleen.\(^17\) Although the current state of knowledge regarding the Ep molecule does not permit definitive identification of the substance present in the plasma of anephric rats as Ep, it is likely to be Ep for the following reasons: (1) it stimulates \(^{59}\)Fe incorporation when injected into polycythemic mice; (2) it stimulates heme synthesis by rat marrow cells in liquid culture; and (3) its biologic activity is neutralized by rabbit serum containing antibodies to human urinary Ep.\(^{21,22}\)

Little is known about Ep metabolism. Some experimental evidence has suggested that the liver is a major site of Ep deactivation,\(^2\, 8\) whereas other data are in opposition to this concept.\(^9,10\) The observation that administration of phenylhydrazine or carbon tetrachloride to experimental animals is followed by an increase in the plasma Ep level has been attributed to a decrease in the rate of inactivation of the hormone because of liver injury.\(^3,5\) Similarly, increased plasma Ep levels have been reported in patients with parenchymal liver disease.\(^11,12\)

The results of the studies which we are reporting here are not readily explained by the concept that Ep is less rapidly deactivated because of a reduction in hepatic mass and function. If this were the case, then one would expect that plasma Ep levels would be highest in rats hepatectomized within 1 day of nephrectomy and exposure to hypoxia.

Numerous investigators have studied the regeneration of liver cells following partial resection of the liver.\(^23,24\) A net gain in cellular RNA content is demonstrable 12 hr postoperatively. The mitotic activity of the hepatocytes is maximal about 24–30 hr after partial hepatectomy, whereas peak mitotic activity of the littoral cells (which comprise 30%–40% of the total hepatic cell population but only 5%–10% of the cellular volume) occurs approximately 1 day later, i.e., at the beginning of the third day after removal of 70% of the liver. The liver weight doubles within 2 days and returns to its preoperative level 1 wk following 70% hepatectomy. In our experiments the liver weight has been restored to preoperative levels 4 days postoperatively; however, only 50%–60% of the liver mass had been removed.

The mechanism by which the plasma Ep titer rises during liver regeneration
is not clear at this time. The increased plasma Ep levels of partially hepa-
tomized rats are unlikely to be due to metabolic changes occurring between 2
and 4 days posthepatectomy that alter the hemoglobin oxygen dissociation
curve, since one would expect this to affect renal as well as extrarenal Ep
production.

One might argue that shifts in the oxygen dissociation curve occur in par-
tially hepatectomized rats only if they are also nephrectomized 2-4 days post-
hepatectomy. However, the \( P_{30}O_2 \) of partially hepatectomized rats was higher
than that of sham operated ones, indicating a lower hemoglobin oxygen affinity.
This, change, if anything, would tend to increase oxygen delivery to the tissues.

Several possible explanations of the phenomenon observed here are offered
for consideration. First, recent studies have reported Ep production to be in-
creased in anephric rats with Kupffer cell hyperplasia induced by administra-
tion of zymosan or colloidal carbon. Accordingly, the proliferation of littoral
cells on the third day after partial hepatectomy may be the factor responsible
for the increased production of Ep observed at that time.

Second, an increase in liver lysosomal enzymes has been reported to follow
immediately the burst of mitotic activity in the regenerating liver. In this re-
gard, the lysosomal fractions of kidney and liver homogenates have been re-
ported to contain Ep, proerythropoietin, and erythrogenin. These lysosomal
enzymes may originate in either hepatocytes or littoral cells.

Third, the regenerating liver has been reported to produce \( \alpha \)-fetoprotein
and, possibly, other growth factors for a variety of tissues. Conceivably, these
substances may stimulate extrarenal Ep production, although this has not been
experimentally tested. Additionally, since both \( \alpha \)-fetoglobulin and Ep are pro-
duced more effectively by fetal than by adult livers, and both appear to be in-
creased in the plasma of animals undergoing liver regeneration, it is conceivable
that regenerating adult liver cells are capable of doing some of the metabolic
functions that are performed by fetal but not resting adult liver cells. Finally,
the actively regenerating hepatic tissue may distort and compress the hepatic
vessels, thereby inducing local hypoxia and acting as a stimulus for Ep produc-
tion.

In a recent clinical study, anephric patients maintained by hemodialysis were
found to have increased hemoglobin levels after a bout of serum hepatitis. In
the light of the results of our experiments, it is possible that this intriguing ob-
servation can be explained by an increase in Ep production by regenerating
liver cells.

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