
By WALTER FRIED, HARRY P. WARD AND ALAN R. HOPEMAN

ERYTHROCYTOSIS has been associated with various types of tumors. In some of these cases, the tumor was found to contain a substance which was similar to erythropoietin both immunochemically and in biologic activity. We shall here describe what we believe to be the first reported case of a patient with a leiomyoma of the esophagus and erythrocytosis. Experiments to determine the etiology of the erythrocytosis suggested that the tumor contained a substance that was not erythropoietin but had the ability to stimulate the production of erythropoietin by the kidneys.

MATERIALS AND METHODS

Assay of Erythropoietin

The in vivo assay for erythropoietin was performed according to the method of DeGowin et al., using the polycythemic mouse. The in vitro assay for erythropoietin has been described recently. In brief: canine bone marrow is suspended in 50 per cent NCTC 109 and 50 per cent fetal calf serum. A volume of 0.2 ml. of the serum to be assayed is added to 2.6 ml. of the culture suspension. After twenty hours of incubation in a water-saturated atmosphere of 5 per cent CO₂ and room air at 37°C, 0.2 ml. of ⁵⁹FeCl suspended in autologous dog serum is added to the culture. The cultures are "harvested" after six additional hours of incubation and ⁵⁹Fe heme is extracted with the method of Teale. The level of erythropoietin is determined by comparing ⁵⁹Fe incorporation into heme in the unknown sample with the log-dose response line obtained with three dilutions of a known erythropoietin-rich serum assayed simultaneously with the unknown serum. An erythropoietin level of 0.05 units/ml. consistently demonstrates an incorporation of ⁵⁹Fe into heme that is 25 per cent greater than the control cultures prepared with pooled normal 0+ serum. The serum erythropoietin level of thirty-four normal patients had a mean value of 5.6 per cent greater than the control pooled serum with a standard deviation of 13.24.

Chemical Extraction of the Tumors

Immediately after surgery, a portion of the tumor was frozen and maintained in that state until the following extraction procedures were performed. A saline extract was prepared by placing the tumor in saline (1 Gm. tumor per ml. of saline) and grinding it in an omnimixer for 3 min. After centrifugation, the supernatant was collected and frozen for use in the experiments which were performed in vivo. In extracts prepared for assay in the in vitro system, the supernatant was dialyzed over night against phosphate buffered saline at 4°C before use.

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Table 1.—Blood Volume Determinations

<table>
<thead>
<tr>
<th>Date</th>
<th>Total Blood Vol. (cc/Kg. wt.)</th>
<th>RBC Vol. (cc/Kg. wt.)</th>
<th>Plasma Vol. (cc/Kg. wt.)</th>
<th>Hematocrit (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>January 29, 1966</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>56</td>
</tr>
<tr>
<td>February 8, 1966</td>
<td>84.0</td>
<td>47.0</td>
<td>37.0</td>
<td>56</td>
</tr>
<tr>
<td>March 14, 1966</td>
<td>72.0</td>
<td>32.5</td>
<td>39.8</td>
<td>43</td>
</tr>
<tr>
<td>April 1, 1966</td>
<td>63.6</td>
<td>29.0</td>
<td>34.6</td>
<td>45</td>
</tr>
<tr>
<td>May 9, 1966</td>
<td>84.8</td>
<td>29.7</td>
<td>55.1</td>
<td>35</td>
</tr>
<tr>
<td>June 26, 1967</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>45</td>
</tr>
<tr>
<td>Normal values</td>
<td>53–78</td>
<td>23–32</td>
<td>30–47</td>
<td></td>
</tr>
</tbody>
</table>

Chloroform-soluble extracts of the tumor were prepared by adding 1 ml. of chloroform per Gm. of residue after removing the supernatant saline extract. This was ground with a mortar and pestle for 15 min. after which the suspension was centrifuged and the supernatant separated. The supernatant was evaporated to dryness in a glass dish under a hood. The residual white powder was then wetted with 2 cc. of Tween-18 per 20 Gm. of original tumor weight and suspended in saline at a concentration of 1 ml. of saline per Gm. of original tumor weight. A control mixture was prepared which contained 1 ml. of Tween-18 per 10 ml. of saline.

Case Report

C. R., a 42 year old white male, was admitted to Fitzsimons General Hospital on January 29, 1966 for evaluation of a right hilar mass noted on a routine chest x-ray that was obtained prior to admission for the surgical repair of inguinal hernias. Except for the presence of bilateral inguinal hernias of eight years duration, there were no complaints. He denied chest pain, cough, shortness of breath, and hemoptysis. There was no history of dysphagia, dysphonia, nausea, vomiting, or weight loss. The patient had smoked cigarettes for twenty years and, during the past ten years, averaged two packs per day. A chest roentgenogram taken nine years prior to admission was unavailable for review but, presumably, had been interpreted as a normal examination. The past history and family history were unremarkable.

On physical examination the patient was a well nourished, muscular, white male with moderate plethora of his head and neck. Other than bilateral inguinal hernias, the physical examination was normal.

The hematocrit was 56 per cent, hemoglobin was 15.7 Gm./100 ml., and the red cell count was 5.97 million/cu. mm. The white blood count was 12,200 with a normal differential, the phase platelet count was 327,000, and the reticuloocyte count was 1 per cent. Radiosotope blood volume studies are summarized in Table 1. Prior to surgery, the 51Cr RBC volume was significantly elevated at a level of 47 cc./Kg. of actual weight.

Roentgenograms of the chest showed a right hilar mass at the level of the aortic arch. Laminograms demonstrated that the mass was located in the mediastinum, and there was no evidence of calcification. A barium swallow showed a smooth, regular mass, most likely intrinsic in the wall of the esophagus.

Normal laboratory values included a urinalysis, blood sugar, urea, Cephalin flocculation, albumin and globulin, alkaline phosphatase, and serology for syphilis. The total bilirubin was 1.2 mg. per cent with a direct fraction of 0.3 mg. per cent. The following pulmonary evaluations were performed and all tests were normal: vital capacity, FEV1, MBC, and arterial blood gas analysis. An intravenous pyelogram was normal. A bone marrow was not performed.

On February 17, 1966, surgery was performed and a large esophageal leiomyoma was resected. The tumor weighed 50 Gm. and showed the typical histologic appearance of a leiomyoma.

The patient had a stormy postoperative course caused by an empyema of the pleural space. Diplococcus pneumoniae and Staphylococcus aureus were cultured from the wound. On March 3, 1966 a thoracotomy with open drainage of the empyema was performed.
Table 2.—The Effect of Tumor Extracts on the Ability of Hypoxic Mice to Produce Erythropoietin

<table>
<thead>
<tr>
<th>Mice No.</th>
<th>Hematocrit</th>
<th>$^{59}\text{Fe}$ Per Cent Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Mixture</td>
<td>9</td>
<td>49±0.2</td>
</tr>
<tr>
<td>Saline Extract</td>
<td>11</td>
<td>49±0.3</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>12</td>
<td>49±0.2</td>
</tr>
</tbody>
</table>

Patient improved rapidly after this procedure and was discharged on May 9, 1966, with a hematocrit of 35 per cent and a red cell volume of 29 cc./Kg wt. (Table 1). At this time the plasma volume was increased to a supranormal level. The reason for this was not clear. The patient had received no intravenous fluids for over ten days prior to this date, making iatrogenic over hydration unlikely. We have been unable to personally evaluate the patient during the past year, but he has been recently contacted and it was learned that his hematocrit had returned to normal and remained so. A hematocrit taken on June 26, 1967 was 45 per cent.

Special Studies

The serum erythropoietin before surgery was elevated at a level of 131 per cent (normal 32 per cent), as measured by the in vitro assay. This percentage increase over the control represented approximately 0.6 erythropoietin units/ml when compared with the log-dose response regression of three dilutions of a known erythropoietin-rich serum previously assayed against Erythropoietin Standard B with the in vivo technic.* Following surgery, the serum was assayed for erythropoietin every other day for ten days and no significant level of erythropoietin could be demonstrated.

A volume of 0.2 ml and 0.4 ml of a saline extract of the tumor failed to stimulate $^{59}\text{Fe}$ incorporation into heme in the in vitro culture system and, in a similar manner, a volume of 1.0 ml failed to stimulate $^{59}\text{Fe}$ uptake into RBC's of plethoric mice in the in vivo assay.

The effect of tumor extracts on the plasma erythropoietin levels of mice which were exposed to hypoxia was determined as follows: three groups of ten mice received five daily 0.5 ml injections, subcutaneously, of either saline extract of tumor, chloroform extract of tumor, or of a control mixture. One hour after the final injection, they were placed in a chamber which was evacuated to one half of normal atmospheric pressure and maintained at this pressure for eight hours. Immediately after removal, the mice were exsanguinated. Their hematocrits were determined and their plasma was collected for assay of erythropoietin by the in vivo method. Table 2 shows the combined results from two experiments performed in this manner. The results are expressed as the percentage $^{59}\text{Fe}$ uptake into RBC's of polycythemic mice after injection of 0.3 cc. of plasma, subcutaneously.

Mice which received saline extract and those which received chloroform extract had higher plasma erythropoietin titers after exposure to hypoxia than those which received the control mixture ($p < .001$). The chloroform extract had the most marked effect.

*Kindly performed by Dr. N. Gesink, National Jewish Hospital, Denver, Colo.
DISCUSSION

Although several patients have been reported who have had leiomyomas of the uterus associated with erythrocytosis, we believe that this is the first reported case of a patient with a leiomyoma of the esophagus and erythrocytosis. The etiologic relationship between the tumor and erythrocytosis is supported by the demonstration of a decrease in the patient's red cell mass and serum erythropoietin levels to normal values after surgical removal of the tumor. A number of cases have been reported of patients who have developed erythrocytosis as a result of ectopic erythropoietin production by a tumor. Using crude saline extracts, we were unable to demonstrate the presence of substances with the biologic activity of erythropoietin in this tumor. Because of the animal work demonstrating the ability of testosterone to stimulate erythropoietin, the possibility of a tumor factor that was not erythropoietin but could stimulate the production of erythropoietin was evaluated. When mice, injected with either saline or chloroform extracts of the tumor, were exposed to hypoxia, their plasma erythropoietin titer rose to a higher level than did that of mice which received a control mixture prior to being made hypoxic. This effect was similar in degree to the previous study with testosterone. Unfortunately, the tumor extract was not evaluated for androgenic activity per se; however, there was no clinical evidence of increased androgen secretion in the patient.

SUMMARY

A patient with a leiomyoma of the esophagus and erythrocytosis is reported. Preliminary studies in mice suggest that the tumor contained a substance which was capable of increasing erythropoietin production by the kidneys. Because of the limited amount of tumor available, we were unable to further characterize this substance. We hope that this report will encourage other investigators to repeat and extend these studies.

SUMMARIO IN INTERLINGUA

Es reportate le caso de un patiente con un leiomyoma del esophago e erythrocytosis. Studios preliminari in muses suggestiona que le tumor contineva un substantia que esseva capace a augmentar le production de erythropoietina per le renes. Viste le restringite quantitate de tissu tumoric disponibile, nos trovava impossibile characterisar ille substantia plus detaliatemente. Nos spera que iste reporto va stimular altere investigatores a repeter e extender ise studios.

REFERENCES

Leiomyoma and Erythrocytosis: A Tumor Producing a Factor Which Increases Erythropoietin Production. Report of Case

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