Erythropoiesis in Rats with Acute Uremia and the Effect of Erythropoietin

By Efstratios S. Kurtides, Walter A. Rambach and Howard L. Alt

THE INCORPORATION of tritiated thymidine by the rat spleen is an accurate and reliable index of erythropoiesis under certain well-defined experimental conditions.1 Since tritiated thymidine uptake by various tissues reflects DNA synthesis, and therefore proliferative activity, changes in the latter will be reflected by parallel changes in the thymidine uptake.

In the current experiments, tritiated thymidine incorporation by spleen, bone marrow, intestine and thymus was studied in normal and acutely uremic rats. In addition, the effect of exogenous erythropoietin on uremic erythropoiesis was investigated in these animals.

MATERIAL AND METHODS

The experimental animals were female, albino rats of a Sprague-Dawley strain, ranging in weight from 190 to 300 Gm. All boiled filtrates of anemic rabbit plasma were prepared according to a previously described method.2 The resultant lyophilizate, hereafter called ESF (erythropoiesis stimulating factor), was solubilized in normal saline in a concentration of 7.0 mg. per ml. Two ml. as a single subcutaneous dose was injected in experimental animals, whereas control animals received an equal volume of normal saline.

Tritiated thymidine with a specific activity of 2.0 mc. per millimole was injected as a single intravenous dose of 20.0 µc. via the tail vein. Animals so studied were sacrificed by aortic exsanguination, under ether anesthesia, 60 minutes after the injection.

Acutely uremic animals were prepared, under ether anesthesia, by bilateral nephrectomy through a midline abdominal incision. Control animals were sham operated. Forty-eight hours later the animals were injected with tritiated thymidine. In those animals given ESF the lyophilizate was injected 30 minutes postoperatively. The spleen, bone marrow content of both femurs and tibias, the thymus and a 1.0 cm. segment of the upper jejunum were removed and weighed. The tissue content of tritium was then determined by a modification of the oxygen combustion-liquid scintillation method of Kelly, et al.3 as previously described.1

In a second group of animals, bilateral ureter ligation was performed at the ureterovesical junction. These and sham operated controls were studied in a manner similar to the nephrectomized group.

In a third group of animals, ferrokinetic determinations were performed. Control and nephrectomized animals, with or without ESF injections, were studied. Fe59 uptake by rat bone marrow and spleen reaches a peak 6 hours after an intravenous injection.2 Accordingly, Fe59 with a specific activity of 4.74 mc. per mg. of elemental iron was injected intravenously in a single dose of 0.2 µc. 42 hours after nephrectomy, and the 6-hour level of the label was determined in the spleen, bone marrow, red cells and plasma.2

Finally, an evaluation was made of the extent to which total tissue uptake of tritiated...
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Table 1.—Effect of ESF and Uremia on H3Tdr Uptake by the Rat Spleen and Bone Marrow

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Hct ( (%) )</th>
<th>Retics. ( % )</th>
<th>Splenic Uptake ( %/Gm. )</th>
<th>Bone Marrow Uptake ( %/Gm. )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>21</td>
<td>41.8 ± 2.8</td>
<td>1.9 ± 0.5</td>
<td>0.97 ± 0.31</td>
<td>21 2.4 ± 0.55</td>
</tr>
<tr>
<td>ESF</td>
<td>17</td>
<td>42.0 ± 3.2</td>
<td>3.5 ± 1.1</td>
<td>2.58 ± 0.81</td>
<td>17 2.4 ± 0.62</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>20</td>
<td>33.3 ± 3.4</td>
<td>2.6 ± 0.9</td>
<td>0.63 ± 0.20</td>
<td>16 1.5 ± 0.50</td>
</tr>
<tr>
<td>Nephrectomy and ESF</td>
<td>12</td>
<td>35.6 ± 2.8</td>
<td>3.9 ± 0.6</td>
<td>1.74 ± 0.50</td>
<td>6   1.9 ± 0.50</td>
</tr>
<tr>
<td>Bilateral Ureteral Ligation</td>
<td>13</td>
<td>41.2 ± 5.2</td>
<td>3.3 ± 0.9</td>
<td>0.82 ± 0.20</td>
<td>7   2.1 ± 0.80</td>
</tr>
<tr>
<td>Bilateral Ureteral Ligation and ESF</td>
<td>4</td>
<td>40.0 ± 4.0</td>
<td>2.5 ± 0.8</td>
<td>3.42 ± 0.90</td>
<td>8   3.8 ± 0.50</td>
</tr>
</tbody>
</table>

Underlined figures are significantly different from their appropriate controls at the 1 per cent level (p < 0.01).

5 Mean ± 1 S.D.

Table 2.—Effect of ESF and Uremia on H3Tdr Uptake by the Rat Thymus and Intestine

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>H3Tdr Uptake ( %/Gm. )</th>
<th>Thymus</th>
<th>No. of Animals</th>
<th>H3Tdr Uptake ( %/Gm. )</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>11</td>
<td>0.62 ± 0.10</td>
<td>13</td>
<td>1.54 ± 0.50</td>
<td>14</td>
<td>1.49 ± 0.83</td>
</tr>
<tr>
<td>ESF</td>
<td>5</td>
<td>0.60 ± 0.07</td>
<td>10</td>
<td>1.31 ± 0.53</td>
<td>15</td>
<td>1.74 ± 0.40</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>10</td>
<td>0.32 ± 0.10</td>
<td>15</td>
<td>1.74 ± 0.40</td>
<td>14</td>
<td>1.49 ± 0.83</td>
</tr>
<tr>
<td>Nephrectomy and ESF</td>
<td>4</td>
<td>0.25 ± 0.08</td>
<td>14</td>
<td>1.49 ± 0.83</td>
<td>14</td>
<td>1.49 ± 0.83</td>
</tr>
<tr>
<td>Bilateral Ureteral Ligation</td>
<td>13</td>
<td>0.41 ± 0.20</td>
<td>10</td>
<td>1.61 ± 0.70</td>
<td>15</td>
<td>1.84 ± 0.29</td>
</tr>
<tr>
<td>Bilateral Ureteral Ligation and ESF</td>
<td>—</td>
<td>—</td>
<td>8</td>
<td>1.84 ± 0.29</td>
<td>8</td>
<td>1.84 ± 0.29</td>
</tr>
</tbody>
</table>

Underlined figures are significantly lower than the normal control (p < 0.01 and p < 0.02).

*Mean ± 1 S.D.

Thymidine reflects actual DNA incorporation. Normal and nephrectomized animals with or without ESF stimulation when studied. The spleen was removed 60 minutes after tritiated thymidine injection, weighed and divided into three parts. In one part the tritium content was determined as described above. From the remaining parts the total nucleic acids (TNA) were extracted by the Schmidt-Thannhauser technic.4 The final KOH-TNA hydrolyzate obtained by this method was volumetrically pipetted into Visking plastic bags and dried under an infrared lamp. The tritium content of these samples was then determined by the method described above. Originally DNA was separated from RNA. However, as was to be expected, no tritium label was found in RNA. Therefore, in simplification, the TNA fraction was studied, and the activity present was considered representative of DNA. Tritium in DNA was expressed as percent of total tritiated thymidine injected per gram of tissue.

At the end of each experiment, blood was collected by aortic exsanguination and reticulocyte counts, microhematocrits and blood urea nitrogen determinations were obtained.

RESULTS

The data on normal and uremic animals is presented in tables 1 and 2. A severe uremia was present in all nephrectomized and bilateral ureter ligated
animals at 48 hours. The blood urea nitrogen values averaged 290 and 320 mg. per 100 ml. of serum, respectively. In nephrectomized animals a significant suppression of splenic, bone marrow and thymic uptake of thymidine was noted. Intestinal incorporation was not affected. In ligated animals, marrow, splenic and intestinal uptake was not suppressed. However, thymic uptake was depressed.

In normal animals a significant rise in reticulocyte counts and splenic uptake of thymidine was noted 48 hours after a single ESF injection. Bone marrow incorporation of thymidine remained unchanged. These results agree with our previously published findings. ESF injection at the time of nephrectomy caused a threefold rise in thymidine uptake by the spleen when compared to nephrectomized controls. No significant effect of ESF was noted on the bone marrow. In ligated animals ESF caused a greater rise in splenic thymidine uptake than it did in nephrectomized animals, whereas bone marrow changes were again insignificant.

ESF failed to increase thymidine uptake by the thymus and intestine in normal and nephrectomized rats. It similarly had no effect on the intestine in ureter ligated animals.

That tissue tritiated thymidine uptake is a reflection of DNA synthesis is confirmed by the results presented in table 3. As the total tissue uptake of tritiated thymidine is altered either by ESF stimulation, nephrectomy or a combination of these procedures, quite similar changes in the appropriate direction are noted in DNA incorporation values.

The 6-hour splenic Fe59 uptake rose more than threefold following ESF administration, while that in the bone marrow remained unchanged (table 4). This measure of increased splenic erythropoiesis is corroborated by a significantly increased red cell Fe59 incorporation (from 7.4 ± 2.2 to 16.3 ± 2.6 per cent; p value less than 0.01), and an accelerated plasma iron clearance (from 6.5 ± 2.7 to 0.8 ± 0.4 per cent of total counts injected remaining in the circulating plasma at the end of 6 hours; p value less than 0.02).

Nephrectomy was followed by a depression in splenic and bone marrow uptake of Fe59, with a return to normal values when ESF was administered at the time of operation.

**Discussion**

Attention was first drawn to the kidney as a source of erythropoietin by Jacobson and associates,5,6 and the literature has been reviewed by Gordon7
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Table 4.—The Effect of ESF on the Ferrokinetics in Normal and Nephrectomized Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Total Splenic Uptake*</th>
<th>Total Bone Marrow Uptake*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25</td>
<td>2.8 ± 1.3f</td>
<td>24.3 ± 8.4f</td>
</tr>
<tr>
<td>ESF</td>
<td>4</td>
<td>10.2 ± 1.8</td>
<td>24.4 ± 3.6</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>13</td>
<td>1.50 ± 0.7</td>
<td>18.2 ± 9.1</td>
</tr>
<tr>
<td>Nephrectomy and ESF</td>
<td>11</td>
<td>2.6 ± 1.2</td>
<td>24.2 ± 6.3</td>
</tr>
</tbody>
</table>

Underlined figures are significantly different from their control with p of 0.05 or less.

*Per cent of total counts injected in whole organ at 6 hours.
† Mean ± 1 S.D.

and Stohlman. In the current experiments an effort has been made to gain further insight into the relationship between erythropoiesis, the kidney, and the pathogenesis of the anemia of renal disease. Whether one may justifiably relate the results obtained in acute uremia after bilateral nephrectomy or ureter ligation to the anemia known to be present in the slowly progressive, chronic renal disease of humans is a moot point. However, until studies on chronic renal disease in animals are completed, the present data may allow some hypotheses to be drawn.

Uremia present in the rat 48 hours after bilateral nephrectomy is associated with a significant suppression of thymidine uptake in the bone marrow, spleen and thymus, but not the intestine. In the equally uremic bilateral ureter ligated animal the thymidine uptake was unaffected in the bone marrow, spleen and intestine, but was significantly depressed in the thymus. Thus, nephrectomy and ureter ligation do not similarly affect all tissues. The intestinal uptake of thymidine is unaffected in uremia irrespective of the presence or absence of the kidneys. The uptake in the thymus is suppressed whether or not kidneys are present. The response of the bone marrow and spleen depends on the presence or absence of renal tissue, for it would appear that in acute uremia renal tissue is singularly essential for bone marrow and splenic proliferative activity. The reasons for such differences in tissue response are not readily apparent.

Though thymidine incorporation by erythroid tissues is increased in the uremic animal after the administration of ESF, the response in the nephrectomized rat differs significantly from that in the ureter ligated animal. This difference is particularly well seen in the spleen, which in the rat has been shown to be an organ which sensitively responds to erythropoietic stimulation. In the ureter ligated rat the response is in excess of that seen in the intact animal. In the nephrectomized rat the absolute response is subnormal, though the magnitude of the increase (factor of 2.7) is the same as noted in the normal animal. The fact that a difference in response to erythropoietin exists between renoprival and ureter ligated animals suggests that the uremic state in these animals, though quantitatively the same, differs in quality. The specific nature of such a postulated difference has been discussed by Jacobson and Reissmann, but no firm conclusions can be drawn. However, it would appear that the kidney, in addition to being a source of erythropoietin, plays additional roles in the control of erythropoiesis. The kidney may activate
circulating erythropoietin, or it might facilitate the end organ response to ESF. Reissmann has suggested the possibility that the kidney inactivates an inhibitor of erythropoiesis.

Our observation of the ability of administered ESF to increase erythropoiesis in the uremic animal agrees with other reported studies. The Fe$^{59}$ data is in particular accord with the Fe$^{59}$ uptake studies of Reissmann, et al., reflecting a normal response to ESF in ureter ligated animals and a subnormal response to a similar stimulus in the renoprival animal. In previously published studies, we have demonstrated that thymidine incorporation by the spleen, following ESF administration, specifically mirrors increased erythropoiesis. In the normal animals in the current study a good correlation is again noted between Fe$^{59}$ and tritiated thymidine incorporation following ESF stimulation. Therefore, it is of interest that in the uremic, ESF-treated animals there exists a discrepancy between Fe$^{59}$ and thymidine uptake values. Unless the approximate threefold increase in thymidine incorporation reflects ineffective mitotic activity, one would expect a near trebling of erythroid production, with an Fe$^{59}$ incorporation consistent with the new population size. Instead, the iron label only increases by 80 per cent. This suggests that in uremia there is a defect in iron utilization which is not corrected by ESF administration. The defect may be due to malutilization of iron by erythrocytic elements, or may indicate a relative unavailability of iron for red cell production. Since the anemia of uremia is normochromic, it is unlikely that the former mechanism is operative. It is more probable that the latter explanation is relevant, for we and others have presented evidence in human uremia which suggests that iron is abnormally shunted to stores.

The current studies have adequately demonstrated that total tissue tritiated thymidine uptake adequately reflects DNA synthesis, since factorial increases in DNA incorporation following ESF stimulation parallel tissue incorporation. In the nonstimulated animal that portion of the label not appearing in the DNA resides in precursor pools of thymidine, nucleosides and nucleotides. The data reveals that under stimulation, there is not only an increase in DNA incorporation, but also the activation of some mechanism which increases the size of the precursor pool to meet the demand of increased cell proliferation.

The suppression of thymic proliferative activity in uremia suggests a possible relationship between a diminished immune response and the increased incidence of infection noted in human uremic populations. The failure of the thymus to respond to ESF has been previously demonstrated by us and Smellie et al., and serves to document the functional difference that may exist between tissue of similar morphology such as the spleen and the thymus.

Summary

The proliferative activity of erythropoietic and other tissues has been studied in normal, nephrectomized and bilateral ureter ligated rats.

Forty-eight hours after bilateral nephrectomy, rats showed a significant suppression of DNA synthesis and Fe$^{59}$ incorporation in the bone marrow and spleen. The administration of erythropoietin prevented this suppression
of Fe\textsuperscript{59} uptake, and produced an increase in splenic DNA synthesis comparable to that noted in the normal, similarly stimulated animal. Bilateral ureter ligation in animals, producing a blood urea nitrogen elevation equal to that induced by nephrectomy, caused no suppression of DNA synthesis in bone marrow or spleen. These latter animals exceeded the normal in their response to erythropoietin administration.

Intestinal DNA synthesis was unaffected in uremia produced by nephrectomy or ureter ligation. Thymic proliferative activity was suppressed in uremia induced by either procedure.

The observations indicate that acute, severe uremia of relatively short duration does not influence the DNA synthetic activity of all tissues in an adverse fashion. What factors may modify tissue responses are unknown. In the case of erythropoietic organs, such factors seem to originate in the kidney.

SUMMARIO IN INTERLINGUA

Le activitate proliferative de tissus erythropoietic e altere esseva studiate in rattos normal, in rattos nephrectomisate, e in rattos con bilateral ligation ureteral.

Quaranta-octo horas post nephrectomia bilateral, le rattos monstrava un significative suppression del synthese de acido deoxyribonucleic e del incorporation de Fe\textsuperscript{59} in le medulla ossee e le splen. Le administration de erythropoietina preveniva iste suppression del incorporation de Fe\textsuperscript{59} e produceva un augmento del synthese splenic de acido deoxyribonucleic comparabile a illo notate in normal animales que esseva stimulates in le mesme manera. Bilateral ligation de ureter, producente un elevation del nitrogeno de urea in le sanguine equal a illo induceite per nephrectomia, causava nulle suppression del synthese de acido deoxyribonucleic in le medulla ossee o in le splen. Le animales in iste ultime gruppo excedeva le normales in lor responsa al administration de erythropoetina.

Le synthese de acido deoxyribonucleic intestinal non esseva afficite in uremia producite per nephrectomia o ligation ureteral. Activitate proliferative de thymo esseva supprimite in uremia inducite per le un o le altere procedimento.

Le observationes indica que acute uremia de grado sever que ha un relativemente curte duration non affice adversemente le synthese de acido deoxyribonucleic de omne le tissus. Qual factores ha le capacitate de modificar le responsas del tissus non es cognoscite. In le caso del organos erythropoietic il pare que tal factores ha lor origine in le ren.

REFERENCES

3. Kelly, R. G., Peets, E. A., Gordon, S., and Buyske, D. A.: Determination of C\textsuperscript{14} and H\textsuperscript{3} in biological samples by Schöninger combustion and liquid

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