The Anemia of Chronic Renal Failure in Sheep

Response to Erythropoietin-rich Plasma In Vivo

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Abstract. The hypoproliferative anemia in chronic renal failure has been assumed to be the result of decreased erythropoietin (Ep) production by the damaged kidney and of the shortening of erythrocyte survival. However, many in vitro studies suggest that erythropoietic inhibitors in uremic plasma may contribute to the anemia. To determine the in vivo relevance of uremic inhibitors, increasing amounts of Ep as Ep-rich plasma were infused into six uremic sheep, and their erythropoietic responses were compared with those of nine normal sheep receiving similar amounts of Ep-rich plasma. Three sheep were studied in both normal and uremic states. Ep-rich plasma was obtained from phenylhydrazine- and phlebotomy-induced anemic sheep. Stable uremia was created by subtotal nephrectomy. Erythropoiesis was quantitated by reticulocyte response, ferrokinetics (plasma iron turnover and marrow transit time), and by hemoglobin C synthesis. Ep-rich plasma stimulated erythropoiesis similarly in uremic and normal sheep, regardless of the degree of uremia. Nondialyzed uremic sheep responded as well as dialyzed animals. The anemia was corrected in the uremic sheep after 15–40 daily infusions of Ep-rich plasma, the total dosage depending on the severity of the anemia. Polycythemia was induced when the infusions were continued. Reticulocytes, plasma iron turnover, and erythrocyte mass changes increased as the amount of Ep-rich plasma was increased. These dose-response effects, coupled with the identical erythropoietic response in normal and uremic sheep given the same amount of Ep-rich plasma, imply that there are no physiologically significant erythropoietic inhibitors in uremia.

Introduction

Erythropoietin (Ep)[1] production is a primary function of the kidney (1), and Ep is secreted in response to complex interactions involved with oxygen delivery. The anatomical site and the cellular or subcellular mechanisms by which the kidney recognizes altered oxygen availability are unknown. But the response to anemia in normal animals and humans is an orderly sequence of increased Ep production followed by increased marrow erythropoiesis and an increase in the effective delivery of new erythrocytes to the circulation. Ep also increases cellular hemoglobin (Hb) synthesis and stimulates the terminal differentiation of committed erythroid progenitors (2). An additional effect, seen in goats and certain sheep, is the switch in the synthesis of the usual adult hemoglobin beta chain, β^A, to the synthesis of a structurally distinct beta chain, β^C (3). Thus, sheep of phenotype HbAA ($\alpha_2\beta_2{}^A$) will change phenotype to HbCC ($\alpha_2\beta_2{}^C$) with erythropoietic stress. Sheep with the other common beta chain (β^B) do not undergo such a change.

Because of the kidney's central role in erythrocyte production, anemia is a major complication of chronic renal failure (CRF). Of the ~70,000 patients on maintenance dialysis in the United States, more than 98% are anemic (4) and more than 60% require periodic erythrocyte transfusions (5). Despite transfusions, anemia persists and remains a major factor in preventing better rehabilitation of chronically uremic patients.

There are two definite mechanisms that contribute to the anemia of renal failure: a decreased Ep production by the diseased kidney, resulting in marrow hypoproliferation (6), and a shortening of erythrocyte survival (7). A third possible mech-

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1. *Abbreviations used in this paper:* BPA, erythroid burst-promoting activity; CRF, chronic renal failure; Ep, erythropoietin; Hb, hemoglobin; MTT, marrow transit time; PIT, plasma iron turnover; RIA, radioimmunoassay.

anism, the depression of bone marrow proliferation by ill defined inhibitors (8), has been suggested by both in vitro and in vivo studies, but the interpretations of the data are controversial. Although many in vitro studies note the presence of inhibitors to erythropoiesis in uremia, the clinical significance of these inhibitors has been difficult to define. Inhibitors of erythropoiesis, if present in CRF, could significantly alter the response to Ep. Therefore, the most critical test of the relevance of uremic inhibitors defined in vitro would be determined by the results of Ep infusions in vivo.

Since not enough purified human Ep is available to study its effects in vivo, we prepared endotoxin-free Ep-rich plasma from sheep made severely anemic by phenylhydrazine and repeated phlebotomy. The use of homologous Ep in a large sheep model of CRF (9) permitted two questions to be addressed: are there inhibitors to erythropoiesis in vivo, i.e., is Ep as effective in CRF as in the normal state; and can the anemia of severe CRF be reversed? By infusing identical amounts of Ep into normal animals and later into the same sheep with CRF, we have shown that graded doses of Ep-rich plasma increase erythropoiesis equally in both states. Furthermore, prolonged treatment completely corrects the anemia. Based on these in vivo responses, the significance of uremic inhibitors to erythropoiesis seems minimal. These results predict that Ep therapy should be effective in treating the anemia of CRF in humans.

Methods

Animal model. Adult female sheep weighing 40–80 kg underwent splenectomy (to exclude splenic erythrocyte pooling and release during studies of erythropoiesis [10]), and the creation of a vascular access before studies in the normal state. A silastic catheter with a Dacron cuff was inserted through the jugular vein and its tip was placed in the right atrium (11). Catheter sterility was maintained by instilling 1 ml of a solution of 0.5% formalin in acid citrate dextrose into the catheter three times a week. 15 min after being instilled, this solution was aspirated and replaced with 1 ml of a heparin solution (1,000 U/ml) to ensure catheter patency (12). The surgical two-stage procedure for creating CRF has been described previously (9). During the second-stage nephrectomy, carotid artery–jugular vein cannulation was performed to expedite hemodialysis if required. If anemia (hematocrit ≤ 20) did not develop within 2 mo after the second-stage nephrectomy, further renal infarction was achieved by either injecting 0.3–0.5 ml of Ivolan pellets (Unipoint Industries, High Point, NC) into the renal artery or by cauterizing approximately one-half of the renal remnant. The histology of the renal remnant at autopsy showed changes of end-stage renal disease: severe, diffuse interstitial fibrosis, tubular atrophy, and periglomerular fibrosis.

The animals were fed a standard sheep pellet, salt, and water. Salt was restricted in those sheep that required dialysis.

Hemodialysis was initiated when either plasma potassium levels rose above 7.0 meq/liter or plasma creatinine/urea nitrogen levels rose above 8/100 mg/dl. Hemodialysis was performed for 4–5 h, 2–3 times/wk with a dialyzer (Ex-85; Extracorporeal Medical Specialties, Inc., King of Prussia, PA, or Travenol 1211; Travenol Laboratories, Inc., Deerfield, IL) with a fluid delivery system (B-D Drake-Willock, Portland, OR). Each liter of dialysate contained 2.4 meq $CaCl_2$, 35 meq Na Acetate, 113 meq NaCl, 0.5 meq $MgCl_2$, and 100 mg of dextrose, but no potassium. 30,000 U of heparin were required to prevent extracorporeal clotting. A saline rinse was used to minimize dialyzer blood loss. Animals were dialyzed in a special metal stanchion which could be lifted off the ground and weighed by an overhead scale. Weights were measured every 30 min during dialysis to monitor ultrafiltration fluid losses. This allowed for the maintenance of a stable circulatory volume in the more severely uremic sheep, despite the infusions of Ep-rich plasma. In those uremic sheep that did not require dialysis, spontaneous diuresis was adequate to prevent volume overload from plasma infusions.

Procurement of Ep-rich plasma. Adult female sheep, 50–70 kg, served as Ep donors. They were crossmatched (13) with study sheep, and a right atrial catheter was then surgically inserted. Phenylhydrazine hydrochloride (16 mg/kg) was injected subcutaneously every other day for three doses to induce hemolysis. The hematocrit was then maintained at 8–12 by phlebotomy of 550 ml once or twice a day for 3 wk. Imferon (Merrell Dow Pharmaceuticals Inc., Cincinnati, OH) (1.5 g) was then given intravenously and 6–8 wk later the animal was again given phenylhydrazine and phlebotomized.

The heparinized blood obtained by phlebotomy was centrifuged and the plasma was separated and stored at −20°C. Approximately 10 liters of Ep-rich plasma was obtained from each cycle. 30 liters was subsequently pooled, divided into 500-ml aliquots, and frozen until used. Ep potency of the pools, as determined by bioassay (14) and radioimmunoassay (RIA) (15), disclosed similar concentrations, ranging from 0.4 to 2.1 U/ml. All pools were free of endotoxin (16).

Study protocol. Three Ep infusion protocols were employed: (*a*) Ep was given at a dosage of 10–27 U/kg; this was administered in two doses 24 h apart, and infused as 500 ml of Ep-rich plasma over 20–30 min. (*b*) Twice the original dosage was given 3 wk later, again in two doses, 24 h apart. (*c*) 3 wk later, one-half of the original dose was given daily for 10 d. The same pool of Ep-rich plasma was used for all studies in a given animal. 500 ml of plasma from donor sheep with a normal hematocrit were infused on two separate days and served as a control for the Ep infusions. Protocols 1–3 were carried out in normal animals, but protocol 2 was omitted for uremic sheep in order to conserve Ep.

After the above studies were completed in the anemic, uremic sheep, the infusions were continued daily until the hematocrit returned to normal. In three such sheep, infusions were continued until polycythemia (hematocrit ≥ 40) occurred.

Laboratory evaluation. The erythropoietic response to Ep-rich plasma infusions was quantitated by reticulocyte count, plasma iron turnover (PIT) (17), marrow transit time (MTT) (17), and the extent of HbC synthesis in HbAA or AB animals (18). A ^{51}Cr erythrocyte mass was obtained before and after the long-term Ep-infusion studies.

Reticulocytes were determined daily by standard techniques and were corrected for the hematocrit. Erythrocyte mass was determined by the injection of autologous erythrocytes labeled with 50 μCi of ^{51}Cr. Ferrokinetics used ^{59}Fe. The PIT, MTT, and percentage erythrocyte utilization (17), plasma iron (19), and total iron binding capacity (20) were done by standard techniques in our laboratory.

Marrow HbC synthesis was determined as follows. Marrow was aspirated from the iliac crest and washed once with Hank's balanced salt solution (HBSS). Approximately 10^6 nucleated marrow cells were incubated in HBSS with 4 mg of human transferrin (Sigma Chemical Co., St. Louis, MO) and 5 μCi of ^{59}Fe for 1 h at 37°C. Excess iron was removed by washing the cells at least three times in 15 vol of HBSS. The cells were then hypotonically lysed in distilled water, 1% KCN was added, and the cells were placed at −20°C overnight. Cell membranes were extracted by CCl_4. Free Hb was then dialyzed against 0.05 M TrisHCl buffer, pH 8.1. Hb separation was performed by DEAE Sephacel (Pharmacia Fine Chemicals, Piscataway, NJ) chromatography by the use of a 20-cm column and a pH gradient of 8.1–7.4. The radioactivity

Table I. Clinical Characteristics

	Weight	BUN	Creatinine	Hematocrit	Erythrocyte mass	Ep* (RIA)
	kg	*mg/dl*	*mg/dl*	*%*	*ml/kg*	*mU/ml*
Normal sheep (n = 9)	68.5‡±15.6‡	23±4	1.1±0.1	32.3±2.1	15.1±5.0	36.4±6.7
Uremic sheep (n = 6)§	58.5‡±17.4‡	74±8	6.5±3.1	19.2±2.2	9.5±2.6	45.9±14.4
P value¶	0.283	0.001	0.008	0.001	0.018	0.228

BUN, blood urea nitrogen. * Average values of 18 observations in seven normal sheep and 28 observations in five uremic sheep. ‡ Mean±SD. § Seven studies in six sheep. ¶ P value estimated by two-sample t test

in the collected fractions was determined with a gamma counter and the hemoglobins were analyzed. Radioactivity in each fraction for hemoglobins A, B, and C (marked by carrier hemoglobins) was then expressed as a percentage of the total counts obtained. Radioactivity already present in the peripheral blood (as a result of prior ferrokinetic studies) was considered insignificant if it was <5% of the total ^{59}Fe uptake by the marrow cells. In a few samples, the amount of radioactivity present in peripheral blood was significant. This was corrected by analyzing HbC from marrow cells both before and after in vitro incubation with ^{59}Fe. The percentage HbC synthesis was then calculated by subtracting the radioactivity present in the original sample from the ^{59}Fe uptake by the marrow cells in vitro.

Ep was measured both by the standard exhypoxic polycythemic mouse bioassay (14) and by RIA (15). The RIA-Ep levels were quantitated by comparison with a simultaneously assayed sheep standard Ep of known activity.

Plasma was analyzed for creatinine, urea nitrogen, potassium, sodium, bicarbonate, chloride, glucose, phosphorus, and calcium with an autoanalyzer.

Statistical analysis. The two-sample t test for unequal variances (21) was used to test for differences between the mean parameter values of normal and uremic sheep in Tables I and II. The linear relationships depicted in Figs. 3–5 were estimated by the method of least squares. To determine the statistical significance of these relationships, the slope coefficients were tested by the ratio of each slope coefficient to its standard error, which has a t distribution. In Figs. 4 and 5, the separate regression lines for normal sheep and uremic sheep were tested for coincidence (equal slopes and equal intercepts) with the dummy variable multiple regression model (22). The significance of the results is expressed as a probability value. Significance is considered ≤0.05.

Results

Nine normal sheep were studied; eight completed all studies and one received only one dose of Ep-rich plasma.

Six sheep were studied when uremic. Three had been studied when normal and three were made uremic without having been studied. One uremic sheep was studied twice and the averages of the observations from each study were used in the analyses. The dialyzed sheep survived for 6, 7, and 8 mo; the nondialyzed uremic sheep survived for 9, 11, and 21 mo. Technical complications of dialysis (water-induced hemolysis) and intractable pulmonary edema accounted for the shorter survival of the dialyzed sheep.

Table I summarizes several characteristics of the nine normal and six uremic sheep. Uremic sheep had significantly higher blood urea nitrogen and creatinine levels than normal sheep and significantly lower hematocrits and erythrocyte volumes. Weights and serum Ep levels were comparable in the two groups.

The RIA Ep levels in normal animals ranged from 20.6 to 56.6 mU/ml (mean 36.4±6.7) at a hematocrit of 32.3±2.1. The RIA Ep levels in uremic, anemic sheep ranged from 12.6 to 103 mU/ml (mean 45.9±14.4), hematocrit 19.2±2.2). As seen in Fig. 1, the uremic sheep had lower Ep levels than normal animals made equally anemic by phenylhydrazine and/or phlebotomy.

Table II details the erythropoietic responses to infusions of normal plasma and various dosages of Ep-rich plasma. Normal plasma infusions had no effect on the four parameters of erythropoiesis in either study state. Not all studies were performed in all animals; some sheep were of HbBB phenotype and in others, marrow could not be aspirated, preventing the measurement of HbC synthesis. There were no statistically significant differences between any of the erythropoietic reponse parameters for normal or uremic animals studied during the baseline period,

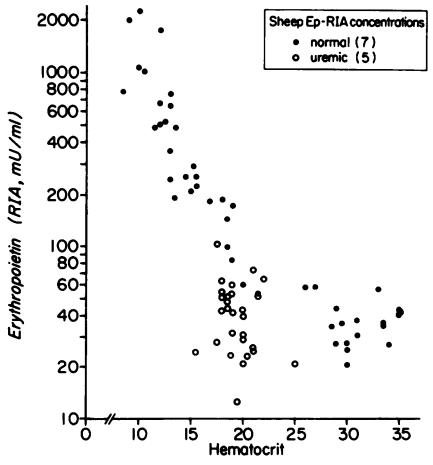

Figure 1. The correlation of serum Ep levels as measured by RIA in seven normal and five anemic, uremic sheep. Anemia was induced in normal sheep by phenylhydrazine and/or phlebotomy.

*Table II. Response to Ep Infusions**

	Dosage Ep	Reticulocyte	PIT‡	MTT	HbC synthesis
	U/kg	%		*d*	%
Baseline studies					
Normal sheep (n = 9)		0.02±0.01§	0.70±0.20	4.4±1.8	1.5±1.7 (4)
Uremic sheep (n = 6)		0.06±0.07	0.60±0.25	4.1±0.8	2.5±1.3 (3)
P value‖		0.216	0.620	0.552	0.632
Normal plasma infusions					
Normal (n = 8)		0.18±0.14	0.72±0.19	ND	0.7±0.0 (2)
Uremia (n = 3)		0.07±0.03	0.61±0.10	4.0 (1)	4.4±0.8 (2)
Ep-rich plasma (2 d)					
Normal (n = 9)	17.8±7.2	1.04±0.36	1.26±0.34	1.6±0.2 (7)	3.7±2.0 (4)
Uremia (n = 5)	24.6±14.4	1.55±1.03	1.41±0.25	1.5±0 (2)¶	11.5±5.1 (2)¶
P value	0.369	0.338	0.382		
Ep-rich plasma, double dose					
Normal (n = 5)	39.8±13.8	1.89±0.79	1.48±0.15	1.4±0.4 (3)	8.2±1.2 (2)
Ep-rich plasma (10 d)					
Normal (n = 8)	85.6±37.1	2.54±0.77	1.90±0.40	1.5±0.7 (5)	14.6±8.6 (4)
Uremia (n = 6)	107.0±25.2	2.69±0.79	2.19±0.38	1.8±0.9 (3)	27.7±16.1 (3)
P value	0.228	0.726	0.197	0.525	0.330
Ep-rich plasma (10 d) − baseline					
Normal (n = 8)		2.52±0.77	1.22±0.32	−3.34±2.12 (5)	15.3±8.9 (3)
Uremia (n = 6)		2.63±0.81	1.55±0.46	−1.77±1.21 (3)	30.4±20.7 (2)¶
P value		0.801	0.181	0.23	

ND, not determined. * As Ep-rich plasma. ‡ PIT as milligrams of iron/100 ml whole blood per day. § Mean±SD. ‖ P value estimated by two-sample t test. ¶ Sample size too small for analysis.

after normal plasma infusion, or after various dosages of Ep-rich plasma. In addition, the differences between the baseline studies and the responses of the 10-d infusion of Ep-rich plasma are shown on the bottom of Table II and their mean values do not differ significantly among the eight normal and six uremic sheep that were studied at both times.

Fig. 2 compares the effect of the same amount of Ep-rich plasma given to a sheep when normal and later, when uremic. The increases in reticulocyte count and PIT were essentially identical regardless of the degree of renal function. Two other animals had similar responses to the same amount of Ep-rich plasma in both study states (data not shown).

Table III shows the results of studies in five anemic, uremic sheep given daily infusions of Ep-rich plasma to normalize their hematocrits. The hematocrit was normalized twice in one animal; after the Ep-rich plasma infusions were discontinued, 2 mo elapsed before the hematocrit spontaneously returned to a stable anemic level. When the increase in erythrocyte mass was compared with the total amount of Ep given, a significant correlation was obtained (Fig. 3). Similar results were observed when the response of normal and uremic sheep to infused Ep-rich plasma was analyzed by correlating Ep dose to the peak change in reticulocyte count or PIT (Figs. 4 and 5). There were significant linear relationships between log Ep dose and reticulocyte count in normal and uremic sheep ($P < 0.002$ and 0.05, respectively) and between log Ep dose and PIT ($P = 0.01$ and 0.05, respectively). However, there were no significant differences in either the slopes or intercepts of the regression lines (coincident lines) between the two groups, which indicates that the amount of response of the reticulocyte count and PIT per unit change in log dose of Ep was the same in normal and uremic sheep.

Three animals continued to receive Ep-rich plasma, which eventually induced polycythemia. Fig. 6 details the correction of the anemia in one animal by daily infusions of Ep-rich plasma. Polycythemia was then induced by infusions of Ep-rich plasma twice a week.

Discussion

The anemia of CRF is a major complication in chronic dialysis patients and contributes to poor patient rehabilitation. Ep deficiency, secondary to damage to the renal secretory site(s), has

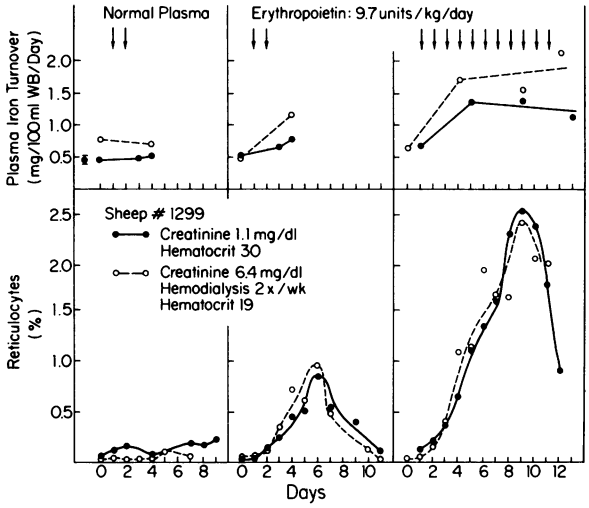

Figure 2. Response to normal plasma and identical amounts of Ep in sheep 1299 when normal and uremic. Each arrow represents 500 ml of infused plasma. WB, whole blood.

been considered a major cause of the associated hypoproliferative anemia. Recently, however, there has been an emphasis on the possible role of uremic inhibitors of erythropoiesis (23–26).

Measurement of Ep in serum has been imprecise, making it difficult to determine the actual role of Ep deficiency in the pathogenesis of the anemia of CRF. With the exhypoxic polycythemic mouse bioassay (27), Ep levels have usually been undetectable in anemic, uremic patients (28, 29), although subnormal levels are difficult to quantitate by this method. An improved bioassay which uses concentrations of plasma (30) disclosed that normal subjects had levels ranging from 3.9 to 15.4 mU/ml, whereas half of the nephric, uremic patients studied had similar levels and the other half had elevated levels. Although no correlative studies of total and effective erythropoiesis were performed, the finding of elevated Ep levels in a substantial

*Table III. Response to Long-term Ep Infusions**

Sheep No.	Creatinine	Days of Ep*	Total dosage Ep	Hematocrit		Increase in erythrocyte mass
				Before	After	
	mg/dl		*U/kg*			*ml*
1037	3.3	15	176	19.5	32	208
1037	3.5	15	159	21	33	305
6055	5.2	27	324	20	33	409
1299	6.4	30	291	18.5	30	368
17	15.0	40	524	15	30	523
2641	8.7	16	194	20	33	398

* As Ep-rich plasma.

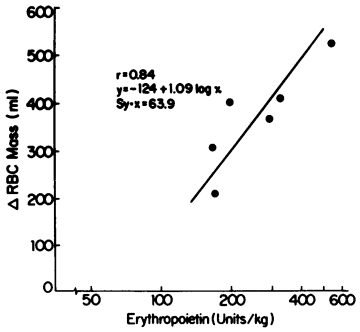

Figure 3. Change in erythrocyte (RBC) mass in uremic sheep as a function of total amount of infused Ep (P = 0.035).

number of anemic uremic patients suggests that inhibitors of marrow function are of consequence.

Consistent with this was the finding of markedly elevated Ep levels in a very anemic anephric dialysis patient (31). Since the purification of Ep (32), which led to the subsequent development of an RIA for Ep (15, 33), levels in normal and uremic subjects can be measured more easily (34). The serum levels of Ep in normal males and females were reported to be 17–19 mU/ml as measured by RIA (34), whereas the mean serum level was 28±6 mU/ml in anemic, uremic dialyzed patients (35) and ~20 mU/ml (15), similar to normal, in anephric patients. The serum Ep levels in the normal and uremic sheep (Table I) were similar to values observed in normal and uremic humans. We interpret these findings to indicate that maximal Ep secretion, in response to anemia, is reduced because of the decreased functional renal mass in CRF. However, it is also conceivable that biologically inactive fragments of Ep retained in CRF could result in apparently normal or even elevated levels of Ep measured by RIA (33).

Studies in animals have also failed to clarify the roles of Ep deficiency and uremic inhibition in the anemia of CRF. Peritoneally dialyzed anephric rats responded to very large amounts of sheep Ep in one study (36) but not in another (37). Rats, uremic from subtotal nephrectomy, responded to rat Ep (38, 39), but stable uremia was not achieved with this model. Mice with hereditary polycystic kidney disease responded to human urinary Ep if they were mildly azotemic but failed to respond when more uremic (23).

Studies in man on the effectiveness of Ep in the anemia of

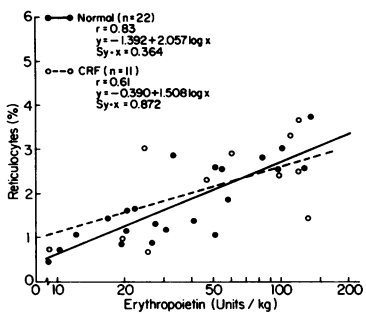

Figure 4. Correlation of maximal reticulocyte response to Ep dose in normal ($P < 0.002$) and uremic sheep ($P < 0.05$).

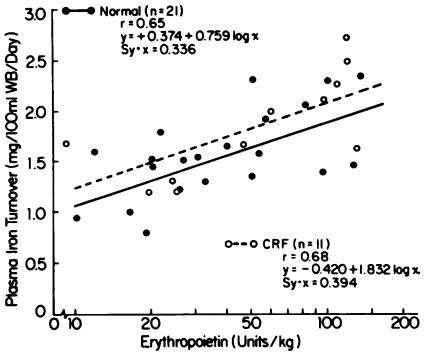

Figure 5. Correlation of plasma iron turnover response to Ep dose in normal ($P < 0.01$) and uremic sheep ($P < 0.05$). WB, whole blood.

CRF have been limited because of the lack of enough of the purified hormone. In 1963, Van Dyke was unable to elicit a reticulocyte or ferrokinetic response when he infused crude Ep over 3 d into a patient with chronic glomerulonephritis and moderate anemia (40). Essers infused Ep-rich plasma from aplastic anemia patients into dialyzed and nondialyzed azotemic patients (41). 500 U of Ep, which elicited a twofold reticulocytosis in three normal subjects, failed to induce reticulocytosis in two patients with marked azotemia (creatinine 7.6 and 16.8 mg/dl, respectively) but did elicit a response similar to normals in two mildly azotemic patients (creatinine 4.2 and 3.5 mg/dl). Larger infusions of Ep (1,870–2,600 U) into dialyzed and nondialyzed azotemic patients produced a 2- to 2.5-fold increase in circulating reticulocytes.

Because previous studies in animals and humans have produced contradictory results, we developed a protocol to determine if Ep is as effective in CRF as in the normal state and if the anemia of CRF can be reversed by Ep. To do this, we employed a previously described sheep model of stable or progressive CRF, which is similar to that seen in uremic humans (42). The similarities include: (*a*) The histology of the kidney

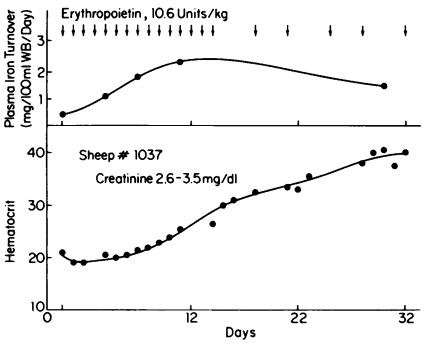

Figure 6. Correction of anemia in a uremic sheep by daily infusions of Ep-rich plasma. Polycythemia was created by continued infusions. Each arrow represents 500 ml of infused Ep-rich plasma. WB, whole blood.

remnant is identical to that seen in end-stage renal disease (43). (*b*) The anemia is hypoproliferative as defined by a normal serum iron, normal-to-decreased reticulocytes, normal-to-decreased PIT, and normal or inappropriately low serum Ep levels (42). (*c*) Hemolysis is not a major component of the anemia (42). (*d*) The severity of the anemia may be independent of renal excretory function (44), although anemia is usually not present unless the serum creatinine is 3.0 mg/dl or greater (45). Since in vitro inhibitors of erythropoiesis in humans have been observed with levels of renal function similar to those observed in our sheep (24), we believe that this model of CRF is comparable to the human with CRF.

Furthermore, the sheep offers advantages over other animals in that: (*a*) Because of its size, large quantities of autologous or homologous Ep-rich plasma can be administered and the animal can be studied in the normal and uremic state. (*b*) The subtotally nephrectomized sheep maintains a stable and/or progressive form of renal failure and can be maintained by hemodialysis, if necessary. (*c*) There is an Ep-dependent activation of a structurally distinct hemoglobin (HbC) in sheep of HbA phenotype, which provides an independent monitor of erythropoietic stimulation.

Using this model, we found that the administration of Ep-rich plasma resulted in indistinguishable physiologic responses in the normal and uremic states as quantitated by increases in reticulocyte counts, HbC synthesis, PIT, and shortening in MTT. The responses were independent of the degree of azotemia, as demonstrated by the linear relationship between Ep dose and the variously analyzed responses (Figs. 3–5). These physiologic similarities were not due to a prolongation of the half-life clearance of the infused Ep (Mladenovic, J., J. W. Eschbach, J. R. Koup, J. F. Garcia, and J. W. Adamson. Unpublished observations). Furthermore, the anemia could be corrected completely by daily infusions of Ep-rich plasma.

Although we have not infused a purified form of Ep, we conclude that it is primarily Ep in the anemic plasma that is producing the erythroid-stimulating effects observed. Plasma from normal sheep failed to elicit any erythropoietic stimulation, and there was the predicted correlation between Ep levels in the infusate, as measured by bioassay and RIA. Furthermore, only Ep is known to produce all of the following: reticulocytosis, increased PIT, shortened MTT, HbC activation, and the production of polycythemia.

Although other hormones such as testosterone (46), serotonin (47), cyclic AMP (48), prolactin (49), and triiodothyronine (50) have been shown to stimulate erythropoiesis in experimental animals, these effects occur by stimulation of Ep production. It seems unlikely that chronic anemia is associated with elevations of these hormones or factors.

Erythroid burst-promoting activity (BPA), defined in vitro, enhances early erythropoiesis separately from Ep (51) and theoretically might contribute to some of the responses to the Ep-rich plasma. However, even if BPA were present, equal amounts were given to both normal and uremic sheep along with the

Ep, and BPA has not been demonstrated to be relevant in vivo. Nevertheless, until purified Ep is available, we cannot conclude unequivocally that it was only Ep that produced the erythropoietic changes noted in our uremic sheep. Since purified sheep Ep from which to make an antibody is unavailable, Ep could not be completely removed from the anemic plasma to serve as a control. Nevertheless, even if stimulating factors in addition to Ep were present in the anemic plasma, they were infused equally into both normal and uremic sheep, and no inhibition of Ep or of any other stimulating factor was observed.

The possibility that erythropoietic inhibitors could be present but masked by excessive amounts of the infused hormone seems unlikely. This is supported by the finding that increasing amounts of plasma resulted in a progressively greater response, as quantitated by ferrokinetics, reticulocytosis, and erythrocyte mass. If inhibitors were present and larger amounts of Ep overcame the inhibition, a linear in vivo dose-response curve would not be predicted.

These animal studies suggest that Ep therapy should correct the hypoproliferative anemia of CRF. The amount required should be less than previously predicted, since uremic inhibition may not be present. In addition, Ep will be required only intermittently since the hematocrit may not return to anemic levels for several months after hormone infusions are stopped. The uremic sheep requires less than 10 U of Ep/kg for 15–40 d (depending on the severity of the anemia) to correct the anemia. It is likely that a smaller, but as yet unknown, quantity of Ep would be required for maintenance of the hematocrit.

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