Hemodialysis and continuous ambulatory peritoneal dialysis effects on erythropoiesis in renal failure

RICHARD J. S. MCGONIGLE, FRED HUSSERL, JOHN D. WALLIN, and JAMES W. FISHER

Department of Pharmacology and Internal Medicine (Nephrology), Tulane University School of Medicine, and Department of Medicine, Section on Renal Disease, Ochsner Medical Institutions, New Orleans, Louisiana

Hemodialysis and continuous ambulatory peritoneal dialysis effects on erythropoiesis in renal failure. Parameters of erythropoiesis were studied in patients with endstage renal disease established on continuous ambulatory peritoneal dialysis (CAPD) and regular hemodialysis treatment (RDT). Serum erythropoietin was measured by radioimmunoassay, and erythroid progenitor cell (CFU-E) formation was assayed in fetal mouse liver cultures. Serum erythropoietin concentrations in both CAPD (35.3 ± 4.0 mU/ml) and RDT (31.9 ± 1.9 mU/ml) patients were significantly higher ($P < 0.01$) than normal values (23.1 ± 1.0 mU/ml). The serum erythropoietin concentration did not correlate with either hematocrit or inhibition of CFU-E formation in either group of dialysis patients. In both CAPD and RDT patients the hematocrit correlated significantly ($P < 0.001$) with the degree of serum inhibition of CFU-E formation. CFU-E formation decreased from 74.5 ± 2.5 to $62.5 \pm 3.5\%$ of control with increasing concentrations of uremic serum in cell cultures from 5 to 20%. In RDT patients a single hemodialysis produced a decrease in the mean serum erythropoietin concentration from 31.8 ± 2.1 to 27.4 ± 1.8 mU/ml ($P < 0.01$) but no significant change in CFU-E formation. In conclusion, although serum immunoreactive erythropoietin levels are elevated above the normal range in dialysis patients, the response remains inadequate for the severity of the anemia, and it is the degree of serum inhibition of erythropoiesis in both CAPD and RDT patients which correlates with and possibly determines the degree of anemia.

Effets de l'hémodialyse et de la dialyse péritonéale continue ambulatoire sur l'erythropoïèse au cours de l'insuffisance rénale. Les paramètres de l'érythropoïèse ont été étudiés chez des malades avec une néphropathie terminale en dialyse péritonéale continue ambulatoire (CAPD) et en hémodialyse périodique (RDT). L'érythropoïétine sérique a été mesurée par dosage radioimmunologique, et la formation de cellules souches érythroïdes (CFU-E) a été dosée dans des cultures de foie foetal de souris. Les concentrations d'érythropoïétine sérique chez les malades en CAPD ($35,3 \pm 4,0$ mU/ml) et en RDT ($31,9 \pm 1,9$ mU/ml) étaient significativement plus élevées ($P < 0,01$) que les valeurs normales ($23,1 \pm 1,0$ mU/ml). La concentration d'érythropoïétine sérique n'était corrélée ni à l'hématocrite, ni à l'inhibition de formation de CFU-E dans aucun groupe d'hémodialysés. Chez les malades en CAPD et en RDT, l'hématocrite était significativement corrélé ($P < 0,001$) avec le degré d'inhibition sérique de formation de CFU-E. La formation de CFU-E diminuait de $74,5 \pm 2,5$ à $62,5 \pm 3,5\%$ du contrôle en augmentant les concentrations de sérum urémique dans les cultures cellulaires de 5 à 20%. Chez les malades en RDT, une hémodialyse unique entraînait une diminution de la concentration d'érythropoïétine sérique moyenne de $31,8 \pm 2,5$ à $27,4 \pm 1,8$ mU/ml ($P < 0,01$), mais pas de changement significatif dans la formation de CFU-E. En conclusion, bien que les niveaux d'érythropoïétine immunoréactive sérique soient élevés au-dessus de la normale chez les malades en dialyse, la réponse reste inadéquate compte tenu de la sévérité de l'anémie, et c'est le degré d'inhibition sérique de l'érythropoïèse chez des malades en CAPD et en RDT qui est corrélée avec et peut-être détermine le degré d'anémie.

Continuous ambulatory peritoneal dialysis (CAPD) has been reported to produce an improvement in the anemia associated with endstage renal disease as indicated by the hematocrit [1–5]. An increase in hemoglobin levels over a period of months to years with regular hemodialysis therapy (RDT) has also been reported [6–8]. Fisher [9] reviewed the major factors involved in the mechanism of the anemia of chronic renal failure including erythropoietin deficiency, inhibition of erythropoiesis, and shortened red cell life span. In vitro membrane dialysis has been shown to partially remove inhibitors of erythroid progenitor cell formation including inhibitors of both CFU-E (colony forming unit-erythroid) and BFU-E (burst forming unit-erythroid) formation [10–12]. The improvement in anemia in patients on CAPD may be related to a more efficient removal of "middle-molecule" uremic toxins [1, 2, 13] which have been considered responsible for the inhibition of erythropoiesis [10, 14]. Alternative explanations include an improvement in red cell survival time in patients on CAPD, since hemolysis persists and may even be exacerbated by hemodialysis in addition to the effects of uremia [15, 16]. The increased blood loss from the dialyzer unit, together with blood loss from various other sources [17, 18] would also tend to aggravate the anemia found in patients on RDT. A recent report showed that the increase in hematocrit of patients starting CAPD reflects a decrease in plasma volume in addition to an increase in red cell mass [19]. Heretofore, serum levels of erythropoietin and inhibitors of erythropoiesis have not been compared in patients on CAPD and RDT. This study was designed to examine the reported erythropoietin deficiency associated with the anemia of renal failure in patients on CAPD and in patients receiving RDT and to assess the effect of the two forms of dialysis on erythropoiesis in the fetal mouse liver cell culture system.

Methods

Patients

Two groups of patients were studied after informed consent had been obtained: *(1)* Forty-six patients established on regular hemodialysis treatment (RDT) were investigated. Their ages ranged from 15 to 79 years (mean ± SEM, 52.5 ± 2.1 years) and they had been receiving hemodialysis for periods ranging from 2 to 94 months (mean ± SEM, 35.7 ± 4.1 months). The majority of patients were on dialysis 4 hr three times per week using a

standard 1 m² dialyzer. The etiology of endstage renal disease in these patients included hypertension, 14 patients; diabetes mellitus, 9; glomerulonephritis, 4; chronic pyelonephritis, 3; polycystic kidney disease, 12; Alport syndrome, 1; nephrocalcinosis, 2; obstructive uropathy, 1; and lupus nephritis, 1. Most patients were receiving oral vitamin supplementation, but no patient was receiving androgen therapy.

(2) Thirty-six patients established on CAPD were studied. Their ages ranged from 20 to 77 years (mean ± SEM, 50.6 ± 2.6 years) and had been on CAPD 1 to 38 months (mean ± SEM, 15.7 ± 2.6 months). Standard 2-liter exchanges were performed four times each day. The etiology of renal disease included polycystic kidney disease, 4 patients; diabetes mellitus, 6; glomerulonephritis, 9; obstructive uropathy, 3; chronic interstitial nephritis, 1; congenital renal dysplasia, 1; hypertension, 6; lupus nephritis, 1; and unknown, 5. Two patients were surgically anephric. All patients were receiving oral vitamin supplementation, but no patient was adminstered iron or androgen treatment.

Blood was drawn immediately before and after hemodialysis or at the time of exchange of peritoneal dialysis bags. The blood was centrifuged and the serum was frozen at −70°C. No patient was studied within 2 weeks of a blood transfusion. Patients on CAPD and RDT had minimal residual renal function with creatinine clearances all less than 5 ml/min. The iron status in 26 patients on RDT and 18 patients on CAPD was assessed by measurement of the serum ferritin concentration.

Investigations

Radioimmunoassay for serum erythropoietin [20]. 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. A human 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 was used for the immunization. The antiserum was absorbed with normal human serum and urinary proteins to increase the specificity for the assay for erythropoietin. A second antibody (goat antirabbit gamma globulin) was used for the separation of bound from free-labeled antigen.

Fetal mouse liver cell culture technique. Mouse (CD-1) liver cells from fetuses 12 to 13 days old, known to be predominantly erythroid committed, were prepared according to the technique of Iscove, Sieber, and Winterhalter [21]. Liver cells were disaggregated and suspended as single cells at a concentration of 10⁵ cells/ml in a culture medium containing alpha modified Eagle's medium with Earle's salts, and without ribosides, deoxyribosides, and 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 strepto-
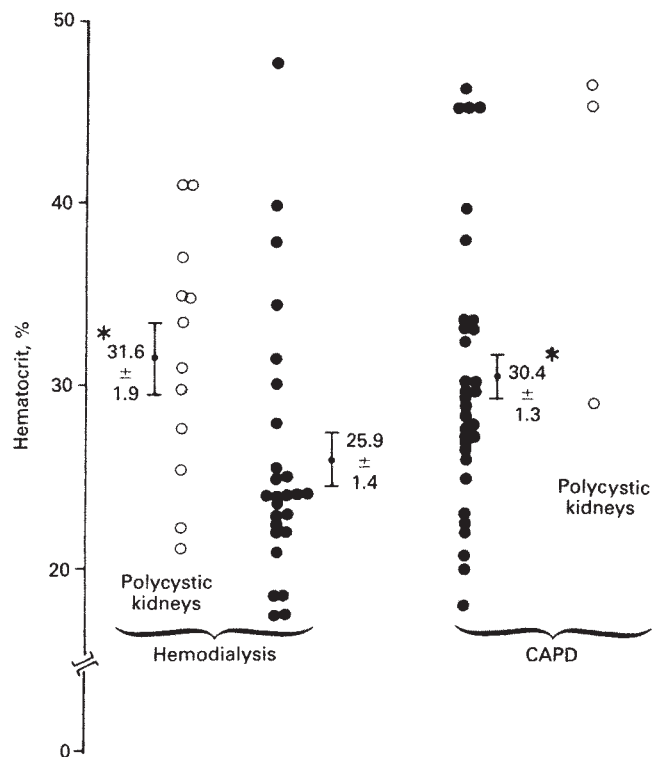

Fig. 1. *Hematocrit levels in patients on continuous ambulatory peritoneal dialysis CAPD and on regular hemodialysis treatment* (RDT). Numbers are means ± SEM. The *asterisk* represents values significantly higher (*P* < 0.05) than RDT patients.

mycin. 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 cultures (1 ml) were plated in 10 × 35 mm petri dishes and incubated for 48 hr at 37°C in a humidified atmosphere at 95% air and 5% CO_2. After staining of the plates with diaminobenzidine according to the method of Ogawa et al [22], 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[1] (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.

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

Results

The age and sex distribution of each group of dialysis patients used in the present studies were similar. Patients had been receiving RDT for a longer period of time than patients on CAPD. No clinical evidence of blood loss or iron deficiency was detected in either group of patients. The mean serum ferritin concentration in RDT patients was 439 ± 77 μg/liter (range, 47 to 1293 μg/liter), and in CAPD patients 190 ± 40
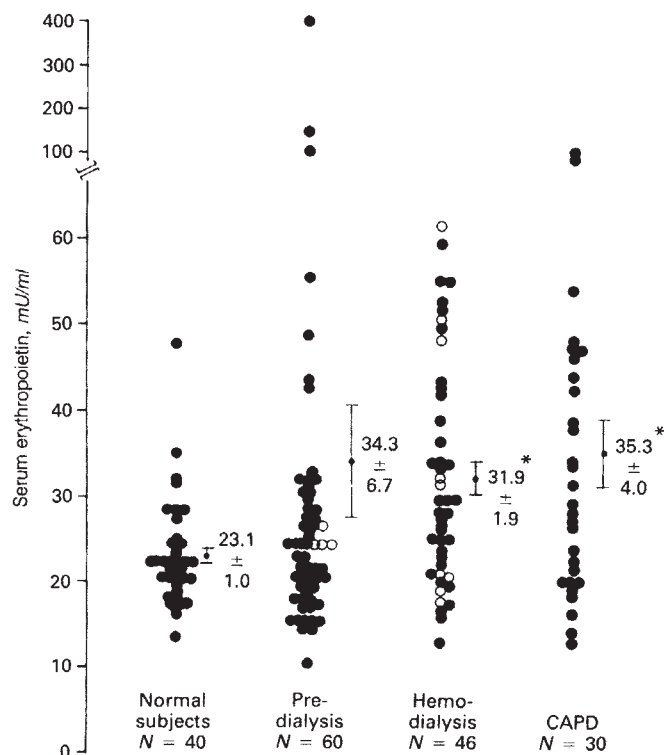
Fig. 2. *Serum erythropoietin concentrations in 40 normal subjects, 60 patients with varying degrees of renal insufficiency not on dialysis, 46 patients receiving regular hemodialysis treatment (RDT) and 30 patients on continuous ambulatory peritoneal dialysis (CAPD). Numbers are means* ± SEM. *The* asterisk *represents values significantly higher (P < 0.01) than the normal range. The* open circles *represent patients with polycystic kidney disease.*

μg/liter (range, 33 to 500 μg/liter). One patient on CAPD with a diagnosis of myelofibrosis was excluded from the study. Hematocrit levels were significantly ($P < 0.05$) higher in patients established on CAPD (mean, 30.4 ± 1.3%) in comparison to patients receiving RDT (mean, 25.9 ± 1.4%) though in both groups a wide range in the severity of anemia was seen (Fig. 1). RDT patients in whom the etiology of renal failure was due to polycystic kidney disease had significantly higher ($P < 0.05$) hematocrit values (mean, 31.6 ± 1.9%) than RDT patients with renal disease due to other causes (mean, 25.9 ± 1.6%). Similarly two of the three patients on CAPD with a diagnosis of polycystic kidney disease had elevated hematocrit levels above 45%.

Serum erythropoietin levels were significantly elevated ($P < 0.01$) above the normal range of 13.5 to 48 mU/ml (mean, 23.0 ± 1.0 mU/ml) in both CAPD (35.3 ± 4.0 mU/ml) and RDT (31.9 ± 1.9 mU/ml) patients (Fig. 2). No significant difference was seen in the mean serum erythropoietin concentration between the two groups of dialysis patients. Patients with polycystic kidney disease receiving RDT had similar serum erythropoietin levels in comparison to patients also on regular hemodialysis with other forms of renal disease (35.7 ± 6.7 vs. 31.6 ± 2.0 mU/ml). Of 60 patients studied with varying degrees of renal insufficiency who had not started dialysis treatment serum erythropoietin levels were normal in 54 patients and elevated in 6 patients. Three of these patients had markedly elevated serum erythropoietin concentrations for which no explanation was found.
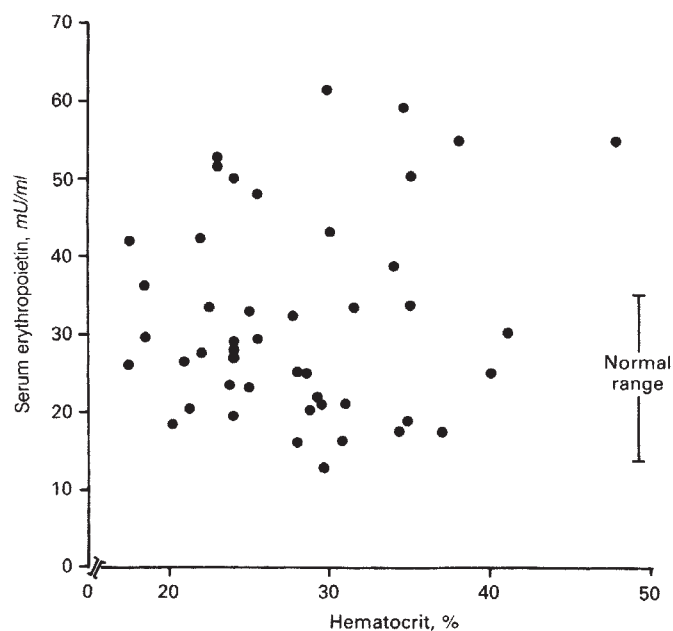

Fig. 3. *Relationship between hematocrit and serum erythropoietin concentration in patients receiving regular hemodialysis treatment (RDT).*
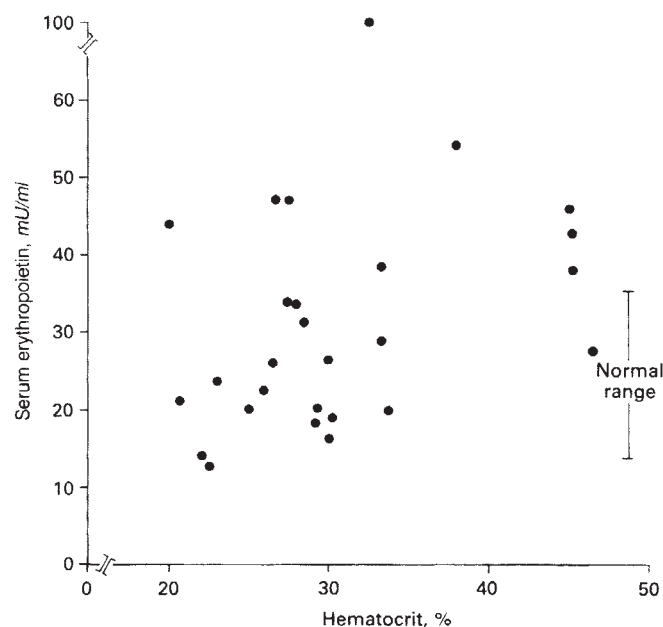

Fig. 4. *Relationship between serum erythropoietin concentration and hematocrit in patients on continuous ambulatory peritoneal dialysis (CAPD).*

A wide scatter of serum erythropoietin concentrations in patients receiving RDT showed no relationship to the patients' hematocrit levels (Fig. 3). Patients on CAPD also showed no significant association between hematocrit and serum erythropoietin concentration. However, there was a trend (r = 0.31) toward a correlation between the hematocrit and erythropoietin levels in that a higher hematocrit level was related to more elevated serum erythropoietin concentrations (Fig. 4). There
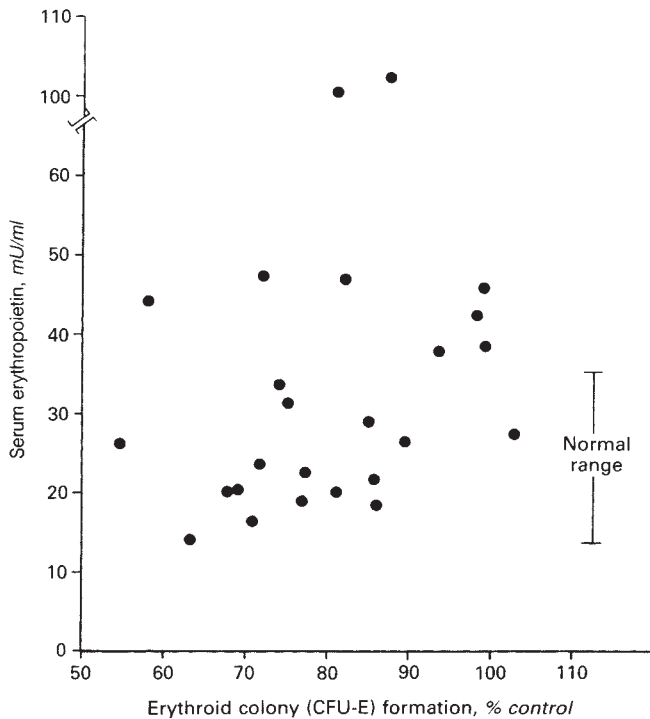
Fig. 5. *Relationship between serum erythropoietin concentration and inhibition of CFU-E formation by sera from patients on CAPD.*
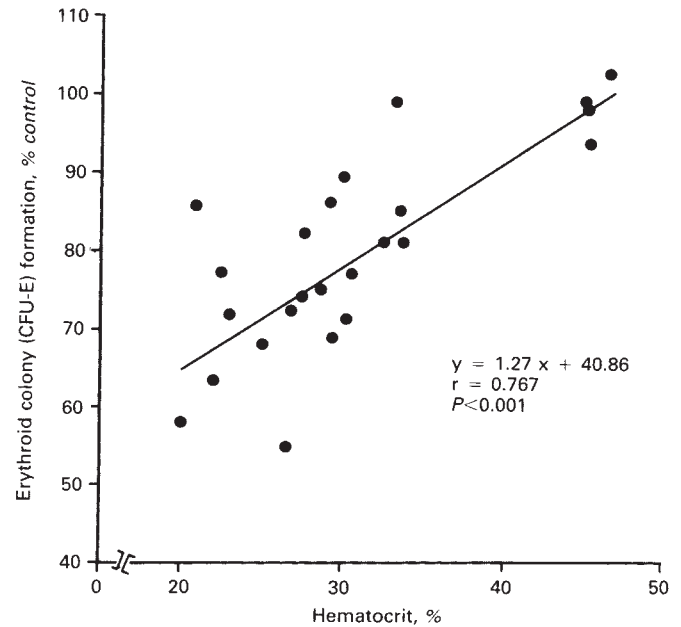

Fig. 6. *Relationship between serum erythropoietin concentration and inhibition of CFU-E formation by sera from patients on RDT.*

was no significant relationship between the serum erythropoietin concentrations and the percentage inhibition of erythroid colony formation by serum in either CAPD patients (Fig. 5) or patients receiving RDT (Fig. 6). Inhibition of erythroid progenitor cell (CFU-E) colony formation was seen in all patients receiving CAPD or RDT in whom the hematocrit was less than

Fig. 7. *The direct relationship between hematocrit and inhibition of erythroid colony formation in CAPD patients.* The concentration of patient's serum in cultures is 5%.
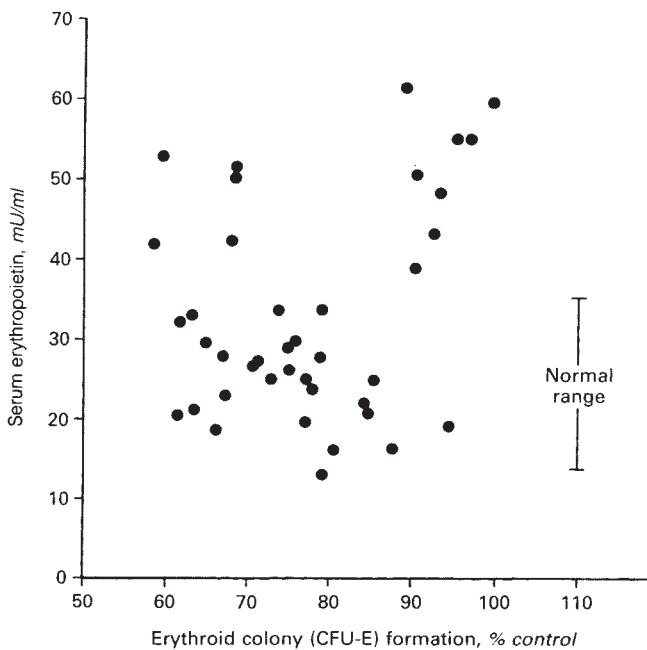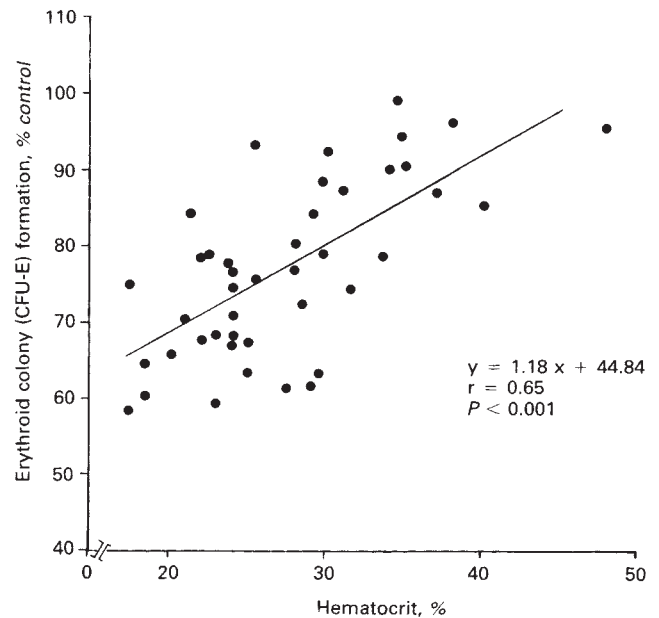

Fig. 8. *The direct relationship between inhibition of erythroid colony formation and hematocrit in patients receiving RDT.* The concentration of patient's serum in cultures is 5%.

35%. The degree of inhibition of CFU-E formation correlated directly with the hematocrit in both CAPD patients (r = 0.767, $P < 0.001$, Fig. 7) and RDT patients (r = 0.65, $P < 0.001$, Fig. 8). The more severe the anemia the greater the degree of inhibition of erythroid colony formation by uremic serum in cell culture. The relationship between the hematocrit value and the effect of serum on CFU-E formation was similar in the two groups of dialysis patients using the method of least squares for
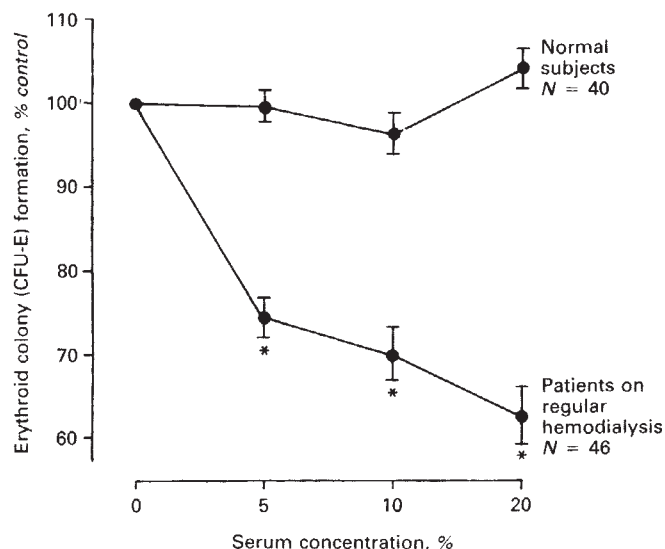
Fig. 9. *Effects of different concentrations of uremic serum from patients receiving RDT on erythroid colony formation in fetal mouse liver cultures.* Values are expressed as means ± SEM. The *asterisk* represents values significantly different ($P < 0.001$) from CFU-E formation in normal sera.

Fig. 10. *Decrease in serum erythropoietin concentration immediately before and after a single 4-hr hemodialysis in patients receiving RDT.* Values are expressed as means ± SEM.

plotting the linear regression line. CFU-E formation was progressively reduced ($P < 0.05$) from 74.5 ± 2.5% to 62.7 ± 3.5% of control when increasing concentrations (5 to 20%) of uremic sera were added to the fetal mouse liver cell cultures (Fig. 9). The difference between the effects of normal serum and uremic serum on CFU-E formation was highly significant ($P < 0.001$) at each of the different concentrations of serum in culture tested.

Serum erythropoietin concentrations measured immediately before and after a single 4-hr hemodialysis showed a significant ($P < 0.01$) decrease from a mean of 31.8 ± 2.1 to 27.4 ± 1.8 mU/ml (Fig. 10). The mean value for erythroid colony formation also showed a slight but not statistically significant decrease from 76.0 ± 1.9 to 73.0 ± 1.3% of control (Fig. 11).

Discussion

Regular hemodialysis therapy (RDT) and continuous ambulatory peritoneal dialysis (CAPD) have both been reported to lead to an improvement in the anemia associated with endstage renal disease [1–8]. However, a large proportion of RDT and CAPD patients remains anemic. The often greater improvement in hematocrit levels during the first 6 months of CAPD treatment in comparison to RDT has been reported to be due to a reduction in plasma volume in addition to an increase in red cell mass [19]. CAPD is associated with a much greater clearance of middle molecular weight substances and a less efficient removal of small molecular weight substances in comparison to hemodialysis [1]. These observations have led several investigators to suggest that uremic toxins responsible for inhibition of erythropoiesis are middle molecular weight in size and that it is the difference in relative clearances of these inhibitors of erythropoiesis by RDT and CAPD which is responsible for any difference in effect of the two forms of dialysis on the anemia of renal failure [2, 3].

In this study we have examined parameters of erythropoiesis in both CAPD and RDT patients. Although significantly elevat-
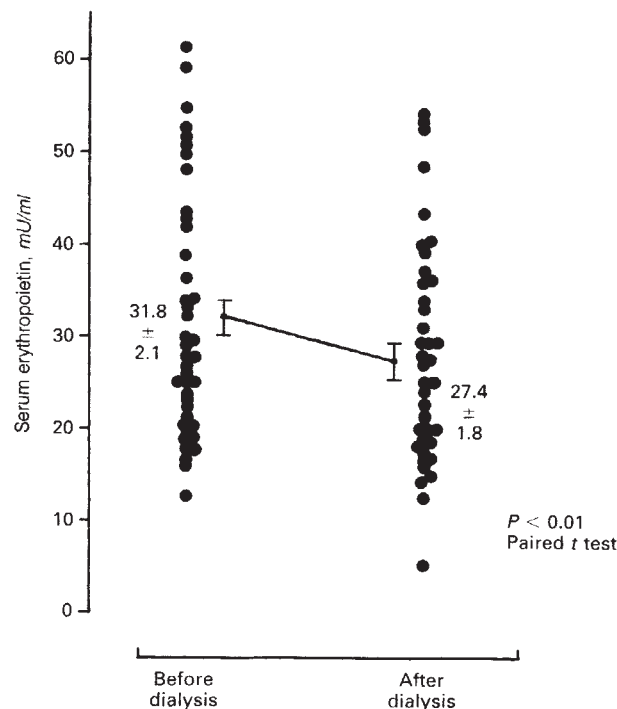
ed above the normal range in the two groups of dialysis patients, the mean serum erythropoietin concentration was inappropriately low for the degree of anemia confirming the importance of a relative erythropoietin deficiency in the anemia of renal failure. Several investigators have measured serum erythropoietin levels in patients with renal disease using a radioimmunoassay [23–25], but only small numbers of patients have been studied. Cotes [23] reported eight anephric patients with serum erythropoietin levels ranging from < 5 to 99 mU/ml. Zaroulis, Hoffman, and Kourides [25] showed a range for serum erythropoietin concentration of 18 to 115 mU/ml (mean ± SD 40.5 ± 30 mU/ml) in 11 patients with renal failure on regular hemodialysis. Utilizing the fetal mouse liver cell bioassay DeKlerk et al [26] found decreased serum erythropoietin levels in nephric patients on hemodialysis, and a significant positive correlation between hemoglobin and serum erythropoietin in nephric dialysis patients who were transfusion independent. Caro et al [27] reported serum erythropoietin levels in 25 patients undergoing chronic hemodialysis using the polycythemic mouse bioassay and a plasma concentration technique. Eight of 11 anephric patients had decreased but detectable levels of erythropoietin. Erythropoietin production was reported to be more variable in the nephric dialysis patients with both normal and elevated erythropoietin levels. The authors suggested that the difference may have been due to different degrees of marrow unresponsiveness related to hyperparathyroidism.

The fetal mouse liver CFU-E culture used in our experiments served as a convenient system to detect inhibition of erythropoiesis by uremic serum and does not necessarily reflect all of the in vivo effects of uremic serum on the erythroid progenitor cells in the marrow. However, the response of fetal mouse liver cell cultures to both erythropoietin and uremic serum does
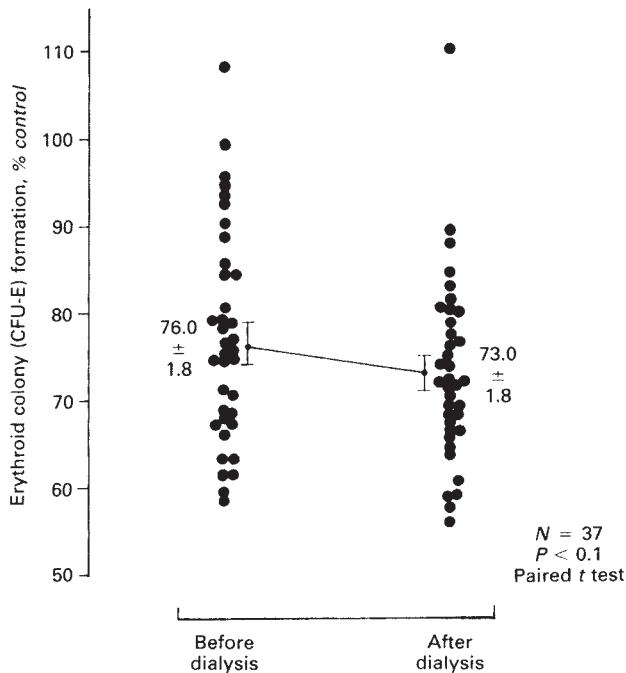
Fig. 11. *The change in effect of serum from patients receiving RDT on CFU-E formation immediately before and after a single 4-hr hemodialysis.* Values are expressed as means ± SEM. The concentration of serum in cultures is 5%.

closely parallel the response of human bone marrow cultures [12].

In the present studies it was the degree of inhibition of CFU-E formation rather than the serum erythropoietin concentration which correlated with the hematocrit. The continuing close association between hematocrit and inhibition of CFU-E formation in patients with endstage renal disease receiving either CAPD or RDT suggests that it is the degree of inhibition of erythropoiesis which determines the severity of anemia. An inverse relationship between hematocrit and both inhibition of heme synthesis in rabbit erythroblasts and mouse marrow CFU-E has been described previously in patients receiving long-term hemodialysis [28]. Neither form of dialysis appeared very efficient in clearing inhibitors of erythropoiesis from the serum of uremic patients, and the degree of abnormal erythropoiesis appeared very similar in the two groups of dialysis patients. The uremic toxins responsible for inhibition of erythropoiesis may be of small molecular weight size but not removed by dialysis due to intracellular or serum protein binding.

Zappacosta, Caro, and Erslev [5] reported that patients on CAPD in whom anemia improved had higher serum erythropoietin concentrations than those patients in whom the hematocrit remained unchanged. This study was retrospective and only involved a small number of patients. We did not confirm these findings in that in our studies the serum erythropoietin concentration was not associated with either the hematocrit nor related to changes in hematocrit in patients receiving CAPD. Patients with polycystic kidney disease receiving RDT had higher hematocrit levels than RDT patients with endstage renal disease due to other causes. This was not due to higher serum erythropoietin concentrations in our patients with polycystic kidney

disease as was proposed by Zappacosta, Caro, and Erslev [5] for the polycystic kidney patients in their studies. Erythrocytosis has been reported in patients on long-term hemodialysis in whom the etiology of renal failure was due to polycystic kidney disease [29], diffuse parenchymal renal disease [30], and acquired cystic disease of endstage kidneys [31]. Elevated erythropoietin levels and erythrocytosis have been reported in dialysis patients with liver disease, in particular, hepatitis [32], but no patient in our studies had clinical or biochemical evidence for hepatocellular dysfunction.

In vitro dialysis has been reported to remove inhibitors of erythropoiesis [10, 11]. It is of interest that we found a reduction in CFU-E formation following a single 4-hr hemodialysis. Although this decrease in CFU-E formation did not achieve significance, it did parallel a significant fall in serum erythropoietin concentration. A decline in serum erythropoietin over a period of months of regular hemodialysis has been reported by Radtke et al [8]. However, Radtke et al used a fetal mouse liver cell assay for erythropoietin which is an in vitro biological assay subject to the variable effects of inhibitors of CFU-E formation also present in uremic serum. In contrast, the radioimmunoassay used in the present study was capable of accurately detecting small changes in serum erythropoietin concentrations. Nevertheless, the fall in serum erythropoietin concentration following a single hemodialysis was surprising in that the molecular weight of erythropoietin has been reported variously between 23,000 to 39,000 daltons [33–35] and would not therefore be expected to be cleared through a cuprophan membrane. It appears possible that the radioimmunoassay detects immunologically reactive fragments of erythropoietin sufficiently small to be removed by hemodialysis. Immunoreactive fragments of erythropoietin considerably smaller than the whole hormone and without biological activity have been postulated to be present in the sera of uremic patients [36]. Other factors affecting erythropoietin production or release may account for the change in serum levels during hemodialysis. A diurnal variation of serum immunoreactive erythropoietin has been described in normal subjects [37]. Postural changes may also possibly affect erythropoietin production since an interrelationship between the renin system and erythropoietin has been demonstrated [38]. It is also possible that a lack of reproducibility in the radioimmunoassay might account for these changes.

In conclusion, this study has demonstrated the continuing presence of inhibition of erythropoiesis in both patients receiving regular hemodialysis treatment and those on continuous ambulatory peritoneal dialysis. In addition, a close relationship between the severity of the anemia of renal failure and the degree of inhibition of CFU-E formation in the two groups of dialysis patients was seen. Serum erythropoietin levels were moderately elevated above the normal range but still represented a relative deficiency and had no positive correlation with the degree of anemia.

Acknowledgments

References

1. POPOVICH RP, MONCRIEF JW, NOLPH KD, GHODS AJ, TWARDOWSKI ZJ, PYLE WK: Continuous ambulatory peritoneal dialysis. *Ann Intern Med* 88:449–456, 1978

2. OREOPOULOS DG, ROBSON M, FALLER B, OGILVIE R, RAPOPORT A, DEVEBER GA: Continuous ambulatory peritoneal dialysis: a new era in the treatment of chronic renal failure. *Clin Nephrol* 11:125–128, 1979

3. GOKAL R, MCHUGH M, FRYER R, WARD MK, KERR DNS: Continuous ambulatory peritoneal dialysis: one year's experience in a UK dialysis unit. *Br Med J* 281:474–477, 1980

4. AMAIR P, KHANNA R, LEIBEL B, PIERRATOS A, VAS S, MEEMA E, BLAIR G, CHISHOLM L, VAS M, ZINGG W, DIGENIS G, OREOPOULOS D: Continuous ambulatory peritoneal dialysis in diabetics with end-stage renal disease. *N Engl J Med* 306:625–630, 1982

5. ZAPPACOSTA AR, CARO J, ERSLEV A: Normalization of hematocrit in patients with end-stage renal disease on continuous ambulatory peritoneal dialysis. *Am J Med* 72:53–57, 1982

6. ESCHBACH JW, FUNK P, ADAMSON JW, KUHN J, SCRIBNER BH, FINCH CA: Erythropoiesis in patients with renal failure undergoing chronic dialysis. *N Engl J Med* 276:653–658, 1967

7. ESCHBACH JW, ADAMSON JW, COOK JD: Disorders of red cell production in uremia. *Arch Intern Med* 126:812–815, 1970

8. RADTKE HW, FREI U, ERBES PM, SCHOEPPE W, KOCH KM: Improving anemia by hemodialysis: effect on serum erythropoietin. *Kidney Int* 17:382–387, 1980

9. FISHER JW: Mechanism of the anemia of chronic renal failure. *Nephron* 25:106–111, 1980

10. GUTMAN RA, HUANG AT: Inhibitor of marrow thymidine incorporation from sera of patients with uremia. *Kidney Int* 18:715–724, 1980

11. OHNO Y, REGE AB, FISHER JW, BARONE J: Inhibitors of erythroid colony forming cells (CFU-E and BFU-E) in sera of azotemic patients with anemia of renal disease. *J Lab Clin Med* 92:916–923, 1978

12. RADTKE HW, REGE AB, LAMARCHE MD, BARTOS D, BARTOS F, CAMPBELL RA, FISHER JW: Identification of spermine as an inhibitor of erythropoiesis in patients with chronic renal failure. *J Clin Invest* 67:1623–1629, 1981

13. MONCREIF JW, POPOVICH RP, NOLPH KD: Additional experience with continuous ambulatory peritoneal dialysis (CAPD). *Trans Am Soc Artif Intern Organs* 24:476–483, 1978

14. WALLNER SF, VAUTRIN RM, KURNICK JE, WARD HP: The effect of serum from patients with chronic renal failure on erythroid colony growth in vitro. *J Lab Clin Med* 92:370–375, 1978

15. SHAW AB: Haemolysis in chronic renal failure. *Br Med J* 2:213–244, 1967

16. GIOVANETTI S, CIONI L, BALESTRI L, BAIGINI M: Evidence that guanidines and some related compounds cause haemolysis in chronic uraemia. *Clin Sci* 34:141–148, 1968

17. CASTALDI PA, ROZENBURG MD, STEWART JH: The bleeding disorder of uraemia. A qualitative platelet defect. *Lancet* 2:66–69, 1966

18. CHENNEY K, BONNIN JA: Haemorrhage, platelet dysfunction and other coagulation defects in uraemia. *Br J Haematol* 8:215–227, 1961

19. DEPAEPE MBJ, SCHELSTRAETE KHG, RINGOIR SMG, LAMEIRE NH: Influence of continuous ambulatory peritoneal dialysis on the anemia of endstage renal disease. *Kidney Int* 23:744–748, 1983

20. REGE AB, BROOKINS, J, FISHER JW: A radioimmunoassay for erythropoietin: serum levels in normal human subjects and patients with hemopoietic disorders. *J Lab Clin Med* 100:829–843, 1982

21. ISCOVE NN, SIEBER F, WINTERHALTER KH: Erythroid colony formation in cultures of mouse and human bone marrow: analysis of requirement for erythropoietin by gel filtration and affinity chromatography on agarose concanavalin. *J Cell Physiol* 83:309–320, 1974

22. OGAWA M, PARMLEY RT, BANK HL, SPICER SS: Human marrow erythropoiesis in culture. I. Characterization of methylcellulose colony assay. *Blood* 48:407–417, 1976

23. COTES PM: Immunoreactive erythropoietin in serum. *Br J Haematol* 50:427–438, 1982

24. SHERWOOD JB, GOLDWASSER E: A radioimmunoassay for erythropoietin. *Blood* 54:885–893, 1979

25. ZAROULIS CG, HOFFMAN BJ, KOURIDES IA: Serum concentrations of erythropoietin measured by radioimmunoassay in hematologic disorders and chronic renal failure. *Am J Hematol* 11:85–92, 1981

26. DEKLERK G, WILMINK JM, ROSENGARTEN PCJ, VET RJWM, GOUDSMIT R: Serum erythropoietin (ESP) titers in anemia of chronic renal failure. *J Lab Clin Med* 100:720–734, 1982

27. CARO J, BROWN S, MILLER O, MURRAY T, ERSLEV AJ: Erythropoietin levels in uremic nephric and anephric patients. *J Lab Clin Med* 93:449–458, 1979

28. WALLNER SF, VAUTRIN RM: Evidence that inhibition of erythropoiesis is important in the anemia of chronic renal failure. *J Lab Clin Med* 97:170–178, 1981

29. ANDERSON ET, WALKER BR: Polycystic kidney disease, polycythemia and azotemia. *JAMA* 208:2472–2473, 1969

30. HOPPIN EC, DEPNER T, YAMUCHI H, HOPPER J JR: Erythrocytosis associated with diffuse parenchymal lesions of the kidney. *Br J Haematol* 32:557–563, 1976

31. SHALHOUB RJ, RAJAN U, KIM VV, GOLDWASSER E, KARK JA, ANTONIOU LD: Erythrocytosis in patients on long-term hemodialysis. *Ann Intern Med* 97:686–690, 1982

32. MEYRIER A, SIMON P, BOFFA G, BRISSOT P: Uremia and the liver. I. The liver and erythropoiesis in chronic renal failure. *Nephron* 29:3–6, 1981

33. MIYAKE T, KUNG CKH, GOLDWASSER E: Purification of human erythropoietin. *J Biol Chem* 252:5558–5564, 1977

34. ESPADA J, BRANDAN N, LI YT, LI SC, FISHER JW: Purification of human urinary erythropoietin *(abstract)*. *Fed Proc* 41:1159, 1982

35. STYKOWSKI AJ: Denaturation and renaturation of human erythropoietin. *Biochem Biophys Res Commun* 96:143–149, 1980

36. GOLDWASSER E, SHERWOOD JB: Radioimmunoassay of erythropoietin. *Br J Haematol* 48:358–363, 1981

37. COTES PM, BROZOVIC B: Diurnal variation of serum immunoreactive erythropoietin in a normal subject. *Clin Endocrinol* 17:419–422, 1982

38. GOULD AB, GOODMAN S, DEWOLFF R, ONEST G, SWARTZ G: Interrelation of the renin system and erythropoietin in rats. *J Lab Clin Med* 96:523–534, 1980