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Pathogenesis of the anemia of chronic renal failure: The role of erythropoietin

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Introduction

In 1836, Richard Bright in his classical monograph on kidney disease [1] recognized the frequent occurrence of anemia in patients with renal failure. Since that time, significant progress has been made in the elucidation of the pathogenesis of the anemia of chronic renal failure (CRF) and more recently in its treatment. The anemia, has been serious problem that limited the physical tolerance of many patients with CRF and prevented rehabilitation of patients receiving chronic dialysis treatments.

In this chapter we review the role of the normal kidney in the regulation of erythropoiesis, the pathogenesis of the anemia of CRF and its treatment.

The role of the normal kidney in the regulation of erythropoiesis

The role of the kidneys in regulating erythropoiesis was not recognized until 1957 when Jacobson and co-workers showed that removal of kidneys nearly abolishes Ep production in rats [2]. Subsequent studies showed that the normal isolated perfused kidney synthesizes Ep when perfused with a serum free medium [3] and that this synthesis is augmented when the kidney is perfused at low pO_2 s54.

Ep production is regulated by the oxygen supply to the kidney relative to its oxygen requirement. The kidney is responsible for sensing oxygen availability to tissues as well as for releasing Ep into the circulation. An oxygen deficit is postulated to release chemical messengers which activate specific receptors in the kidney to trigger Ep production [5], but the mechanism for day to day control of Ep production still remains obscure [6].

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Why the kidney should serve as the erythroid master organ may be related to the kidney's unique adjustment of oxygen demand to oxygen supply [7]. Oxygen is consumed by the kidney primarily to fuel sodium reabsorption, which in turn depends on glomerular filtration rate (GFR) and renal blood flow. Thus, a reduction in oxygen supply due to decreased renal blood flow also decreases GFR and oxygen demand; this explains why decreased renal blood flow from multiple causes per se does not increase Ep production [7]. However, renal ischemia beyond a critical degree does lead to increase in Ep production [8].

The site of Ep generation within the kidney is a matter of debate. Peritubular interstitial cells, peritubular capillary endothelial cells, mesangial cells and juxtaglomerular apparatus have all been considered as possible sites of renal Ep generation [9-13]. Ep has been extracted from isolated glomeruli of hypoxic rats [13b].

The liver has been identified as the primary site of Ep production during fetal life [14]. However, after birth, Ep generation is shifted to the kidney, and hepatic Ep contributes only 10 % to 15 % of total Ep in normal adults [15].

FUNCTION OF ERYTHROPOIETIN

Ep induces red cell formation by stimulating proliferation, downstream differentiation and maturation of erythroid progenitors and precursors [16]. In high titers Ep causes early release of reticulocytes into the circulation [16]. Figure 1 provides a schematic diagram of the sites of Ep action in the bone marrow. Red cells are thought to originate from a primitive

ABBREVIATIONS

CRF -	Chronic Renal Failure.
Ep -	Erythropoietin.
CAPD -	Continuous Ambulatory Peritoneal Dialysis.
RIA -	Radioimmunoassay.
GFR -	Glomerular Filtration Rate.
BFU-E -	Burst Forming Unit - Erythroid.
CFU-E -	Colony Forming Unit - Erythroid.
rHuEp -	Recombinant Human Erythropoietin.

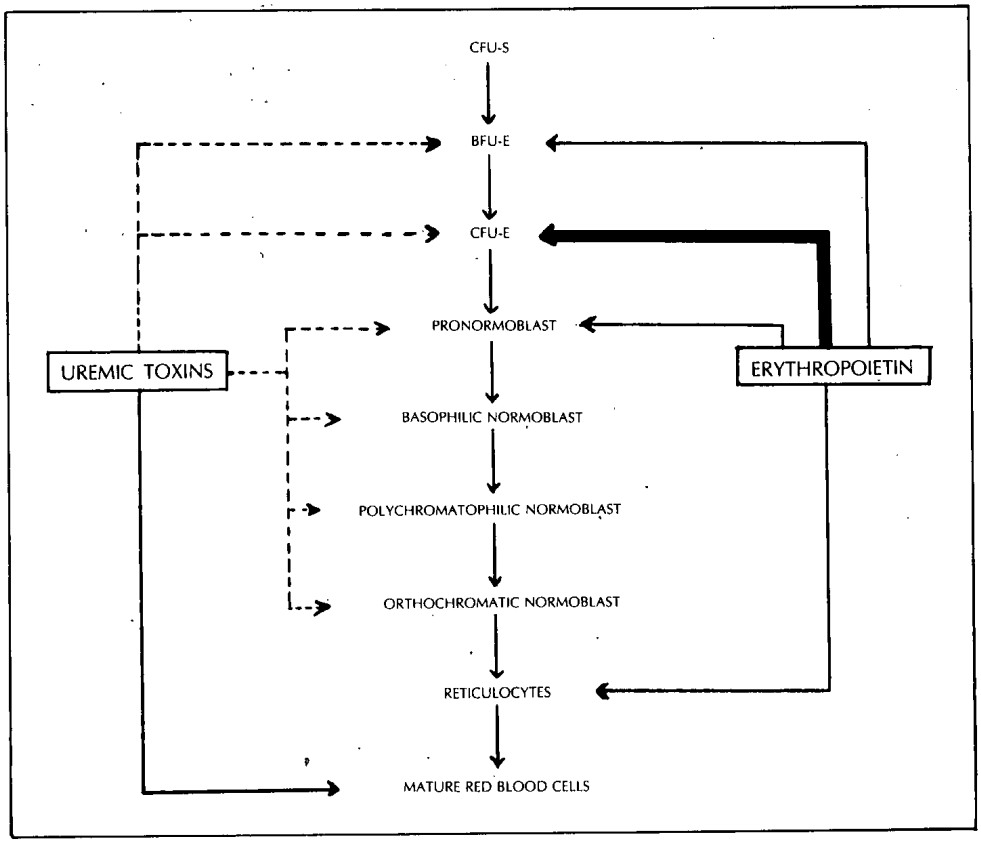

Fig. 1.—Schematic diagram from the sites of action of Ep and uremic toxin during erythroid differentation. The broken lines represent sites where uremic toxins inhibit erythtopoiesis in vitro studies; their role in vivo is not clear. The heavy line denotes exquisite sensitivity of the target cell to erythropoietin. CFU-S = colony forming unit-spleen; BFU-E = burst Forming unit-erythroid; CFU-E = colony forming erythroid.

hematopoietic cell capable of producing progenitors of all types of blood cells. This stem cell has been termed the colony forming unit-spleen (CFU-S) because transplantation of these cells into spleens of heavily irradiated mice gives rise to erythroid, granulocytic and megakaryocytic colonies[17]. CFU-S has the unique capacity of nearly indefinite self replication. The mechanism by which CFU-S is committed to the erythrocytic pathway has not yer been clarified[18]. The most primitive erythroid progenitor cell responding to Ep is called the burst forming unit-erythroid (BFU-E) and the more nature, colony forming unit-erythroid (CFU-E). Proliferation of BFU-E requires a specific growth factor generated by lymphocytes or monocytes; BFU-E will die if further differentiation does not occur. BFU-E are minimally responsive to Ep[19-21]. Ep CFU-e is more sensitive and specific for Ep, action and the response to low concentration of Ep produces small clusters of hemoglobin synthesizing cells[22]. CFU-E is similar to the basophilic normoblast found in the bone marrow.

Structure of erythropoietin and development of biosynthetic human erythropoietin

In 1977, Miyake et al. isolated a relatively large quantity of Ep from the urine of severely anemic patients and purified it to apparent homogeneity[23]. This material served as a source of pure Ep for the study of Ep structure. Ep is a glycoprotein with a molecular weight of approximately 34,000 daltons and contains approximately 25 % carbohydrate consisting mostly of sialic acid[24]. The carbohydrate moiety is not critical for the erythropoietic action of Ep, but prevents its rapid clearance[25].

In recents years, polyclonal and monoclonal antibodies were raised against purified Ep. This led to the development of radioimmunoassay (RIA) of Ep[26]. The most important contribution of the RIA was that it became possible to monitor the isolation and fractionation procedures needed to determine the amino acid sequence of Ep. Part of this sequence was

then used to predict the base composition of a corresponding cDNA, which in turn was used as a probe to identify the entire Ep gene [27-28]. The Ep gene is encoded as a single copy on chromosome 7 [29]. Molecular biologists have isolated the gene and inserted it into mammalian cells capable of synthesizing unlimited quantities of Ep [27]. It was necessary to use animal cells rather than bacteria because Ep, with its 166 amino acids, is highly glycosylated and only animal cells provide the necessary sugar components.

The biological activity and immunological properties of the human purified urinary Ep and recombinant human Ep (rHuEp) have not been distinguishable.

Clinical and laboratory characteristics of the anemia of chronic renal failure

The drop in hematocrit in patients with CRF is generally proportional to the decline in GFR [30]. Severe anemia is generally not noted until the GFR falls below 20 ml/min/1.73 ^{2} [30]. A wide scatter of hematocrit values is noted for the same level of renal function [30, 31] which reflects the multiple causes of renal failure as well as the multifactorial origin of the anemia.

The anemia of chronic renal disease is generally milder in patients with polycystic kidney disease [32] and is more severe for the level of renal dysfunction in patients with medullary cystic disease, nephrotic syndrome, bilateral nephrectomies, and those with severe hyperparathyroidism.

The anemia of CRF is typically normochromic, normocytic. The absolute reticulocyte count is low. The leukocyte and platelet counts are usually normal. The peripheral smear may show burr cells, their frequency is roughly proportional to the severity of uremia. The bone marrow in patients with CRF is characteristically normocellular; the erythroid/granulocyte ratio is either normal of slightly reduced. Ferrokinetic studies show decreased plasma iron turnover, decreased rate of red cell iron utilization, and increased marrow transit time [33b].

Recent studies have shown that the correction of the anemia of CRF with rHuEp results in improvement in generalized coldness, anorexia, fatigue, exercise tolerance, depression, sexual disinterest and dysfunction. These observations suggest that the anemia contribute significantly to the symptomatology of CRF.

Pathogenesis of the anemia of chronic renal failure

A) DECREASED RED CELL SURVIVAL

Decreased red cell survival to approximately half normal is seen in all patients with advanced renal insufficiency [34, 35]. Hemolysis, however, is mild enough that a normal hematopoietic system should be able to compensate for it. The hemolysis is caused by substances in uremic plasma that interfere with the RBC membrane's ability to effectively pump sodium from the cells [36]. The transfusion of normal red cells into uremic patients results in a shortening of the survival of the transfused cells, while transfusion of red cells from patients with CRF into nonuremic subjects is associated with normal red cell survival [34]. Hemodialysis or CAPD does not significantly improve red cell survival [35, 37].

B) INHIBITION OF ERYTHROPOIESIS

Several lines of evidence suggest that abnormal metabolites or substances retained in patients with CRF interfere with bone marrow function: (10) A number of patients with CRF have remained anemic despite the presence of elevated serum Ep levels on bioassay [38] or RIA [31, 32] suggesting that the marrow has decreased sensitivity to circulating Ep in these patients. (2) In nondialysis patients with declining renal function progressive anemia is noted despite no decrease in serum Ep levels [30, 31] suggesting that erythropoietic tissues may be less sensitive to Ep in CRF. (3) Some patients placed on hemodialysis manifest improvement in hematocrit in the absence of significant changes in plasma Ep levels, suggesting that an inhibitor was removed by dialysis [39]. A significant correlation between the degree of in vitro inhibition of erythropoiesis and the hematocrit level has been noted in patients with CRF [31]. (4) Inhibition of erythropoiesis has been detected in the presence of sera from uremic patients in several tissue culture systems employing both human and animal bone marrow cells, and also in fetal mouse liver cultures [40]. Uremic sera inhibit proliferation of BFU-E and CFU-E, hemo synthesis and marrow thymidine incorporation [40].

The identity or specificity of the uremic inhibitors of erythropoiesis has not been established. No inhibitor of erythropoiesis identified to date has demonstrated specificity or a significant differential effect on erythropoiesis. It is possible that the uremic milieu may nonspecifically impair cell proliferation in several tissues. Interference with cell proliferation has been shown in the granulocytes [41] intestinal epithelium [42], granulation tissue [43], lymphocytes [44], as well as male germinal ephithelium [45].

C) ERYTHROPOIETIN DEFICIENCY IN CHRONIC RENAL FAILURE

The most important cause of the anemia of CRF is decreased Ep production from the diseased kidneys. When in vivo bioassays utilizing radioactive iron incorporation in polycythemic mice were used to measure Ep concentration, serum Ep levels in uremic

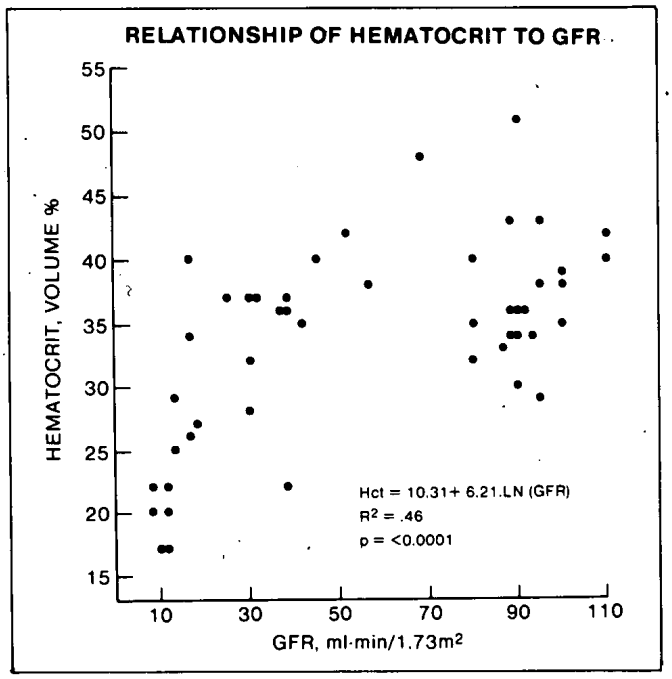

Fig. 2.—Relationship of serum Ep levels to the GFR in patients with chronic renal failure excluding dialysis patients. (From Chandra et al.: ref. 30.)

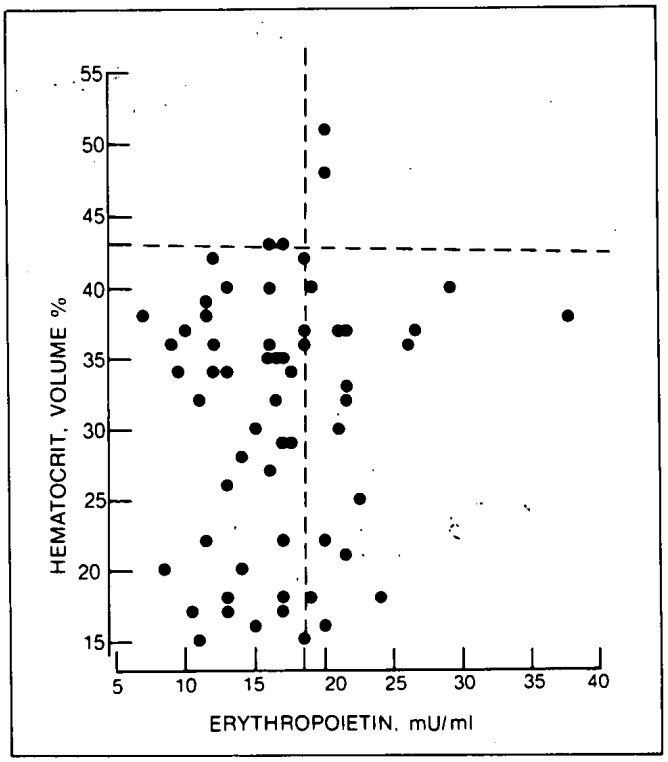

Fig. 4.—Relationship of hematocrit to serum Ep levels in 48 patients with CRF. The broken lines represent the mean values of Ep and hematocrit in normal controls. (From Chandra et al.: ref. 30.)

patients were either undetectable or well below those of comparably anemic patients who have normal renal function[46, 47]. With the purification of human Ep, valid RIAs for Ep were developed[26] that measure serum Ep levels as low as 0.4 mU/ml as compared to the sensitivity of the in vivo bioassay in unextracted plasma of about 50 mU/ml. Using the RIA, mean values for serum Ep were 18.8 mU/ml for normal females, 17.2 mU/ml for normal males, 97 mU/ml for iron deficiency anemia patients and greater than 1000 mU/ml for patients with anemia secondary to bone marrow failure[48]. Using the RIA we and other investigators have found serum Ep levels to be low

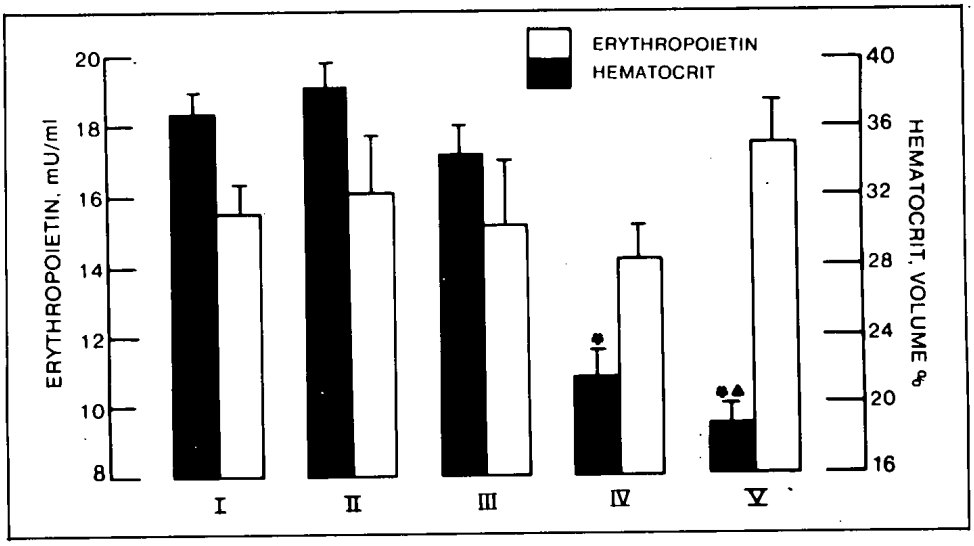

Fig. 3.—Serum Ep levels and hematocrit in 48 patients with chronic renal failure. Groups I-V represent following range of GFR in ml/min/1.73 m², respectively, 790, 40-89, 20-39, 5-19, Hemodialysis patients.
** $p < 0.005$ Groups IV and V vs Groups I-III.*
o $p < 0.005$ Groups V vs Groups I-IV.

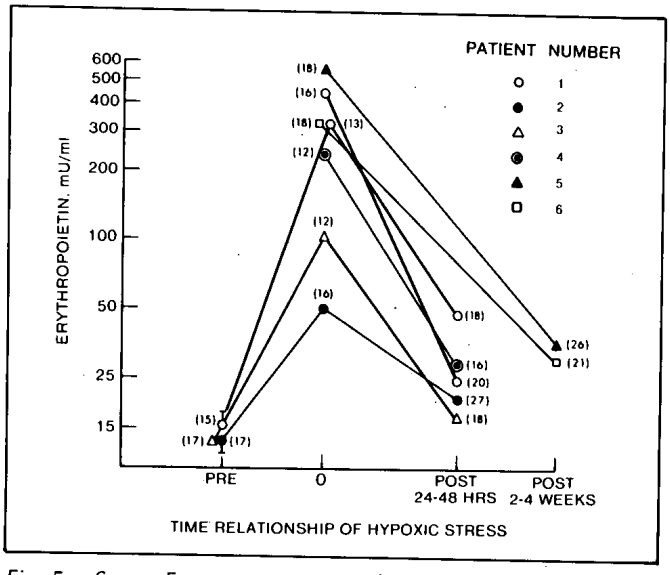

Fig. 5.—Serum Ep response to acute hypoxic stress in six patients.
The number in parenthesis represent the hematocrit value corresponding to the Ep concentration. In patients 1 and 2 the Ep values prehypoxia represent means ± SEM of 4 and 5 earlier determinations respectively. (From Chandra et al.: ref. 30.)

relative to the degree of anemia in patients with renal failure[32, 33a]. The usual correlation between the degree of anemia and elevated levels of serum Ep as would be expected if renal function were normal[48] was not seen in patients with CRF.

1. *Relationship of serum erythropoietin levels and hematocrit to the level of renal excretory function*

We evaluated the relationship of serum Ep levels measured by RIA to GFR and hematocrit in 48 patients 4 to 22 years of age with chronic renal disease of varying severity and of varying etiologies to evaluate if serum Ep levels change with progressive renal failure[30]. In these patients with chronic renal disease, significant anemia was noted only when GFR fell below 20 ml/min/1.73 m^2 (Fig. 2). Serum Ep levels, however, did not change with the decline in GFR despite the development of severe anemia (Fig. 3). Serum Ep levels did not correlate with hematocrit (Fig. 4) or with GFR. McGonigle et al. also found no relationship of serum Ep levels to serum creatinine or hematocrit in 60 patients with varying degree of renal insufficiency[31]. Radtke et al. measured serum Ep levels in 117 adults with renal failure[49]. Serum Ep levels did not consistently decrease in parallel with the decrease in GFR. Patients with GFR of 0-9 ml/min had higher mean serum Ep level than patients with GFR of 10-19 ml/min[49a].

2. *Is hematocrit-tissue oxygenation-erythropoietin feedback mechanism operative in renal failure?*

We quantitated serum Ep response to acute hypoxia in six children with GFR < 20 ml/min/1.73 m^2 during spontaneous episodes of acute hypoxic stress[30]. These clinical events included acute pulmonary edema, acute hemolysis, congestive heart failure, and sepsis with hypotension. Figure 5 shows the serum Ep levels of these six patients during the hypoxic episode (time 0), and either 24-48 hours or 2-4 weeks after such stress. Hematocrit was measured in the same blood sample as Ep. In three patients, serum Ep had been measured during the hypoxic stress. In patient 1, serum Ep levels were measured during two episodes of pulmonary edema. Serum Ep values were several times higher during the acute hypoxic stress as compared to serum ep values during stable state prior to or after such stress (Fig. 5). After the amelioration of the hypoxic stress, serum Ep values fell to levels that were inappropriately low for the degree of anemia in all 6 patients.

Our findings in patients are similar to those reported previously in sheep[33]. Eschbach et al. showed that phlebotomy of uremic sheep resulted in increase in serum Ep and increased erythropoiesis, while administration of blood transfusions to these uremic sheep resulted in a decrease in plasma iron turnover and increase in marrow transit time despite achievement of a final hematocrit that was lower than in sheep[33]. Walle et al. more recently showed a rise in serum Ep in hemodialysis patients in response to spontaneous hemorrhage and suppression in serum Ep levels following blood transfusion[49b].

3. *Is there lowered set point for erythropoietin production in renal failure?*

Though Ep is produced mainly in the kidneys, extrarenal foci of Ep production also exist, the most notable of which is the liver[15]. Ep deficiency is, however, seen in renal failure despite availability of the extrarenal site of Ep production. The possible mechanisms for the inappropriately low serum Ep levels in renal failure include: (1) decreased Ep production capacity due to damage of the major Ep producing site, i.e., the kidney; (2) a decrease in the affinity of hemoglobin for oxygen[50] resulting in increased efficiency of oxygen delivery to tissues and consequently to decreased severity of tissue hypoxia relative to the degree of anemia; (3) unavailability of critical amino acids that are essential for Ep synthesis due to protein malnutrition or abnormal protein metabolism[51-53], and (4) abnormally low set point for Ep production in relation to tissue oxygenation.

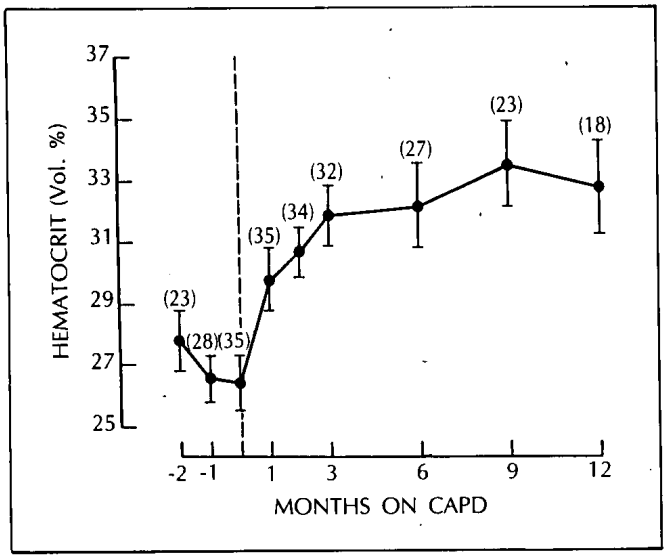

Fig. 6.—Relationship between hematocrit and duration of CAPD (means ± SEM): Numbers in parentheses represent the number of patients. (From Chandra et al.: ref. 59.)

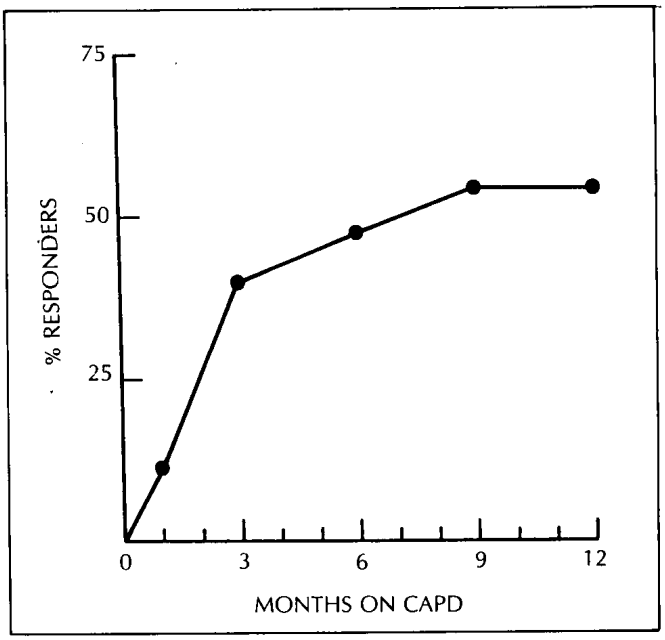

Fig. 7.—Relationship between incidence of hematocrit response and duration on CAPD. (From Chandra et al.: ref. 59.)

The lack of correlation between serum Ep levels and GFR in nondialysis patients with variable degrees of renal insufficiency observed by Chandra et al. and McGonigle et al. [30, 33a], suggests that decreased Ep production is not merely related to the loss of renal parenchyma. The observations that patients with renal failure are able to elevate serum Ep levels in response to acute hypoxia [30, 49], indicate that lack of availability of critical amino acids for Ep synthesis or lack of Ep production capacity are not the major causative factors for Ep deficiency in renal failure. Uremic patients manifest inappropriately low serum Ep levels during stable steady state deposite the potential ability to increase Ep production with acute hypoxia [30]. These observations suggest that either increased Ep production cannot be sustained in uremia or the tissue oxygenation-Ep feedback system operated at a lower set point of tissue oxygen delivery in uremics than in

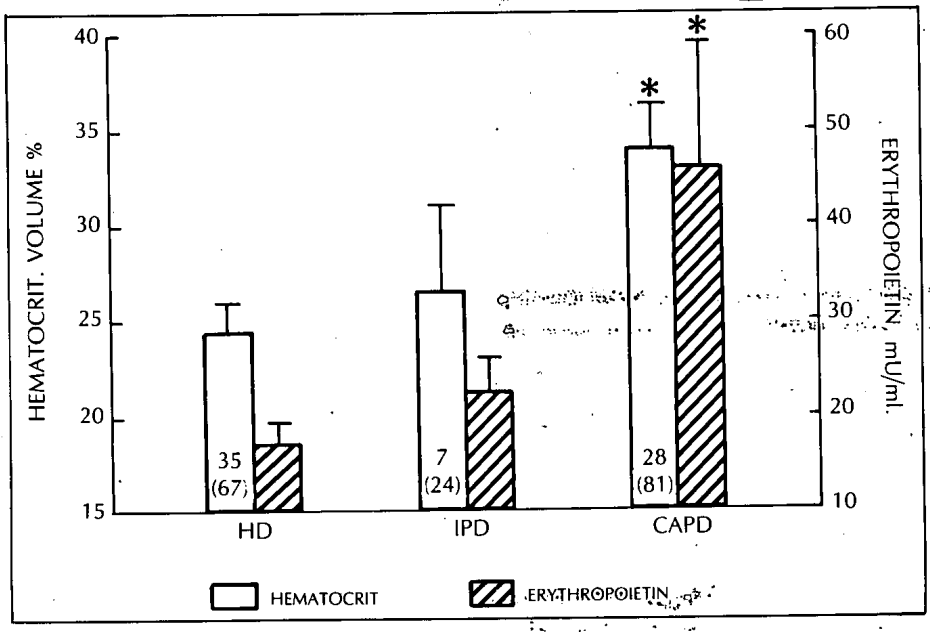

Fig. 8.—Hematocrit serum Ep levels in patients on different modes of dialysis (means ± SEM); the numbers in parenthesis represent the number of determinations and the upper numbers in the bars represent the numbers of patients.
** $p < 0.05$ for CAPD vs HD group. (From Chandra et al.: ref. 59.)*

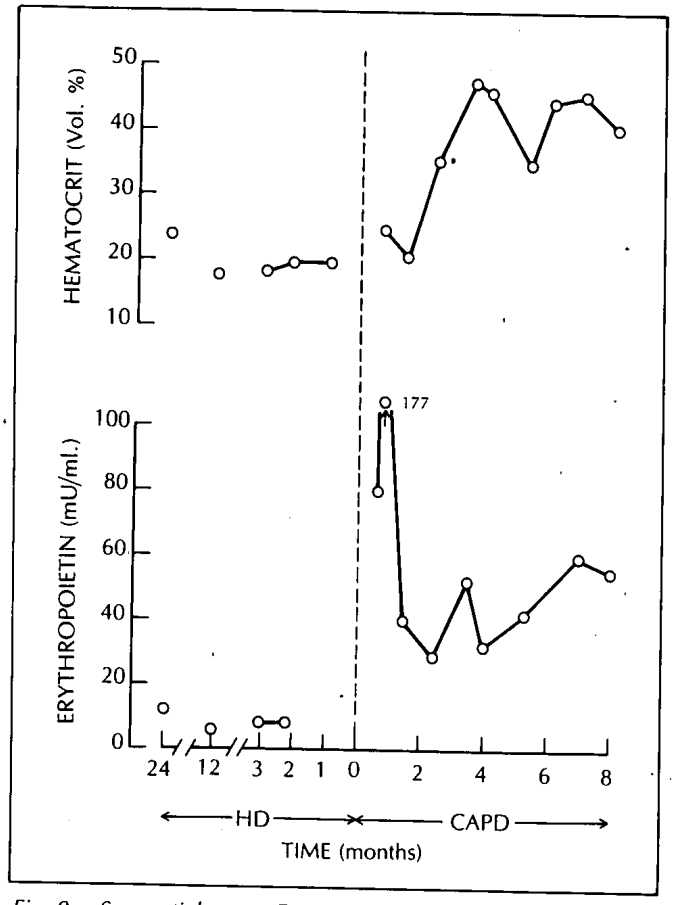

Fig. 9.—Sequential serum Ep concentration and hematocrit in a patient who switched form hemodialysis to CAPD treatments. (From Chandra et al.: ref. 58.)

normal individuals. Sufficient observations indicate that a high rate of Ep production can be sustained in some uremic patients. Several hemodialysis patients have been reported who achieved and maintained normal or close to normal hematocrit in response to high Ep production from either diseased native kidneys[32, 54] rejected renal allograft[55, 56] or regenerating liver[57]. In addition, we have reported high serum Ep levels in CAPD patients in stable state[58, 59].

The available evidence suggests that the hypoxia-Ep-hematocrit feedback system functions at a lower set point of tissue oxygenation in patients with renal failure than in normal individuals. This may be due to either decreased sensitivity of the renal hypoxia sensor from renal parenchymal damage or the uremic biochemical milieu, or because of inherently higher threshold for stimulating Ep production from extrarenal sites of Ep synthesis.

4. *Influence of the mode of dialysis on the anemia of renal failure and serum erythropoietin levels*

An improvement in ferrokinetic studies as well as hematocrit has been noted in patients with end stage renal disease after initiation of hemodialysis treatments. This improvement in hematocrit, however, is not associated with elevation in serum Ep levels[39]. The improvement in anemia may be related to an increase in hemoglobin-oxygen affinity due to removal of retained phosphates, correction of hyperparathyroidism, better nutrition, or to possible removal of inhibitors of erythropoiesis by hemodialysis.

We evaluated the effect of initiation of CAPD on hematocrit in 35 patients with end stage renal disease[59]. Figure 6 illustrated the significant increase in hematocrit that was noted in CAPD patients within one month of starting CAPD ($P < 0.001$). The hematocrit values obtained at 3, 6, 9, and 12 months after starting CAPD were all significantly higher than the pre-CAPD hematocrit ($P < 0.001$). In some patients, hematocrit did not change at all after starting CAPD while some patient increased their hematocrit by as much as 17 % within 2 months. Three patients manifested a rise in hematocrit to values > 50 volume % at 3 weeks, 6 weeks, and 12 months of starting CAPD. Figure 7 illustrates the percentage of CAPD patients who «responded» with increase in hematocrit by at least 10 volume percent over the pre-CAPD hematocrit or to normal hematocrit (> 38 % in females and < 42 % in males). This response was evident within 1 month of starting CAPD in 11 %, within 3 months in 40 %, and within 9 months in all 54 % of patients who did so. The increase in hematocrit in CAPD patients is predominantly due to an increase in red cell mass and to a lesser extent due to a reduction in the excess plasma volume. Red cell survival is not found to be significantly altered with CAPD treatment[37].

We measured serum Ep levels by RIA in 70 patients on different dialysis treatments to evaluate the influence of the mode of dialysis on the serum Ep levels relative to the severity of anemia. Thirty-five patients were on hemodialysis, 7 were on intermittent peritoneal dialysis, and 28 were on CAPD. A total of 172 serum Ep measurements were performed since 42 of the 70 patients had from 2 to 8 blood samples drawn for Ep. Figure 8 illustrates the serum Ep levels and hematocrit in these three groups of dialysis patients. Compared to hemodialysis patients, CAPD patients had higher serum Ep levels and hematocrit ($P < 0.05$). The Ep and hematocrit values of intermittent peritoneal dialysis patients were intermediate between those of hemodialysis and CAPD groups. Our findings suggest that CAPD provides a biochemical milieu more condusive for Ep production than hemodialysis treatment and that the improvement in anemia in CAPD patients may be related to a greater availability of Ep.

Serum Ep levels were found to be higher in six CAPD

patients when measured in the first 4 weeks of initiation of CAPD (144 ± 35 mU/ml) (mean ± SEM) as compared to values obtained in 24 patients later in the course of CAPD treatment (39 ± 6.4 mU/ml)[59]. A significant elevation in serum Ep levels after starting CAPD was noted in two patients who had serum EP levels measured while on a differente mode of dialysis earlier. We have reported[58] a 21-year-old female with sickle thalassemia who manifested severe anemia in association with low serum Ep levels of 8.0 ± 1.2 mU/ml while on hemodialysis for 5 years (Fig. 9). Serum Ep levels increased to 80 and 177 mU/ml respectively 2 and 3 weeks after starting CAPD. The elevation in serum Ep levels was followed by a rise in reticulocyte count from 3.9 % to 22 % and in hematocrit from 19 % to 48 %. The other patient was switched from intermittent peritoneal dialysis to CAPD treatment. His serum Ep levels ranged from 15-26 mU/ml while on IPD for 3 months. Serum Ep values increased to 212 mU/ml within 3 weeks of starting CAPD. This was followed by a rise in hematocrit from 30 % to 35 % six weeks after starting CAPD.

Increased serum Ep levels in CAPD as compared to hemodialysis patients are most likely related to increased production, since Ep catabolism or clearance is not significantly influenced by the mode of dialysis. Liver, reticuloendothelial system, and erythropoietic tissues are the major sites of Ep catabolism[60]. Increased Ep production in CAPD compared to hemodialysis patients may be related to achievement of better clearance of uremic toxins[61] that may inhibit Ep production by blunting the sensitivity of hipoxia sensor mechanism. Alternatively the improved state of protein metabolism associated with CAPD[62], or Ep production by dialysate stimulated peritoneal macrophages may contribute to higher Ep levels.

5. *Possible role of peritoneal macrophages in erythropoietin in CAPD patients*

The significant rise in hematocrit noted after starting CAPD treatment is often not sustained[60] suggesting that factors other than improved biochemical milieu contribute to increased Ep production in CAPD patients. Since peritoneal macrophages have the potential to produce Ep[63] we evaluated the possibility that the repeated infusion of hypertonic and relatively acidic dialysate in the peritoneal cavity of CAPD patients may stimulate these cells to produce Ep.

Ep concentration was measured by RIA in the dialysis effluent of 13 CAPD patients after an overnight 9-12 hour exchange. The Ep concentration in the dialysate was 2.99 ± 0.24 mU/ml with simultaneous serum Ep concentration of 26.6 ± 4.2 mU/ml (unpublished observation). The calculated Ep excretion rate in the dialysate was 560 ± 36 mU/hour of CAPD treatment. Since the volume of distribution of Ep is reported to be equal to the plasma volume[60] the total Ep body pool in these 13 adult CAPD patients was calculated to be 70,224 mU at any given time based on their mean hematocrit of 34 %, mean serum Ep concentration of 26.6 mU/ml, estimated blood volume of 4 l, and plasma volume of 2.64 l. We calculated the mean Ep excretion rate to be 13,440 mU/24 hours that would represent about 20 % of the Ep pool of the body. Since Ep is a relatively large molecule (molecular weight 34,400 dalton), its clearance from the blood in the peritoneal dialysate is not expected to be this high. The large excretion rate of Ep in the dialysate suggests that Ep may be produced in the peritoneal cavity by peritoneal macrophages. Wideroe et al. showed an increase in the erythropoiesis-stimulating activity in the peritoneal dialysis effluent of CAPD patients as early as 2 days after starting CAPD[63]. We have cultured macrophages obtained from peritoneal dialysis effluent of CAPD patients; macrophage conditioned medium contained Ep at a concentration of 3.5 ± 0.3 mU/ml (unpublished observation). We hypothesize that Ep produced by the peritoneal macrophages may get into the blood stream to a certain extent and contribute to the elevation of serum Ep levels in CAPD patients. In patients treated with intermittent peritoneal dialysis, however, because of the short dwell time and frequent peritoneal lavages, the Ep produced in the peritoneum may not enter the blood stream in significant quantities.

6. *Fluctuations in serum erythropoietin and hematocrit levels in dialysis patients*

We noted significant fluctuations in hematocrit and serum Ep values in 42 dialysis patients, when 2 to 8 blood samples were obtained at different time intervals[59]. The mean coefficient of variation for serum Ep values was 57 % for hemodialysis patients, 43 % for patients on intermittent peritoneal dialysis, and 108 % for CAPD patients. The fluctuation in Ep balues was not due to methodologic problems, because in a normal human serum pool, the intraassay and interassay coefficient of variation for Ep values were 8.4 % and 9.7 % respectively. Our findings suggest that a single determination of Ep in a dialysis patient may not accurately reflect the true erythropoietic status of the patient.

Multiple factors may influence Ep production and its serum concentration in dialysis patients. These factors include (1) plasma volume contraction; (2) changes in hemoglobin-oxygen affinity due to fluctuations in acid base status and serum posphate concentration[64] (3) status of protein malnutrition[51, 52] (4) serum levels of renin, angiotensin II and aldosterone[65, 66] (5) extent of hemolysis[67] (6) renal tissue concentration of

calcium and certain prostaglandins[68]. Since the biochemical environment of the body changes from day to day in dialysis patients, wide fluctuations in serum Ep levels can be expected.

We and others have observed poor correlation between serum Ep levels and hematocrit in predialysis patient groups with varying levels of renal function[30, 31] and in hemodialysis patients[33a, 59]. When data from patients treated with different modes of dialysis were analysed together (Fig. 8) a weak correlation between serum Ep and hematocrit was found ($r - 0.36$, $P < 0.005$) with CAPD patients having the higher hematocrit and Ep and hemodialysis patients having lower Ep and hematocrit[31]. This poor correlation between hematocrit and serum Ep appears to be related to the multifactorial origin of the anemia of renal failure and to several factors that may affect Ep production without influencing hematocrit[64-68]. Moreover, Ep production at a site not governed by the hematocrit-tissue oxygenation-Ep feedback regulation e.g., peritoneal macrophages may contribute to the lack of correlation between hematocrit and Ep.

7. *Response to endogenous and exogenous erythropoietin in chronic renal failure*

Several observations suggested that Ep is capable of stimulating erythropoiesis despite the uremic milieu. (1) Ep levels are lower in anephric patients than in nephric uremic patients. Bilateral nephrectomies in a stable dialysis patient result in a further decrease in erythropoiesis and a hematocrit[46, 69]. (2) Patients with hemolytic uremic syndrome often manifest a high rate of erythropoiesis despite advanced renal failure. (3) Hemodialysis patients with polycystic kidney disease manifest higher erythropoiesis and higher serum Ep levels than patients with renal failure from other renal diseases[32]. (4) CAPD patients manifest higher hematocrit in association with higher serum Ep levels than do hemodialysis patients[58, 59]. (5) Several hemodialysis patients have improved or normalized their hematocrit in association with increased serum Ep levels[54-57].

Several investigators examined the response of uremic animals to Ep administration. In 1958, Naets showed that erythropoiesis was restored to near normal in eight anephric dogs by infusion of large amounts of Ep[70]. In two subsequent studies, the anemia of chronically uremic rats was corrected as well as that in nonuremic rats by daily administration of Ep[71, 72]. Eschbach et al. infused Ep-rich sheep plasma into subtotally nephrectomized uremic sheep, some of whom were maintained on hemodialysis[73]; identical erythropoietic responses were elicited by the same doses of Ep-rich plasma in both normal and uremic sheep.

Ep replacement therapy had not been possible in the past because of difficulties involved in purifying large amounts of natural material. With the availability of large quantities of pure human Ep derived from recombinant DNA technique, clinical trials of Ep replacement in hemodialysis patients were launched in the late 1985[74, 75]. Virtually all hemodialysis patients treated with 50-300 U/kg Ep IV twice weekly have increased their hematocrit. Transfusions are eliminated, iron overload is reduced and quality of life improves. These clinical trials have extended and confirmed our expectations[76] that Ep administration can totally correct the anemia of renal failure. These trials showed that the uremic inhibitors of erythropoiesis do not preclude an erythropoietic response.

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