Experimental Studies on Erythropoietin. II. The Relationship between Juxtaglomerular Cells and Erythropoietin

By KUNITAKE HIRASHIMA AND FUMIMARO TAKAKU

SINCE the study of Jacobson1 in 1957, the renal production of erythropoietin has been suggested by many investigators.2-5 However, the exact site of production in the kidney has remained unsettled.5,7 On the other hand, from their morphologic characteristics, some endocrine function has been ascribed to the juxtaglomerular cells (JG cells).8 Secretory-like granules of JG cells were especially stained by the method introduced by Wilson.9 Using this staining method, attempts have been made to correlate the granularity of the JG cells, which has been reported to be especially striking in renal ischemia, whether in man or experimental animals.10,11

In our previous studies,12 the erythropoiesis assessed by changes in reticulocyte counts and red cell incorporation of radioiron, and plasma erythropoietin levels as determined with the bioassay method using starved rats as described by Fried et al.,13 were markedly increased in animals with constricted renal arteries.

From these results, suggesting the production of erythropoietin from the ischemic kidney, the following experiments were undertaken in order to study the relationship between erythropoiesis and the granularity of the JG cells in detail.

METHODS

Wistar female rats weighing about 150 Gm. were used throughout the experiments. All rats were fed "Oriental" rat chow.

Normal Control

Seven normal rats served as controls (Group I).

Unilateral Constriction of the Renal Artery

Five rats (Group II) were operated according to the method described previously.12 Through an abdominal approach, the left renal artery was separated from surrounding tissues. A silver ring, 0.3 mm. or less in diameter and 1-1.5 mm. in width, was applied around the artery approximately midway between the aorta and renal pelvis. After the application, the ring was pressed with a forceps so that an apparent narrowing took place.

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in the distal part of the artery. On the 7th day of operation, animals were sacrificed and the kidney examined.

**Bleeding**

From seven rats (Group III), blood corresponding to two per cent of body weight was withdrawn by cardiac puncture.

On the 20th hour of bleeding, animals were sacrificed. To investigate the time course of the change in JG cells after bleeding, each group of rats, consisting of three or five rats, was sacrificed and examined on the 3rd, 7th, and 20th hour and on the 4th, 7th and 10th day.

**Phenyldrazine Induced Anemia**

To induce severe anemia, 0.5 ml. of 5 per cent acetylphenylhydrazine solution was injected subcutaneously in five rats (Group IV) on four successive days. On the 5th day, the animals were sacrificed.

**Transfusion-Induced Polycythemia**

Five ml. of packed washed red cells was injected intraperitoneally in five rats (Group V) on two successive days. On the 3rd day, the animals were sacrificed.

**Histologic Procedure**

All animals were anesthetized with ether to remove both kidneys, which were fixed in Bouin's fluid immediately. Specimens were imbedded in paraffin in the usual manner. Three sections were stained according to Harada instead of the commonly used Wilson's method.

**Staining Procedure (Harada's Method)**

(1) The paraffin section was passed rapidly through xylol and alcohol, and washed in running tap water. (2) The section was immersed overnight in a 4 x 10^-6 gr./dl-aqueous gentian violet solution, (3) differentiated in a 1:2 mixture of aniline and xylol in less than three minutes; (4) rinsed in two changes of xylol, and mounted with balsam.

**Indices of Granulation of Juxtaglomerular Cells (JGI)**

The granularity of JG cells was estimated with the juxtaglomerular index (JGI) according to the method described by Hartroft. Two separate sections, prepared from each kidney, were systematically scanned and the number of glomeruli encountered is related to the granularity of the JG cells seen. The JGI thus expressed the granularity of the JG cells per 100 glomeruli.

**RESULT**

Utilizing the staining method of Harada, the granules in JG cells were intensely stained deep purple and were detectable under lower power (fig. 1). According to the Hartroft method of estimating granularity of JG cells, table 1 shows the average and standard error of JGI in each group.

**JGI of Rats with Stimulated Erythropoiesis**

Following unilateral constriction of the renal artery, "clipped" kidneys tended to increase their juxtaglomerular granulation while the granulation in "untouched" kidneys was reduced (Group II).

When rats were rendered anemic, whether by bleeding or by administration
Fig. 1.—Microphotographs of juxtaglomerular cells stained by the Harada method (x1000). JG cells in the afferent arterioles are shown. The arrows point to JG granules. Above: JG cells packed so densely with granules that the ground substance of the cytoplasm is obscure and the cells appear swollen. Below: JG cells showing a great number of granules scattered throughout the cytoplasm.

of phenylhydrazine (Group III or IV), the JG index increased significantly \( (p < 0.02) \) (table 1).

Twenty hours after bleeding, the mean red blood cell count was reduced from 751 to 471 x 10^4 cu. mm. in Group III, with the JGI 40.3.
Table 1.—The Changes of JGI on Various Hematopoietic Conditions

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Procedure</th>
<th>Indices of granularity of JG cells—JGI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7</td>
<td>normal control</td>
<td>25.3 ± 1.5*</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>Unil. const. of renal artery</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&quot;clipped&quot; kidney</td>
<td>43.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&quot;untouched&quot; kidney</td>
<td>19.1</td>
</tr>
<tr>
<td>III</td>
<td>9</td>
<td>bleeding</td>
<td>40.3 ± 2.9</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>phenylhydrazine-induced anemia</td>
<td>41.3 ± 3.7</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>transfusion-induced polycythemia</td>
<td>8.8 ± 0.8</td>
</tr>
</tbody>
</table>

*Mean ± standard error.

After the injection of phenylhydrazine, the mean red blood cell count was reduced from 599 to 358 x 10⁴ cu. mm. in Group IV with the mean reticulocyte count elevating from 2.6 to 71.5 per cent and the JGI 41.3.

**JGI of Rats with Depressed Erythropoiesis**

When rats were made polycythemic by transfusion, the JGI was significantly reduced (p < 0.01), giving a sharp contrast to the increase in the anemic state. After the transfusion of red blood cells, the mean red blood cell count of Group V increased from 751 to 930 x 10⁴ cu. mm. with the mean reticulocyte count decreasing from 4.2 to 2.4 per cent and the JGI 8.8.

**Time Course of JGI After Bleeding**

Figure 2 illustrates the changes in granularity of JG cells at various intervals after single massive bleeding (two per cent of body weight).

Red blood cell and reticulocyte counts were also followed in another group of five rats similarly bled. The JGI rose sharply at about 20 hours after bleeding, reaching the maximum at the 4th day and thereafter declining sharply.

On the other hand, the increase of reticulocytes started on the 1st day of bleeding, reaching the maximum on the 4th day. The red blood cell count recovered rapidly and reached the prebleeding value around the 7th day after hemorrhage.

**DISCUSSION**

The juxtaglomerular cells first described by Ruyter in 1925 consist of several epitheloid cells near the glomerular hilus, and in close proximity to the macula densa. The JG cells and macula densa together compose the juxtaglomerular apparatus. The cells described in this paper do not include the macula densa.

Regarding the function of JG cells, Goormaghtigh was the first to speculate on the endocrine function of JG cells secreting vasopressor substance—renin. Early works in this field were concerned primarily with the phenomenon of hyperplasia rather than hypergranulation. Since the discovery of the new staining method of JG cell granules, their endocrine function has been widely studied. Through the method estimating the granularity of JG cells, attempts have been made recently to correlate the JG cells with hypertension, blood electrolytes and the effects of mineralocorticoids.
Comparing the staining method of Wilson, the gentian violet staining method by Harada which was used in this investigation was recommended by its simplicity and high specificity for JG granules. The JGI of Hartroft is considered a usual and practical method of estimating relative differences in numbers of juxtaglomerular granules, though an exact quantitative method of assessing degrees of juxtaglomerular granulation has not been reported. This study was performed by the combination of the Harada method for staining and the Hartroft method for estimating the granularity of JG cells.

Based on our previous observation of an increased erythropoiesis and erythropoietin production in animals with ischemic kidneys, the production of erythropoietin by ischemic kidneys was postulated. Through the histologic studies on the ischemic kidneys, we confirmed the hypergranularity in the clipped kidneys which suggested the production for erythropoietin by JG cells. Based on this assumption, further studies were carried out on the changes of JG cell granularity in other states of stimulated erythropoiesis—after bleeding and phenylhydrazine injection.

The JGI of the kidneys in these states was markedly increased to the level in the clipped kidneys. On the contrary, on the state of depressed erythro-
poiesis and erythropoietin production—the polycythemic state after hyper-transfusion—the JGI was significantly decreased. Furthermore, the JGI was found to be changing in parallel with erythropoiesis and/or the production of erythropoietin throughout the course of anemia after bleeding.

Although the changes in blood volume, blood pressure and electrolytes in bleeding, phenylhydrazine-induced anemia and transfusion-induced polycythemia might also be responsible for changes in the granularity of JG cells, it is quite possible that such changes in the granularity of JG cells may be related to procedures which would alter the production of erythropoietin as shown in our experiments.

In 1958, Osnes suggested, without detailed data, that the JG cells secreted erythropoietin. His assumption was based on his observation of a distinct reduction of the number of granules in JG cells in mice with anemia produced through daily bleeding—a result at variance with our present observation. On the relationship between the JG cell function and granularity, a body of available evidences favors the hypothesis that hypergranulation is present during periods of rapid secretion, and degranulation during periods of very slow or absent secretion.

Rats bled repeatedly for a prolonged period showed a hyperplasia of the JG cells according to the experiments by Bohle. Since hyperplasia as a rule represents an increased secretory activity in endocrine organs, such hypergranulation is probably accompanied by hypersecretion. Further investigation to isolate the substance represented by the JG granules is required to establish the physiologic significance and validity of our assumptions.

**Summary**

The relationship between erythropoietin production and the juxtaglomerular (JG) cells of the kidney were studied using Harada’s method for staining and Hartroft’s method for estimating JG granularity.

In stimulated erythropoiesis (after bleeding and phenylhydrazine injection), the JG cells also showed hypergranularity. In depressed erythropoiesis, on the contrary, the JG cells showed hypogranularity. Furthermore, from the observation of the chronological change of the JG cell, granularity tended to change in parallel with erythropoietin production. This study gave an indirect evidence of the secretion of erythropoietin by juxtaglomerular cells of the kidney.

**Summario in Interlingua**

Esseva studiate le relation inter le production de erythropoietina e le cellulas juxtaglomerular (JG) del ren con le uso del metodo de Harada pro le tincturation e del metodo de Hartroft pro le estimation del granularitate de cellulas JG.

In statos de stimulate erythropoiese (post sanguination e injection de phenylhydrazina) le cellulas JG etiam monstrava hypergranularitate. In erythropoiese deprimite, del altere latere, le cellulas JG monstrava hypogranularitate. In
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plus, observationes serial del cellulas JG revelava alterationes de granularitate con un tendentia de occurrer in parallela con le production de erythropoietina. Iste studio fcrni evidentia indirecte del secretion de erythropoietina per le cellulas JG del ren.

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