Proceedings of the Society for Experimental Biology and Medicine

Vol. 126 | December 1967 | No. 3

SECTION MEETINGS

DISTRICT OF COLUMBIA
National Naval Medical Center — December 12, 1967
IOWA
University of Iowa — October 31, 1967

Serum and Urinary Levels of Erythropoietin in Iron Deficiency Anemia.* (32520)

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Increased amounts of erythropoietin have been detected in the plasma and urine of humans and several other animal species when subjected to hypoxia, acute bleeding or when severely anemic. During the past decade, erythropoietin has been reported to be elevated in a variety of primary hematologic disorders characterized by chronic anemia but was not detected in normal non-anemic humans until recently.

For this reason it was proposed that the usual physiological control of normal erythropoiesis was not mediated by erythropoietin but that it was a response called upon in extreme stress(1). In recent years, the development of more sensitive bioassay systems and various means of concentrating urine prior to assay, has led to the demonstration of erythropoietin in the urine of normal non-anemic humans(2-5). In addition it has been shown that administration of antiserum against erythropoietin to normal animals will cause anemia(6). Thus, the fundamental role of erythropoietin in controlling erythropoiesis in normal individuals has been elucidated.

Data reported thus far indicate that iron deficiency anemia is not associated with increased erythropoietin production unless the anemia is quite severe(1,7). Having detected increased erythropoietin in the serum and urine of some children with iron deficiency anemia of only moderate severity, we investigated the relationship between severity of anemia and erythropoietin activity in a larger number. By collecting serum and urine specimens concomitantly we were able to compare the activity in serum with that excreted in the urine at various levels of anemia.

Materials and methods. Clinical. Fifty-five unselected infants and children with iron deficiency secondary to inadequate dietary iron intake or chronic blood loss were the subjects of this study. The diagnosis in each case was established by routine

* Supported by USPHS Grants AM 04048, HE 10880 and 2 T1-HD 48.

hematologic study, supplemented in those with only mild to moderate anemia by determination of serum iron and iron binding capacity (22 cases) and determination of stainable marrow iron (14 cases). The anemia in all cases responded adequately to therapeutic administration of iron.

Samples of serum (48 cases) and of urine (26 cases) were obtained prior to any therapy and were promptly frozen until assayed for erythropoietin activity. From 22 patients, samples of serum and urine were obtained concomitantly so the relationship between serum erythropoietin level and urinary erythropoietin excretion could be studied.

Assays. Erythropoietin activity was measured by quantitating the increase in ^{59}Fe incorporation into circulating RBC of mice made polycythemic by chronic hypoxia and which were given injections of the material to be assayed.

The method employed was basically that of Cotes and Bangham(8) as modified in this laboratory. Virgin female B_6DF_1 mice,† specific pathogen free, and weighing 18 to 22 g were placed in a hypobaric chamber and maintained at ½ atmospheric pressure for 8 of each 24 hours for 14 days.

On the fourth and again on the fifth day after completing this treatment, they were given intraperitoneal injections of 0.2 or 0.4 ml of the material to be assayed. Whole unmodified serum and urine were assayed without concentrating or modifying them in any way. On the sixth day, 0.2 microcurie of $^{59}Fe\ Cl_3$ in phosphate buffer, pH 3.5, was injected into the tail vein. On the eighth day a sample of blood was obtained by cardiac puncture. Heparinized blood, 0.5 ml, was placed in a glass vial, hemolyzed by addition of 3 ml distilled water and its radioactivity determined. A portion of blood was also used for duplicate determination of hematocrit by the micro method. Animals with hematocrit below 52% were discarded from the experiment.

The ^{59}Fe uptake into newly circulating RBC was calculated as a percentage of the total dose given using a formula in which the blood volume was assumed to be 7% of body weight.

Groups of 5 mice were used for assay of each specimen. Each assay procedure included a group of mice given injections of a laboratory standard plasma from rabbits made anemic with phenylhydrazine and previously standardized against the international reference preparation of erythropoietin.‡ Each assay also included a group of mice given injections of a single pool of normal nonanemic human plasma as a negative control. This has been preferred to saline injections as a more physiological control although negative control values obtained with normal plasma are usually slightly higher than those produced by saline. In experiments in this laboratory involving several hundred mice and extending over a period of several years the negative control gave a mean ^{59}Fe uptake of $0.5 \pm 0.2\%$ of the tracer dose.

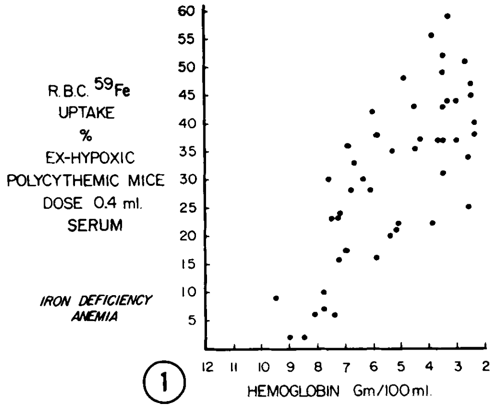
Results. The results of erythropoietin assays of the sera of 48 patients with varying degrees of mild to severe iron deficiency anemia plotted against the patients hemoglobin concentration are shown in Fig. 1.

There is a roughly linear direct relationship between the level of serum erythropoietin and the severity of anemia. Of interest is the finding of increased erythropoietin activity in the serum of patients with only mild iron deficiency. One patient with hemoglobin of 9.5 g/100 ml had an activity of 9%. This patient had chronic mild iron deficiency due to bleeding intestinal lesions. The possibility of a small bleed prior to obtaining the sample cannot be excluded.

Fig. 2 shows the results of erythropoietin assays of urine from 26 patients plotted against their hemoglobin concentration. Again, the activity of urinary erythropoietin increased with severity of the anemia. Increased activity was found in the urine of all patients studied including 2 with hemoglobin higher than 9 g/100 ml. One of these was the patient previously mentioned with chronic intermittent intestinal blood loss. The other was an 11-month-old infant with

† Simonsen Laboratories, Gilroy, Calif.

‡ Kindly supplied by Drs. Mary Cotes and D. R. Bangham, Dept. of Biological Standards, Medical Research Council of Great Britain.



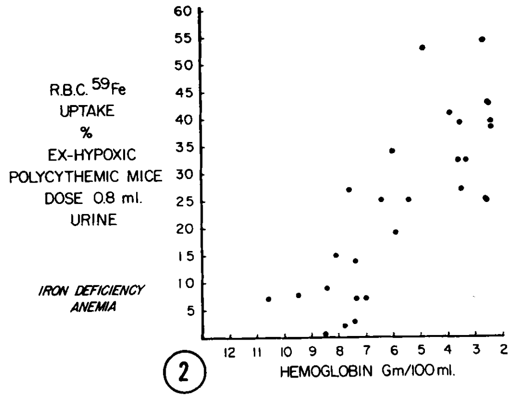


FIG. 1. Erythropoietin activity of serum from 48 patients with iron deficiency anemia related to their hemoglobin concentration at time of sampling.

FIG. 2. Erythropoietin activity in urine specimens from 26 patients with iron deficiency anemia related to their hemoglobin concentration at time of sampling.

nutritional iron deficiency, a serum iron level of 37 γ/100 ml and 11% saturation of iron binding protein.

In Fig. 3 the erythropoietin activity in serum of 22 patients is plotted against the activity found in a urine specimen obtained concomitantly. Serum was assayed in a total

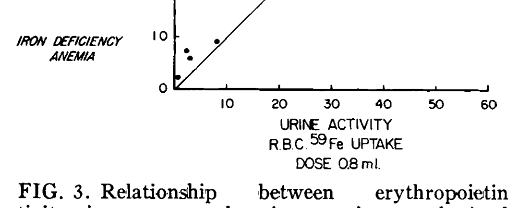


FIG. 3. Relationship between erythropoietin activity in serum and urine specimens obtained concomitantly from 22 patients with iron deficiency anemia. Bioassay of uriine was at adose twice that used for serum assay.

dose of 0.4 ml/mouse and urine in a dose of 0.8 ml/mouse.

Serum activity was generally greater than urine activity despite the larger dose employed for assays of urine. The data show a direct relationship between serum and urine erythropoietin at all levels of activity.

Discussion. Data on erythropoietin in patients with anemia due to iron deficiency are quite limited.

Van Dyke(9) using a starved rat assay initially reported finding increased erythropoietin activity in plasma or urine of only one of seven patients with iron deficiency anemia. He found no evidence of activity in either plasma or urine from 4 patients with hemoglobin between 3.5 and 6.5 g/100 ml secondary to chronic hookworm infection. Later Van Dyke *et al*(1) studied the relationship between degree of anemia and erythropoietin level in 11 adults with severe iron deficiency secondary to hookworm infestation and chronic blood loss. They found an increase in serum erythropoietin in 5 specimens from patients with hemoglobin concentration below 5 g/100 ml and had no increased activity over control values in 5 specimens from patients with hemoglobin between 5 and 10 g/100 ml.

Urine specimens from 11 patients showed increased erythropoietin activity only in 3 who had hemoglobin concentration of 4.1 g/100 ml or less.

In another group of patients with anemia due to a variety of "primary" hematologic disorders, they found increased erythropoietin activity in urine of patients with hemoglobin concentration up to 8 g/100 ml.

On the basis of these data it was suggested that in normal individuals who develop iron deficiency anemia due to chronic blood loss, erythropoietin acts to increase erythropoiesis only in extreme circumstances. It was postulated that in patients with "primary" hematologic disease, the increased erythropoietin activity found at more mild degrees of anemia may result from some other physiologic alteration.

Gallagher *et al*(7) assayed extracts of plasma of 3 patients with iron deficiency anemia. Erythropoietin activity was increased in 2 with hematocrits below 21% and not increased in one with a hematocrit of 29%. Starved or polycythemic rats were used for bioassays.

Iaroshevskii and Shekhter(10) reported finding a reticulocytosis stimulating effect in plasma from 3 to 8 cases of gastrogenic iron deficiency anemia when it was injected into rabbits. The severity of anemia was not stated.

Thus, it has been assumed from the limited data published that in iron deficiency anemia the level of plasma and urine erythropoietin is not significantly increased unless the anemia is quite severe.

The data reported here demonstrate that increased erythropoietin activity can be consistently detected in unconcentrated serum and urine from such patients even when the hemoglobin concentration is as high as 9-10 g/ml. Furthermore, the relationship between erythropoietin activity and the severity of anemia was roughly linear at all degrees of anemia studied. Thus, it does not appear that increased erythropoietin is found in this condition as a secondary erythropoietic mechanism called upon only when the anemia is severe.

The relationship between serum and urine erythropoietin activity was found to be directly proportional at all levels of anemia. The amount excreted in the urine therefore reflects the level in serum and from these studies appears to be approximately ⅓ to ½ that of the serum erythropoietin activity.

Various sources of erythropoietin of human origin have been sought to supply material for further research on its biochemical nature, mechanism of action and physiology. Erythropoietin from the urine of patients excreting significant amounts has been mentioned as probably the most convenient and least costly source(1) but such patients have been generally thought to be limited, for practical purposes, to those with erythroid hypoplasia and certain types of chronic hemolytic anemia(11).

It would appear that human sources of erythropoietin may be found among patients with moderate to severe iron deficiency anemia, a condition encountered not uncommonly in most clinics.

Summary. Study of the erythropoietin activity in unconcentrated serum and urine of 55 children with iron deficiency anemia revealed increased activity in both at hemoglobin levels up to 10 g/100 ml. The erythropoietin activity was directly related to the severity of anemia. There was direct correspondence between serum and urine erythropoietin activity of all degrees of anemia studied, and serum showed greater activity than urine obtained at the same time. It would appear that patients with moderate to severe iron deficiency anemia may provide an important source of human erythropoietin for use in research.

The authors would like to recognize the capable technical assistance of Mr. Charles Greene and Mr. Curtiss Polk.

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Received July 5, 1967. P.S.E.B.M., 1967, v126.