Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency

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Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. The relative importance of erythropoietin (Ep) and inhibition of erythropoiesis in the anemia of chronic renal insufficiency has been investigated. Sixty patients with varying degrees of renal insufficiency, 40 normal subjects and 40 patients with anemia and normal renal function, were studied. Erythroid (CFU-E) and granulocytic (CFU-GM) progenitor cell colony formation were assayed in fetal mouse liver and human bone marrow cultures, respectively. Erythropoietin was measured by radioimmunoassay. Hematocrit and plasma creatinine concentration correlated with the degree of serum inhibition of CFU-E formation (r = 0.69, $P < 0.001$, and r = 0.62, $P < 0.001$, respectively). Serum erythropoietin levels in patients with renal insufficiency (34.4 ± 6.7 mU/ml) were slightly higher than normal values (23.1 ± 0.98 mU/ml), but showed no relationship to plasma creatinine, hematocrit, or inhibition of CFU-E formation. In contrast, serum erythropoietin concentrations increased exponentially as the hematocrit decreased below 32% (r = 0.61, $P < 0.001$), and CFU-E formation was stimulated by serum in anemia patients with normal renal function. Studies of granulopoiesis showed uremic sera supported in vitro CFU-GM growth more efficiently than sera from normal subjects. These results suggest that inhibition of erythroid, but not granulocytic, progenitor cell formation, in addition to a relative erythropoietin deficiency, are the primary factors responsible for the anemia of chronic renal failure.

Déficit en érythropoïétine et inhibition de l'érythropoïèse au cours de l'insuffisance rénale. L'importance relative de l'érythropoïétine (Ep) et l'inhibition de l'érythropoïèse lors de l'anémie de l'insuffisance rénale chronique ont été étudiées. Soixante malades avec des degrés variables d'insuffisance rénale, 40 sujets normaux et 40 malades avec une anémie et une fonction rénale normale, ont été étudiés. La formation de colonies cellulaires souches érythroïde (CFU-E) et granulocytaire (CFU-GM) ont été mesurées dans du foie foetal de souris et dans des cultures de moelle osseuse humaine, respectivement. L'érythropoïétine a été mesurée par dosage radioimmunologique. L'hématocrite et la concentration plasmatique de créatinine étaient corrélées avec le degré d'inhibition sérique de la formation de CFU-E (r = 0,69, $P < 0,001$; et r = 0,62, $P < 0,001$, respectivement). Les niveaux d'érythropoïétine sérique chez les malades atteints d'insuffisance rénale (34,4 ± 6,7 mU/ml) étaient légèrement plus élevés que les valeurs normales (23,1 ± 0,98 mU/ml), mais il n'y avait pas de relation avec la créatininémie, l'hématocrite, ou l'inhibition de formation de CFU-E. A l'opposé, les concentrations d'érythropoïétine sérique s'élevaient exponentiellement au fur et à mesure que l'hématocrite diminuait en dessous de 32% (r = 0,61, $P < 0,001$), et la formation de CFU-E était stimulée par du sérum chez des malades anémiques avec une fonction rénale normale. Des études de la granulopoïèse ont montré que les sérums urémiques supportaient la croissance in vitro de CFU-GM de façon plus efficace que les sérums de sujets normaux. Ces résultats suggèrent que l'inhibition de formation de cellules souches érythroïdes et non granulocytaires, en plus d'un relatif déficit en érythropoïétine, sont les facteurs primaires responsables de l'anémie de l'insuffisance rénale chronique.

Patients with chronic renal failure invariably suffer from a normocytic normochromic anemia associated with a hypoproliferative bone marrow. The primary site of production of erythropoietin is the kidney although serum erythropoietin is detectable in surgically anephric patients. In these cases extrarenal erythropoietin is most probably produced in the liver. Inadequate erythropoietin production to sustain erythropoiesis has been considered as the primary etiologic factor in the anemia of renal disease. However, erythropoietin levels have been reported to be elevated, normal, and decreased in the serum of renal failure patients [1–4]. The relatively insensitive bioassay systems for serum erythropoietin are subject to considerable variability. The in vivo bioassay in polycythemic mice requires the concentration of large quantities of serum to detect normal levels; the various in vitro bioassays may be affected by inhibitors or stimulators of erythropoiesis also present in the serum. The radioimmunoassay for erythropoietin used in the present study has been validated [5], showing a high degree of correlation with the polycythemic mouse bioassay.

It seems probable that the deficiency of erythropoietin is relative to the increased demands made on the kidney in the uremic state created by shortened red cell life span [6], increased blood loss from various sources, and inhibition of erythropoiesis by the accumulation of retained uremic toxins [7]. These inhibitors of erythropoiesis include substances that effect both the progenitor cell compartments (CFU-E, colony-forming unit-erythroid; BFU-E, burst-forming unit-erythroid) [8–10] and heme synthesis [11–12]. It is possible that the decrease in heme synthesis is secondary and merely reflects inhibition of the more primitive erythropoietin responsive cell compartment. Inhibition of marrow thymidine incorporation by sera from patients with uremia has also been reported [13].

The identification of potential inhibitors of erythropoiesis has not been fully established. Urea, creatinine, and guanidinosuccinic acid are all potential inhibitors that are elevated in uremic sera, however, these compounds are not inhibitory to heme synthesis or CFU-E growth [8, 11] in the concentration range seen in renal failure. In vitro effects of PTH on erythroid

progenitor cells vary considerably; both stimulatory and inhibitory effects have been reported [14–16]. The association between hyperparathyroidism and anemia in renal failure patients may be related to bone marrow fibrosis [17]. The polyamine, spermine, inhibits CFU-E formation in both fetal mouse liver and human bone marrow cultures at concentrations similar to that found in uremic serum [9]. In addition, an antibody to spermine was found to remove the inhibitory effect of uremic serum on erythroid progenitor cells [9].

In the present study we have examined the effect of several factors on erythropoiesis in patients with varying degrees of impaired renal function. Firstly, serum erythropoietin levels were measured in renal failure patients and compared to those from patients with similar degrees of anemia with normal renal function. Secondly, serum inhibitors were measured by the CFU-E technique and were correlated with the degree of suppression of erythropoiesis.

Methods

Patients

(1) Sixty patients with varying degrees of renal insufficiency from the Tulane Medical Center Hospital, Veterans Administration Hospital, and Charity Hospital of New Orleans were studied after informed consent had been obtained. Their ages ranged from 17 to 94 years (mean ± SEM, 54 ± 2.0 years). Eighteen patients were female and 42 male. No patient had received any form of dialysis prior to the study. The majority of patients were receiving oral vitamin supplementation. No patient was receiving oral androgen therapy. The etiology of renal disease covered a wide spectrum of diseases including hypertension, 19 patients; diabetes mellitus, 9; glomerulonephritis, 12; polycystic kidney disease, 3; obstructive uropathy, 4; chronic pyelonephritis, 4; analgesic nephropathy, 1; Alport syndrome, 2; multiple myeloma, 1; gout uropathy, 1; congenital dysplasia, 1; unknown, 3. Four patients had evidence indicating other possible causes of their anemia apart from renal disease: Two patients had active peptic ulceration confirmed by endoscopy, one suffered from sickle cell anemia, and one patient had multiple myeloma.

(2) Forty normal subjects were selected from volunteer blood donors at the Ochsner Clinic, New Orleans, Louisiana. The ages of these subjects ranged from 23 to 63 years (mean ± SEM, 41 ± 2 years) and consisted of 12 females and 28 males. Hemoglobin concentrations ranged from 13.3 to 17.4 g/dl (mean ± SEM, 15.7 ± 0.17 g/dl). Two patients were receiving thiazide diuretic treatment for mild hypertension, and one patient was administered thyroid replacement therapy. All patients had normal renal function as measured by serum creatinine concentration.

(3) Twenty-seven female and 13 male patients were studied with varying degrees of anemia. These patients were attending the Hematology Clinic, Charity Hospital in New Orleans. All had normal renal function as measured by urinalysis and serum creatinine. Hematocrit levels ranged from 17.8 to 52.0%. Most of the patients were anemic with sickle cell anemia, 11; chronic gastrointestinal bleeding, 2; iron deficiency anemia, 8; thalassemia, 3; vitamin B_{12} deficiency, 4; folate deficiency, 1; methyldopa induced hemolysis, 1; aplastic anemia, 2; idiopathic thrombocytopenic purpura, 3; and polycythemia vera, 3. Their

Table 1. Effect of normal and uremic serum on granulocytic colony growth[a]

Experiment	Control	Normal serum	Uremic serum
1	86.6 ± 8.1	77.9 ± 4.7 (N = 8)	96.1 ± 3.4[b] (N = 14)
2	160.0 ± 5.8	110.9 ± 3.1 (N = 9)	135.6 ± 3.7[b] (N = 30)
3	70.7 ± 3.9	48.4 ± 2.3 (N = 9)	38.7 ± 4.0[c] (N = 15)

[a] The values represent the number of colonies/2×10^5 human marrow cells; numbers are means ± SEM.
[b] The value is significantly ($P < 0.01$) higher than in normal subjects.
[c] The value is significantly ($P < 0.05$) less than in normal subjects.

ages ranged from 15 to 81 years (mean ± SEM, 41.8 ± 3.2 years). After informed consent, 25 ml of blood were drawn from each patient and centrifuged. The serum was stored at −70°C in respective aliquots until the various assays were performed.

Investigations

Radioimmunoassay for serum erythropoietin [5]. Highly purified erythropoietin (70,400 U/mg protein) obtained from the National Heart, Lung and Blood Institute, Bethesda, Maryland, and prepared by Dr. Eugene Goldwasser's laboratory at the University of Chicago was labelled with iodine-125 by the chloramine T method of Greenwood and Hunter [18]. Erythropoietin antiserum was prepared in rabbits. A human urinary erythropoietin preparation with a specific activity of 80 U/mg protein obtained from the National Heart, Lung and Blood Institute[1] was used for the immunization. The antiserum was absorbed with normal human serum and urinary proteins to increase the specificity of the assay for erythropoietin. A second antibody (goat antirabbit gamma globulin) was used for the separation of bound from free labelled antigen.

Fetal mouse liver cell culture technique. Liver cells from 12 to 14-day-old fetuses of CD-1 mice were prepared according to the technique of Iscove, Sieber, and Winterhalter [19]. Liver cells were disaggregated and suspended as single cells at a concentration of 10^5 cells/ml in a culture medium containing alpha modified Eagle's medium with Earle's salts without ribosides and deoxyribosides and without glutamine, in methylcellulose, 30% fetal calf serum, 100 mU of human urinary erythropoietin[1], 0.1 μM mercaptoethanol, 100 mU of penicillin, and 100 μg of streptomycin. Human serum samples were heat-inactivated at 56°C for 30 min and sterile-filtered before being tested at concentrations of 5, 10, and 20%; the amount of fetal calf serum was altered accordingly to maintain a total serum concentration of 30%.

Cell culture (1 ml) was plated in 35×10 mm petri dishes and incubated for 48 hr at 37°C in a humidified atmosphere at 95% air and 5% CO_2. The pH was maintained constant as indicated by the color of the phenol red indicator present in the Eagle's
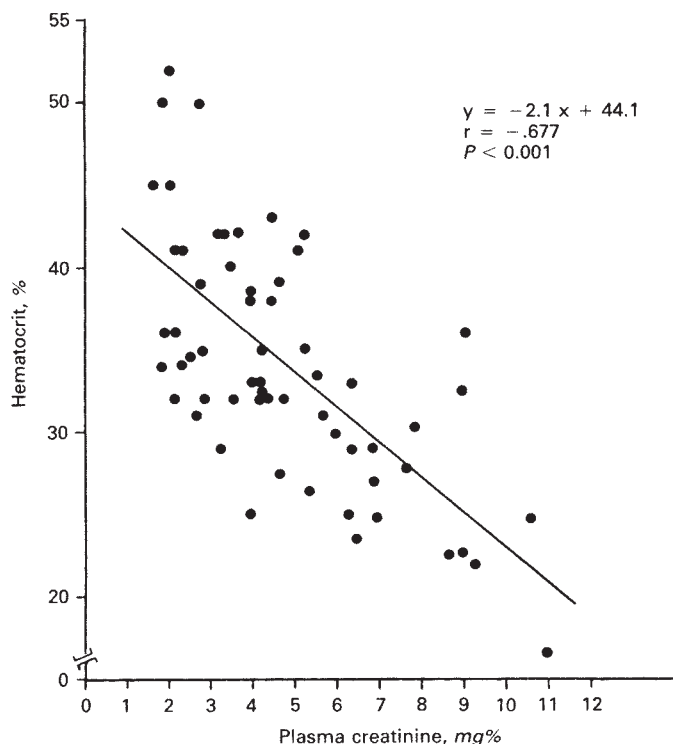
Fig. 1. *Relationship between hematocrit and plasma creatinine concentration in 60 patients with varying degrees of renal insufficiency.*

Fig. 2. *Comparison of hematocrit and inhibition of fetal mouse liver erythroid colony formation by sera from patients with renal insufficiency.* The *triangle* ▲ represents patients in whom another cause for anemia in addition to renal insufficiency was present. The concentration of serum in culture is 5%.

medium. After the plates were stained with diaminobenzidine according to the method of Ogawa et al [20], CFU-E of eight or more cells were counted in four replicate plates using an inverted microscope. One sixteenth of the plate area was counted and considered representative of the total plate area since very large numbers of colonies were present (1000 to 5000 colonies/plate). Only one batch of fetal calf serum (Flow Laboratories 29101607) and one batch of erythropoietin (sp act 10 U/mg protein) were used to minimize variations in erythroid colony formation. Human serum from one subject was used as a standard control. CFU-E formation was expressed as a percentage of the control.

Human bone marrow culture for granulocytic colony formation. The effect of serum on in vitro granulocytic progenitor cells was evaluated using an in vitro granulocyte colony-forming technique [21]. Following informed consent, bone marrow was aspirated from the posterior iliac crest from normal volunteers into heparinized syringes. After sedimentation at room temperature, the buffy coat was aspirated and washed twice in modified McCoy's medium. Each culture was prepared such that 2×10^5 marrow cells were immobilized in 1 ml of supplemented McCoy's medium containing 15% fetal calf serum in 0.3% agar. Cultures were plated in 10×35 mm tissue culture petri dishes (Corning stock #25000, Corning, New York). Five percent human fibroblast conditioned medium served as a source of colony-stimulating factor (CSF) [22]. Serum inhibitor activity was assessed by the addition of 10% normal serum or uremic serum to the cultures prior to the addition of the agar-cell mixture. Cultures were allowed to gel for 20 min and subsequently incubated in a well humidified 7.5% CO_2, 37°C atmosphere for 7 days. Colonies of more than
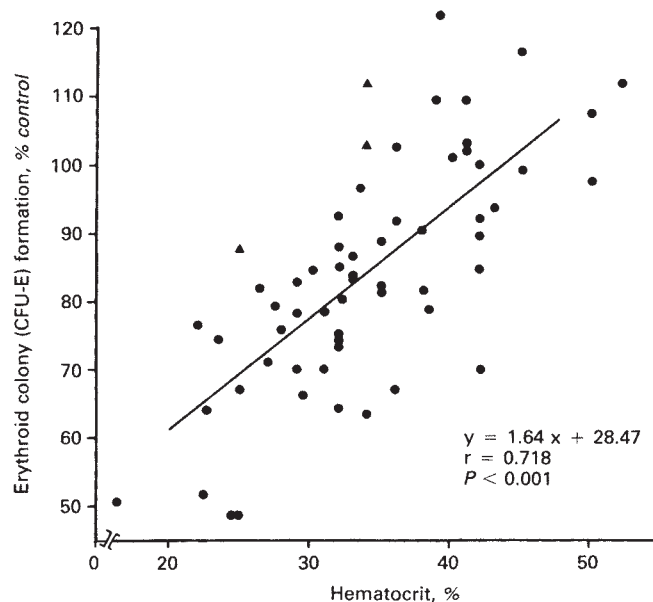
50 cells each were counted with the aid of a dissecting microscope [23]. Five cultures were prepared for each sample; values are expressed as means $\pm$ 1 SEM.

Serum CSF activity was measured using 2×10^5 non-glass adherent human bone cells [24]. This measure was used to remove endogenous CSF-producing cells from the marrow thereby eliminating spontaneous colony formation. Serum CSF was assessed by addition of 10% patient or control sera to the agar cultures.

Statistical analysis. Student's t test and linear regression analysis were used for statistical comparisons.

Results

In patients with impaired renal function the serum creatinine concentration ranged from 1.6 to 10.9 mg% and the hematocrit from 16.5 to 52%. The severity of anemia was found to be related inversely to the degree of impaired renal function as indicated by the hematocrit and serum creatinine concentration ($r = 0.677$, $P < 0.001$; Fig. 1). However, a wide scatter of hematocrit values was seen for the same level of renal function reflecting the multiple causes of renal failure and their numerous complications. In four patients subsequent investigations showed other causes for their anemia in addition to renal disease. One patient had sickle cell anemia, another patient had multiple myeloma, and two patients had active peptic ulcer disease. Figures 2 and 3 show the relationship between both hematocrit and serum creatinine concentration, and the degree of inhibition of erythroid progenitor cell (CFU-E) formation by uremic sera at a concentration of 5% in cell culture ($r = 0.72$, $P < 0.001$; and $r = -0.63$, $P < 0.001$, respectively). The lower the hematocrit and the higher the serum creatinine concentration, the greater the degree of inhibition of CFU-E formation. The four patients with additional causes for their anemia showed
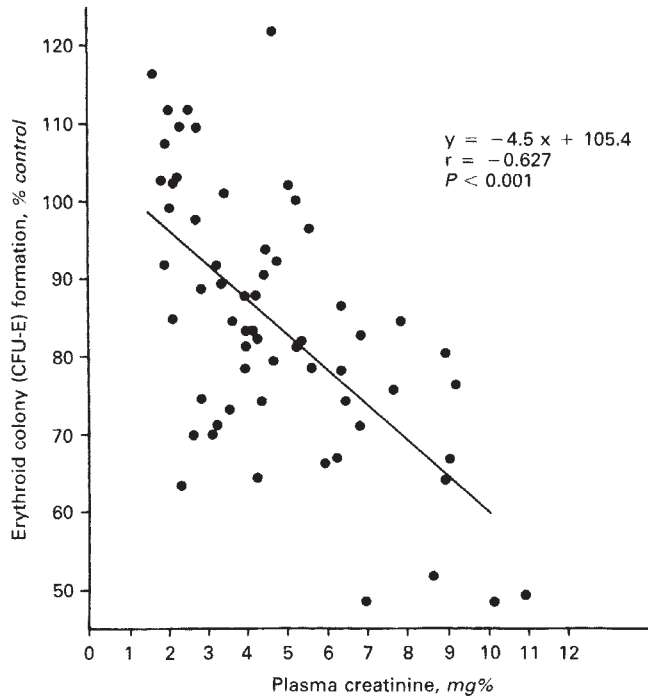
Fig. 3. *Relationship between plasma creatinine concentration and inhibition of erythroid colony formation in patients with renal insufficiency.* The concentration of patient's serum in culture is 5%.

Fig. 4. *Effect of different concentrations of uremic sera on erythroid colony formation in fetal mouse liver cultures.* Values are expressed as means ± SEM. The *asterisk* represents values significantly different ($P < 0.001$) from CFU-E formation in normal sera.

less inhibition of CFU-E formation than would be expected for the degree of anemia due to renal disease. CFU-E inhibition correlated with the plasma urea concentration in addition to the serum creatinine concentration. This association was significant in 5, 10, and 20% concentrations of uremic serum in fetal mouse liver cell cultures. Sera from the 60 patients with renal disease progressively reduced the growth of CFU-E from 83.5 ± 2.0% (mean ± SEM) to 68.5 ± 3.7% ($P < 0.05$) as the concentration of uremic serum in culture was increased from 5 to 20% (Fig. 4). This contrasted significantly ($P < 0.001$) from the 40 normal subjects studied whose serum did not demonstrate significant stimulation or inhibition of CFU-E formation at 5, 10, or 20% concentrations in culture.

The serum erythropoietin concentration in the renal failure patients did not correlate with the plasma creatinine concentration (Fig. 5), hematocrit (Fig. 6), or the degree of inhibition of erythroid colony formation (Fig. 7). Mean serum erythropoietin levels were elevated above the normal range in the 60 patients with renal disease although the difference between 34.67 ± 6.7 (mean ± SEM) and 23.1 ± 0.98 mU/ml was not significant and is mostly due to the elevation above normal values in seven patients and a marked elevation in serum erythropoietin concentrations in three patients. No specific cause for these markedly elevated levels of erythropoietin could be found in these three patients whose renal failure was due to focal segmental glomerulonephritis secondary to heroin addiction, hypertensive nephrosclerosis, and chronic glomerulonephritis.

In the 40 patients with hematologic disorders and normal renal function the serum erythropoietin concentration increased exponentially from 20 to 320 mU/ml with the decline in hematocrit from 52.0% to 17.8% ($r = -0.61$, $P < 0.001$) (Fig. 8).
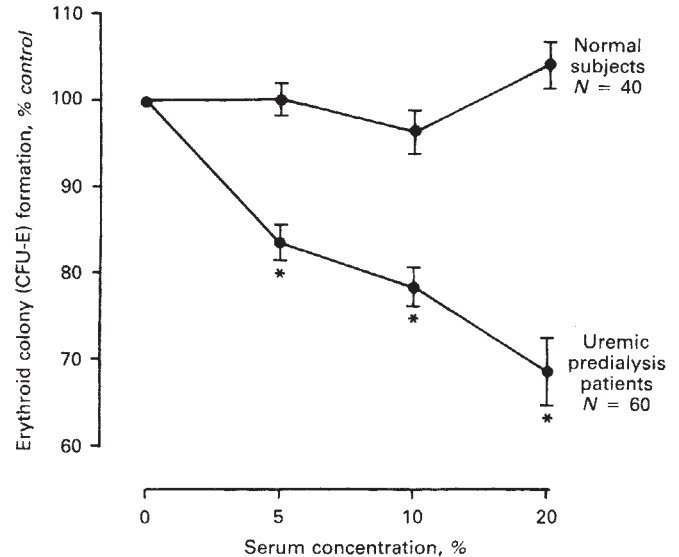
However, the increase in serum erythropoietin in response to anemia was not seen until the hematocrit decreased below a level of 32%. Furthermore, serum erythropoietin levels showed a significant positive correlation with stimulation of CFU-E formation ($r = 0.54$, $P < 0.01$) in the patients with anemia and normal renal function (Fig. 9). Anemic patients with normal renal function showed increasing stimulation of CFU-E formation with decreasing hematocrit values ($r = -0.73$, $P < 0.001$), which was the opposite to that found in patients with anemia and renal disease (Fig. 10). Three patients had a diagnosis of polycythemia vera. Their hematocrit ranged from 40.1 to 52% and their serum erythropoietin levels ranged from 23.6 to 41.2 mU/ml.

Studies of the effects of sera from patients with renal insufficiency on granulopoiesis in normal human bone marrow cultures were performed in three separate experiments. Considerable variation in baseline control granulocyte colony (CFU-GM) formation was seen between different experimental groups. Sera from normal human subjects inhibited CFU-GM formation in comparison to controls. In two of our experiments the degree of inhibition of CFU-GM formation was less in patients with renal insufficiency than in normal subjects. In the third experiment the sera from patients with renal insufficiency produced a greater degree of inhibition of CFU-GM formation than did normal sera. However, of the 15 sera evaluated in the third experiment, four were markedly inhibitory thereby affecting the mean. The overall inhibition of CFU-GM formation by sera from 26 normal subjects was significantly higher ($P < 0.05$) than that of the 59 patients with renal insufficiency. No correlation was seen between CFU-GM formation in patients with renal insufficiency and hematocrit, plasma creatinine concentration, of CFU-E formation. In a further study, no colony-stimulating activity (CSA) was detectable in the sera from 15 patients with renal insufficiency or sera from nine normal subjects.
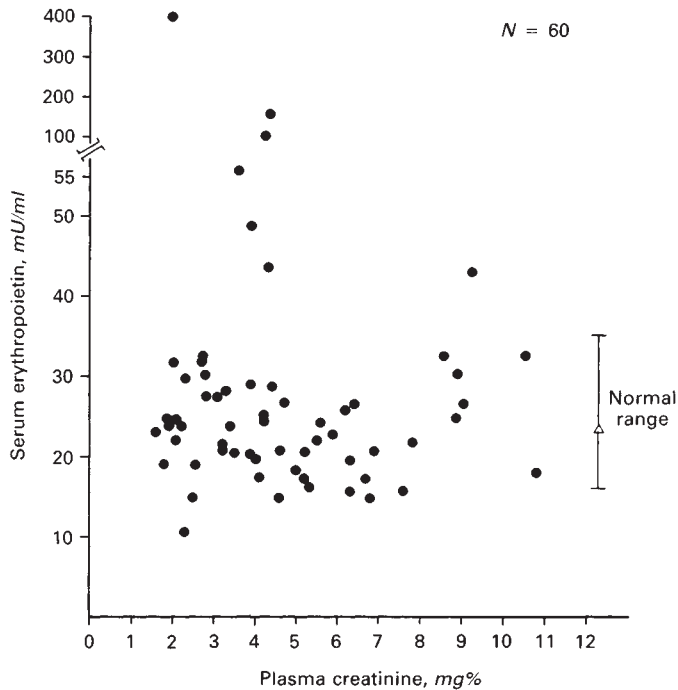
Fig. 5. *Relationship between serum erythropoietin and plasma creatinine concentrations in 60 patients with renal insufficiency.*

Fig. 6. *Relationship between serum erythropoietin concentration and hematocrit in 60 patients with varying degrees of renal insufficiency.*

Fig. 7. *Relationship between serum erythropoietin concentration and the inhibition of erythroid colony formation by sera from patients with renal insufficiency. The concentration of serum in culture is 5%.*

Discussion

Anemia of varying severity is a frequent complication in patients suffering from chronic renal failure. Available evidence suggests that two factors play a major role in the pathogenesis of this anemia. The first factor and probably the most important is a failure to produce sufficient quantities of erythropoietin to correct the anemia and the second is the presence of uremic toxins retained in the serum of patients with renal disease which inhibit erythropoiesis. Inhibition of erythropoiesis has been detected with sera from uremic patients in a number of different tissue culture systems employing both human and animal bone marrow cells and also in fetal mouse liver cultures. The mechanism of this inhibition is not fully understood but appears to be due to interference with the formation and subsequent proliferation of the erythroid-committed progenitor cell compartment. Several investigators have reported that uremic serum inhibits CFU-E and BFU-E formation, and heme synthesis [8, 12]. In addition to these two factors, abnormal bleeding from the uremic gut and shortened red cell life span [6] play a variable role in the anemia of renal disease; circulating red cells in uremia are often distorted in both shape and size, but hemolysis is usually mild or absent until the later stages of endstage renal disease. Guanidine derivatives, including methylguanidine, have been implicated in the etiology of this hemolysis [25].

A wide variety of procedures have been utilized to assay erythropoietin including the exhypoxic polycythemic mouse bioassay and fetal mouse liver cell assay. The polycythemic mouse assay is generally accepted as the international standard assay for erythropoietin. However, these biological assays for erythropoietin are hampered by their low level of sensitivity and the possible interference by other inhibitory and stimula-
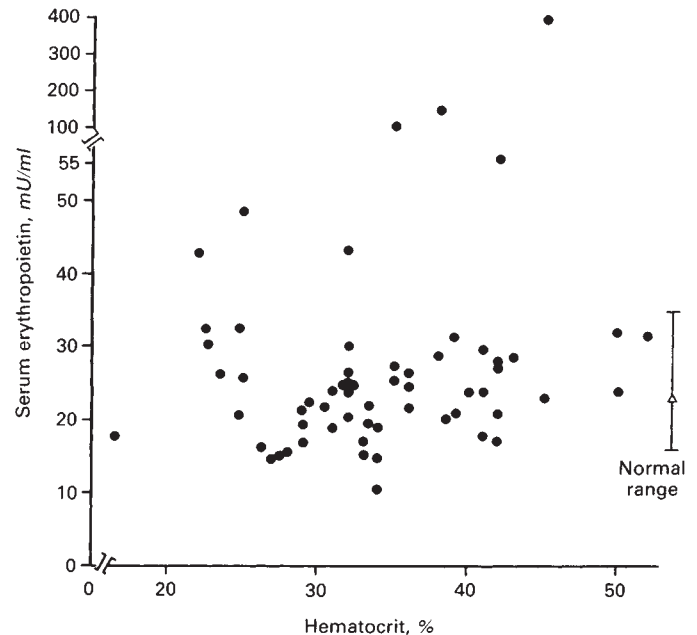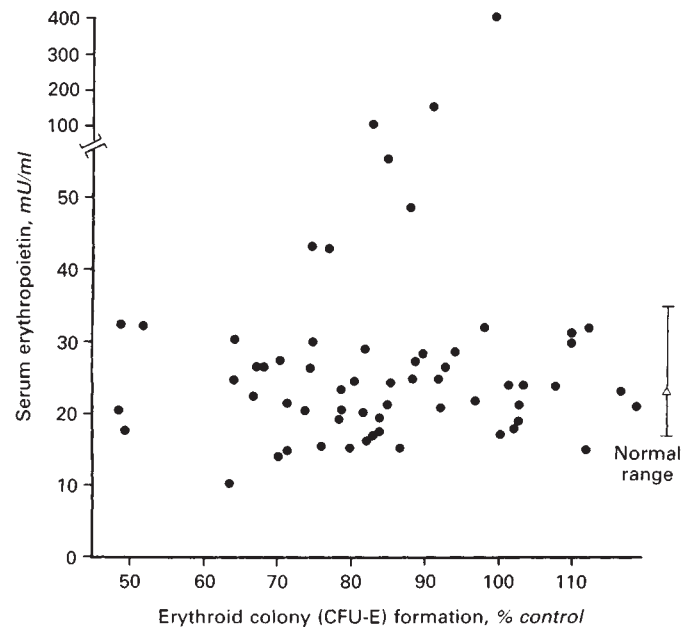
tory substances present in the uremic serum. Early studies showed that the serum erythropoietin concentrations as measured by the polycythemic mouse bioassay were rarely elevated in uremic patients. Erythropoietin has now been purified to homogeneity [26] thereby providing purified hormone for use in a radioimmunoassay which is both specific and highly sensitive [5, 27–30]. Lange, Chen, and Dunn [31] summarized several studies of in vitro and in vivo assays for erythropoietin and
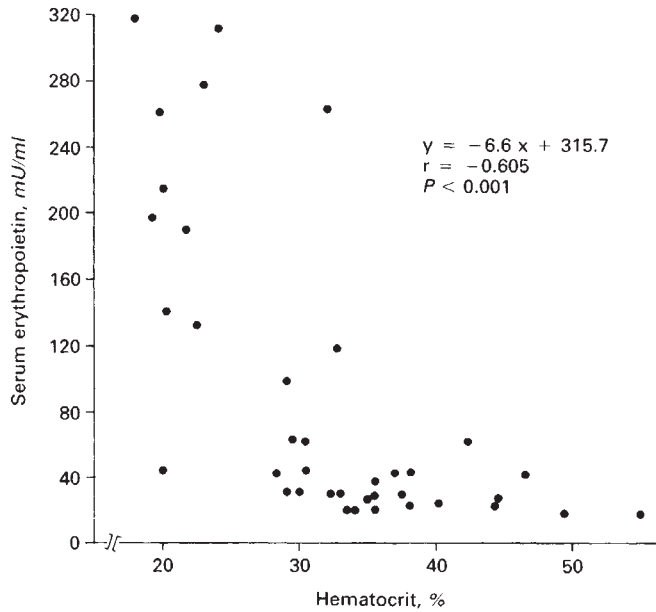
Fig. 8. *Effect of decreasing hematocrit levels on serum erythropoietin concentration in 40 patients with varying degrees of anemia and normal renal function.*
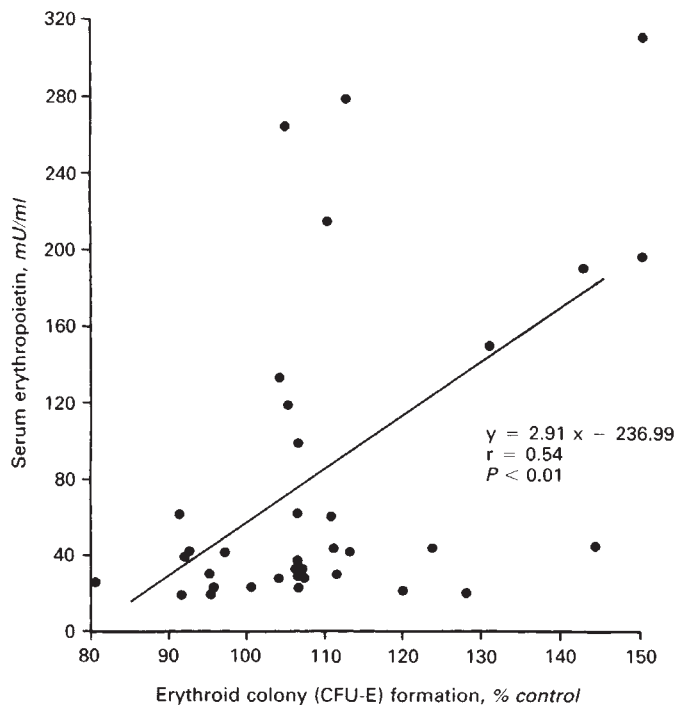

Fig. 9. *Relationship between serum erythropoietin concentration and stimulation of erythroid colony formation in patients with varying degrees of anemia and normal renal function.* The concentration of patient's serum in culture is 5%.

Fig. 10. *Inverse relationship between stimulation of erythroid colony formation and decreasing hematocrit levels in 40 patients with varying degrees of anemia and normal renal function.* The concentration of patient's serum in culture is 5%.

estimated normal human serum levels to be approximately 30 ± 10 mU/ml varying with the method of assay used. Reports of serum erythropoietin values measured by a radioimmunoassay in renal failure patients [5, 28, 30] have only included small numbers of patients. Normal levels of erythropoietin using the
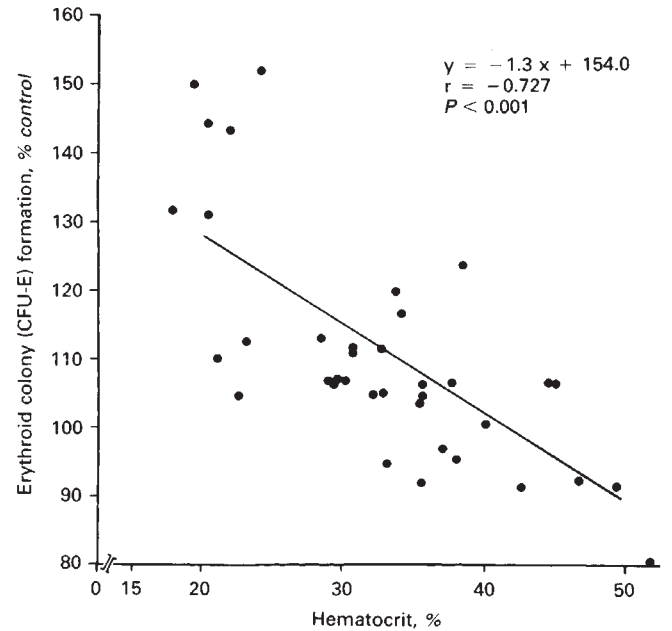
radioimmunoassay range between 14.9 and 29.0 mU/ml [5, 27–30]. Although most investigators have reported elevated serum erythropoietin levels utilizing various assays in patients with anemia and renal insufficiency, a recent investigation by De-Klerk et al [32] using a fetal mouse liver cell bioassay for erythropoietin reported reduced serum erythropoietin concentrations in uremic predialysis patients in comparison to normal subjects. It has been suggested that a sustained regulatory feedback mechanism between hematocrit and serum erythropoietin persists in patients with renal failure but is at a lower basal level [4]. Positive correlations of hemoglobin or hematocrit with serum erythropoietin measured by various bioassays [4, 32] in uremic patients may represent an association between anemia and inhibitors of erythropoiesis rather than with the serum erythropoietin concentration.

In this study we have utilized an erythropoietin radioimmunoassay and the fetal mouse liver cell culture system to compare the relative roles of erythropoietin deficiency and inhibitors of erythropoiesis in the development of anemia in a large number of nondialyzed patients with varying degrees of renal insufficiency and compared the results with anemic patients with normal function. Serum erythropoietin levels were only elevated in seven of the 60 patients with renal insufficiency. The presence of elevated serum erythropoietin levels in uremic patients with anemia in other studies has prompted the question of the exact role of erythropoietin deficiency in the pathogenesis of the anemia of chronic renal failure. Our observations indicate that as renal function deteriorates and anemia develops, the serum erythropoietin concentration remains unchanged. In contrast, in anemic patients with normal renal function there was an exponential increase in serum erythropoietin concentration associated with increasing severity of the anemia. The reponse of increased erythropoietin production to anemia occurred only when the hematocrit declined below 32%.

This inverse relationship between serum erythropoietin and hematocrit in anemic patients with normal renal function supports the findings of other investigators using different assays for erythropoietin [33, 34]. Thus, although serum erythropoietin levels were slightly elevated above the normal range in patients with renal failure, the titers were not sufficiently increased to correct the anemia and therefore reflect a relative deficiency of erythropoietin in comparison to patients with anemia and normal renal function.

It was the degree of serum inhibition of CFU-E formation rather than the serum erythropoietin concentration which correlated with both the degree of anemia and level of renal function. This contrasted with the stimulation of CFU-E formation by serum from patients with anemia of similar severity and normal renal function. Wallner and Vautrin [35] used two different in vitro culture systems (heme synthesis in rabbit erythroblasts and erythroid colony formation in mouse marrow cells) to study inhibition of erythropoiesis by serum from uremic patients. They found that the levels of inhibitor increased as renal failure worsened and hematocrit fell. The severity of anemia for the same level of renal function varied widely in the patients included in our study. Similarly, the degree of inhibition of erythroid colony formation also showed a wide scatter for both the serum creatinine and hematocrit. The variation in CFU-E inhibition for the same degree of anemia may be explained, at least in part, by other causes of anemia in addition to renal failure. Four such cases were identified in our group of patients in which blood loss or hemolysis was present, both of which would tend to increase erythropoietin production and stimulate rather than inhibit CFU-E formation.

The uremic inhibitors responsible for the suppression of erythropoiesis appear to be specific for the erythroid progenitor cell compartment (CFU-E, BFU-E) since only minimal effects were noted on granulocytic colony formation. Indeed CFU-GM formation in the presence of colony-stimulating activity (CSA) was enhanced in two of three studies by uremic serum as compared to normal control serum. The explanation for this phenomenon is unclear; effects on CFU-GM formation did not correlate with the white cell count, hematocrit, or level of renal function. Vincent et al [36] similarly reported an absence of inhibitory effect of uremic serum on CFU-GM formation when preformed CSA was added in vitro, although inhibition of CSA production by leucocytes in human marrow in vitro was seen.

The possibility must be considered that uremic serum lacks a stimulator or a factor which supports growth of erythroid progenitor cells (CFU-E) rather than containing an inhibitor of erythropoiesis. The possibility was also considered that the growth factor(s) in fetal calf serum, not present in human sera, is diluted by the reciprocal dilution of fetal calf serum in our system. However, increasing concentrations of normal adult human sera and decreasing concentrations of fetal calf serum did not produce a significant change in CFU-E in our fetal mouse liver cultures (Fig. 4). Earlier work [9] demonstrating that an antibody to spermine removed the inhibitory effects of uremic serum on erythroid colony formation and the demonstration that spermine itself is an inhibitor of CFU-E is the best support for a role of inhibitors rather than the lack of a stimulator of erythropoiesis in the mechanism of the anemia of uremia. However, until further work is carried out to purify the uremic toxin(s) which might be responsible for the suppression of erythropoiesis, this question cannot be completely resolved.

In conclusion, we have shown that the degree of inhibition of erythroid colony formation by uremic serum correlates closely with the level of renal function and the severity of anemia. The presence of normal or slightly elevated serum erythropoietin levels indicates a relative erythropoietin deficiency and reflects an inadequate erythropoietin response for the degree of anemia. Thus, it is the degree of inhibition of erythropoiesis which appears to determine the severity of anemia in patients with renal disease who have not yet started dialysis treatment. Furthermore, the uremic toxins or inhibitors responsible for inhibition of erythropoiesis appear to be specific for the erythroid progenitor cells.

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