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EDITORIALES

Erythropoietin in the treatment of uremic anemia

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The use of recombinant DNA technology for the large-scale production of erythropoietin is a major advance in the treatment of patients with hypoproliferative anemia [1, 2] and in particular those with renal insufficiency, both in the predialytic phase as well as during dialysis [3, 4].

The existence of an organic substance capable of stimulating the bone marrow to produce erythrocytes has been known since the beginning of the century, but its isolation was possible only in the 70s, at which time American [5] and European [6] reaserchers used the urine of patients with aplastic anemia.

We reported in 1976 at the National Italian Congress of Nephrology that from the urine (3-5 liters) of women with severe post-partum bleeding, it was possible to extract by means of numerous passages through particular filters a modest quantity of a substance which, when injected into anemic uremics, caused an increase in the number of circulating erythrocytes [7]. It was evident, however, that this method was not practical for the production of sufficient quantities of material for the systematic treatment of these patients.

The isolation of this substance was, however, extremely useful as its availability to molecular biologists led to the identification of the chemical structure of erythropoietin, right down to its amino-acid sequence.

Knowledge of this complex molecule allowed two of its constituent peptides to be used as oligonucleotide DNA probes for the localization of the gene codifying erythropoietin in a phagic genome [8]. After the identification of the «erythropoietin» gene, using recombinant DNA technology, a fragment of bacterial DNA was extracted and substituted with the erythropoietin gene. To clone the gene, this system was inoculated into a culture of Chinese hamster ovarian cells which were found to be the most available for maintaining the activity of the gene and for the subsequent indefinite production of the recombinant erythropoietin molecules, whose activity is identical to that of human endogenous erythropoietin [9].

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The function of erythropoietin is the activation of erythropoiesis. It is known that all blood cells derive from a totipotential stem cell located essentially in the bone marrow. Subsequent to a feedback mechanism these cells differentiate into unipotential cells which are oriented toward the various series. This is the first phase of erythropoiesis. This orientation is modulated by the presence in the microenvironment of lymphocytes and monocytes and their secretory products such as IL-1, 2, 3, 4, 5, 6; CSA, CSF, BPA, IFN, etc. [10, 11].

In the second phase of erythropoiesis the cells which are oriented toward the erythroid series give rise to the more mature Burst Forming Unit Erythroid (BFU-E), and to the Colony Forming Unit Ery- throid (CFU-E) which represents the true target of erythropoietin. Erythropoietin, by stimulating the synthesis of the most important nuclear components such as DNA and RNA from which in turn hemoglobin is derived, provokes the proliferation and the maturation of the erythroid cells toward the normoblastic phase. This is followed by an arrest of the synthesis of nuclear factors and of cell division, the disappearance of the nucleus and thus the formation of erythrocytes.

In the fetus erythropoietin is formed in the liver, while in the adult it is produced nearly exclusively in the kidney.

Stimuli originating in the tissues in relationship to the degree of the oxygen supply appear to be able to activate the microreceptors probably located on the endothelial cells of the peritubular capillary, thus leading to the production of erythropoietin [12].

When renal tissue is damaged in the course of various nephropathies this mechanism is progressively interrupted and erythropoietin formation is reduced; this lead to a decrease in erythropoiesis and, subsequently, a normochromic-normocytic hypoproliferative anemia.

The correction of anemia which, as we shall see, follows the administration of recombinant human erythropoietin (rHuEPO), indicates that a deficiency of erythropoietin is, at least partly, responsible for the anemia in uremia. This anemia is not affected by blood purification procedures, though it improves following transplantation. Although hemodialysis reduces the blood levels of toxic inhibitors of erythropoiesis, it does

COMPOSICION: UROBACTAM 500. Cada vial contiene: Aztreonam (D.C.I.), 500 mg. Excipiente, c.s. Cada ampolla contiene: Agua p.i., 4 ml. UROBACTAM 1000. Cada vial contiene: Aztreonam (D.C.I.), 1.000 mg. Excipiente, c.s. Cada ampolla contiene: Agua p.i., 4 ml. **PROPIEDADES:** Aztreonam constituye un medicamento adecuado para el tratamiento de las infecciones sistémicas ocasionadas por las cepas susceptibles de bacterias aeróbicas gramnegativas (incluyendo Pseudomonas aeruginosa). *Microbiología:* En los ensayos «in vitro» han mostrado sensibilidad al Aztreonam los siguientes microorganismos gramnegativos: Escherichia coli, Enterobacter, Klebsiella pneumoniae, Klebsiella spp., Proteus mirabilis, Proteus indol-positivo (P. vulgaris, P. rettgeri, P. morganii), Pseudomonas aeruginosa, Pseudomonas spp., Serratia marcescens, Providencia spp., Salmonella y Shigella spp., Neisseria meningitidis, Haemophilus influenzae (incluyendo cepas productoras de penicilinasa), Citrobacter. Aztreonam es activo, también frente a algunas cepas de Acinetobacter. **INDICACIONES:** Las indicaciones terapéuticas de Aztreonam, si bien vienen determinadas por la actividad antibacteriana y las características farmacocinéticas del nuevo medicamento antibiótico, se fijan teniendo en cuenta la posición del Aztreonam dentro del arsenal de medicamentos antibacterianos disponibles en función de las evidencias demostradas en los ensayos clínicos realizados. En el adulto las indicaciones terapéuticas son: • Infecciones de las vías urinarias altas y bajas complicadas o no. • Prostatitis agudas. • Uretritis gonocócicas. Aunque la sensibilidad al Aztreonam del microorganismo causante de la infección debe ser determinada mediante antibiograma, la severidad de la infección en muchos casos determina que no se requieran los resultados del mismo para iniciar la terapia. **POSOLOGIA Y MODO DE EMPLEO:** Aztreonam inyectable puede ser administrado por vía intramuscular. *Adultos:* Infecciones urinarias altas y/o complicadas: 1 gramo cada 12 horas. Prostatitis agudas: 1 gramo cada 12 horas. Infecciones urinarias bajas no complicadas y gonorrea aguda no complicada: dosis única de 1 gramo. *Ajuste de la dosificación en ancianos:* El estado renal es el factor de mayor importancia en la determinación de la dosis. Deberá usarse el aclaramiento de creatinina para fijar la dosificación apropiada, ya que la creatinina sérica no mide adecuadamente la función renal en estos pacientes. Los ancianos con un aclaramiento de la creatinina superior a 20 ml/minuto pueden recibir la dosis normal recomendada: si la cifra de aclaramiento de la creatinina es inferior, la dosis debe ajustarse siguiendo las indicaciones que se describen en este prospecto en el párrafo que sigue. *Ajuste de la dosificación en pacientes con insuficiencia renal:* Puesto que Aztreonam se elimina principalmente por el riñón, se recomienda la reducción de dosis en caso de insuficiencia renal. En los pacientes que tienen un aclaramiento de creatinina entre 10 ml y 30 ml por minuto, puede administrarse una dosis inicial de 1 g o 2 g seguida de dosis de mantenimiento mitad de la recomendada en pacientes con función renal normal. Cuando sólo se dispone del dato del nivel de creatinina en el suero, puede usarse la siguiente fórmula (basada en el sexo, el peso y la edad de los pacientes) para calcular el aclaramiento aproximado de creatinina. La creatinina en suero debe reflejar una situación estable de la función renal. EN HOMBRE: Peso (en kg) × (140 − edad) / 72 × creatinina en el suero (mg/dl). EN MUJERES: 0,85 × el valor calculado para hombres. En pacientes con insuficiencia renal grave, con valores de aclaramiento de creatinina menor que 10 ml/minuto (por ejemplo sometidos a hemodiálisis), deberán darse inicialmente las dosis usuales de 0,5 g, 1 g ó 2 g. Las dosis de mantenimiento deberán ser de una cuarta parte de la dosis usual, administrándose a intervalos fijos de 6, 8 ó 12 horas. En infecciones severas, además de las dosis de mantenimiento señaladas deberá darse un octavo de la dosis inicial después de cada hemodiálisis. **NORMAS PARA LA CORRECTA ADMINISTRACION:** La solución inyectable se obtiene inyectando asépticamente en el vial el volumen adecuado de agua para inyección, agitando hasta obtener una solución completamente transparente. Dependiendo de la concentración de Aztreonam y del solvente usado, el producto preparado para ser inyectado, es una solución incolora o de color amarillo pajizo pálido, que con el reposo puede desarrollar un tinte ligeramente rosáceo. El pH de las soluciones varía entre 4,5 y 7,5 dependiendo del tipo y de la cantidad de solvente utilizado. En el caso de que el contenido total del frasco no se utilice en una dosis única, la porción de la solución sobrante deberá ser desechada. La solución inyectable de Aztreonam no deberá mezclarse con ningún otro medicamento, incluyendo antibióticos, salvo instrucción específica. *Solución para administración intramuscular:* Se recomiendan los siguientes volúmenes de diluyente para preparar la solución: Volumen del diluyente: UROBACTAM 500, 1,5 ml. UROBACTAM 1000, 3 ml. Las soluciones preparadas para ser administradas por vía intramuscular, deberán inyectarse antes de transcurridas 48 horas desde su preparación, cuando se hayan mantenido a temperatura ambiente (15° a 30° C); este plazo de uso se prolonga hasta 7 días cuando se hayan mantenido en nevera (entre 2° y 6°C). Se administra en inyección intramuscular profunda en una de las masas musculares grandes (tal como cuadrante superior externo de la región glútea o en la parte lateral del muslo). La tolerancia es buena haciendo innecesario el uso de anestésicos locales (la compatibilidad no ha

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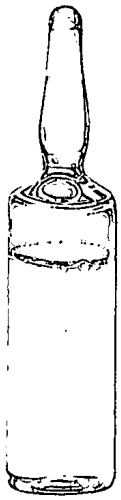

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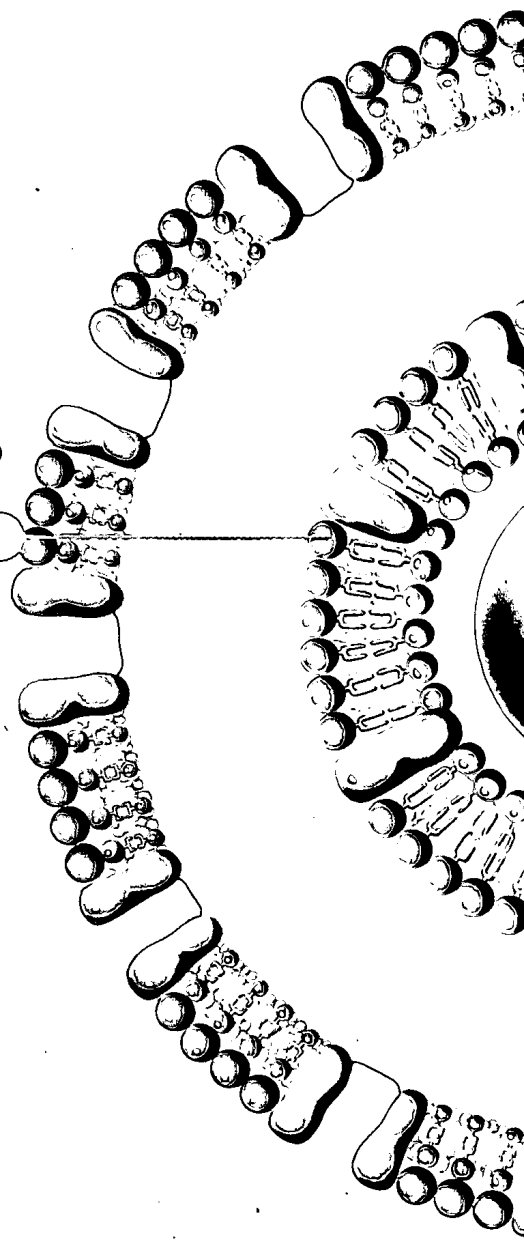

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sido estudiada). **CONTRAINDICACIONES:** Aztreonam está contraindicado en pacientes con alergia conocida a este medicamento. Los estudios actuales señalan que no se produce reacción de hipersensibilidd cruzada con antibióticos betalactámicos; sin embargo como medida de precaución en pacientes con historia de reacciones de hipersensibilidad inmediata (anafiláctica o urticaria) a penicilinas o cefalosporinas, sólo se administrará cuando el beneficio que se espera obtener justifique el riesgo de una hipotética reacción alérgica grave. **PRECAUCIONES:** Los pacientes con disfunción renal conocida o sospechada deben someterse a una cuidadosa observación clínica y a los estudios de laboratorio adecuados, debido a que Aztreonam, puede acumularse en suero y en los tejidos. En estos casos las dosis deben reducirse en la forma descrita en el apartado «Ajuste de la dosificación en pacientes con insuficiencia renal». La experiencia del empleo de este medicamento en pacientes con insuficiencia hepática es limitada. Se recomienda vigilar de manera adecuada la función hepática en tales pacientes, durante todo el tratamiento. El tratamiento con Aztreonam puede dar oportunidad a que se desarrollen microorganismos no susceptibles a este antibiótico y qué sea necesario instaurar un tratamiento adecuado para su control. *Uso durante el embarazo, lactancia y niños:* No se han hecho estudios con este medicamento en mujeres embarazadas. El Aztreonam no debe usarse durante el embarazo, salvo que el beneficio potencial del tratamiento justifique los posibles riesgos. En la leche de las madres sometidas a tratamiento con Aztreonam pueden encontrarse concentraciones del medicamento inferiores al 1% del nivel del medicamento en el suero materno. La seguridad y eficacia del uso del medicamento en niños no ha sido establecido. **EFECTOS SECUNDARIOS:** Generalmente es bien tolerado. En los estudios clínicos, los efectos adversos fueron poco frecuentes. Sólo fue necesario suspender por ese motivo el tratamiento en menos de un 2% de los pacientes. Los efectos indeseables que se consideraron relacionados o posiblemente relacionados con el tratamiento fueron los siguientes: *Dermatológicos:* Rara vez erupciones cutáneas, prurito, urticaria, púrpura, eritema, petequias y dermatitis exfoliativas. *Hematológicas:* Eosinofilia transitoria, aumentos transitorios en el tiempo de protrombina y en el tiempo de tromboplastina parcial (sin anormalidades de sangrado); rara vez alteraciones en el número de plaquetas y anemia. *Hepatobiliares:* Elevaciones transitorias en las transaminasas hepáticas y en la fosfatasa alcalina, sin manifestaciones de signos o síntomas de disfunción hepatobiliar. Rara vez ictericia y hepatitis. *Gastrointestinales:* Diarrea, náusea y/o vómito, cólicos abdominales, úlcera en la boca y alteraciones en el gusto. *Reacciones locales:* Malestar en el sitio de la inyección endovenosa y flebitis; ligero malestar en el sitio de la inyección intramuscular. *Otros efectos indeseables:* Vaginitis, candidiasis, hipotensión arterial, debilidad, confusión, embotamiento, vértigo, diaforesis, cefalea, sensibilidad en los senos, halitosis, dolores musculares, fiebre, malestar, estornudos y congestión nasal. En raras ocasiones aumento transitorio en la creatinina en suero. **INCOMPATIBILIDADES:** La solución de Aztreonam es incompatible con nafcilina sódica, con cefradina y con metronidazol. **INTOXICACION Y SU TRATAMIENTO:** Debido a su escasa toxicidad, es muy improbable ,la posibilidad de intoxicación por Aztreonam, principalmente si la dosificación se realiza de acuerdo con las normas señaladas. No obstante, en caso de producirse se suspenderá su administración y se instaurará tratamiento sintomático. **PRESENTACION:** UROBACTAM 500 Caja con un vial de 500 mg y ampolla disolvente. P.V.P. 1.640,— ptas. (IVA incl.) UROBACTAM 1000 Caja con un vial de 1.000 mg y ampolla disolvente. P.V.P. 3.069,—ptas. (IVA incl.).

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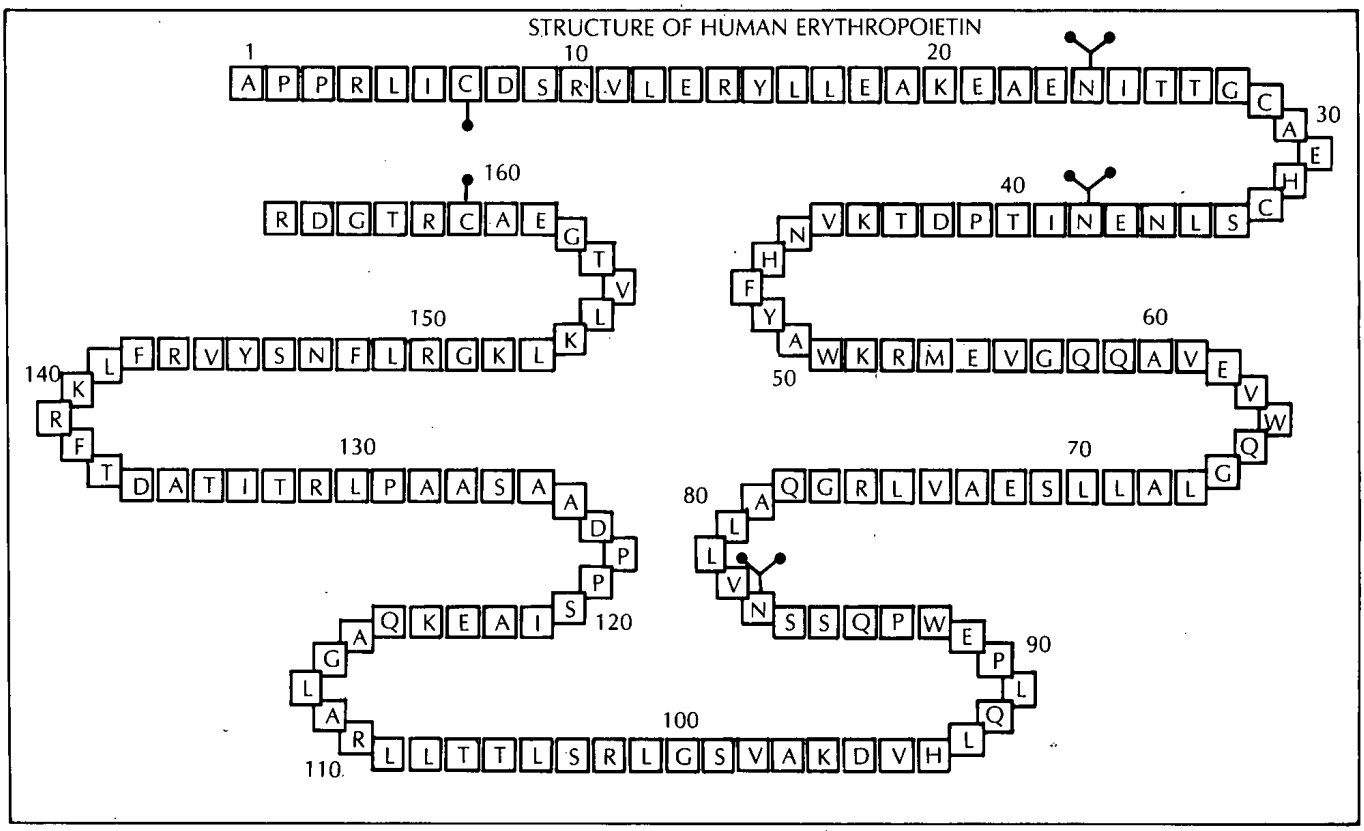

Fig. 1.—The structure of human erythropoietin.

not increases plasma erythropoietin levels and, in fact, may even accentuate the deficit so that the anemia remains unchanged or even worsens as a result of this treatment [13]. There may, however, be some amelioration with CAPD due to a smaller degree of blood loss, as well as to improved interaction between accessory and erythroid cells in the first phase of erythropoiesis which favors an increase in the cells which are the target of the residual erythropoietin [14]. The results, however, with this therapy are neither satisfactory nor long-lasting.

Due to the resistence of uremic anemia to the therapies up to now available, the only possible way of neutralizing its devasting effects was through blood transfusions.

Transfusions are necessary when there is a severe reduction of the hematocrit and hemoglobin levels (below 16 % and 4 g/dl, respectively), as this may cause myocardial or cerebral ischemia. Blood transfusion, however, must be undertaken with great care as it can cause a series of side effects. The use of erythropoietin can eliminate the need for this type of therapy.

Studies of the effects of rHuEPO have begun only recently, in 1985, when it was administered to animals (mice and dogs) and shown to stimulate erythropoiesis to a degree which was proportional to the amount given, without toxic side effects.

Subsequently, rHuEPO was studied in normal humans: low doses had no effects, while higher doses led to an increase in hematocrit and hemoglobin to above normal levels, especially when an iron supplement was given. This showed that the effect of exogenous erythropoietin was added to that of endogenous erythropoietin, this hyperstimulating the erythroid bone marrow cells to a degree of activity potentially greater that that occurring in normal conditions.

Since 1986 there have been numerous clinical trials in the United States and in Europe to evaluate the effects of this hormone in patients with severe renal insufficiency and secondary anemia in the course of chronic dialysis [15, 16].

The characteristics of the subjects chosen for these studies were more-or-less the same in the various centers: hemodialysis patients with hemoglobin values below 7 g/dl, hematocrit below 28 % and a decreased reticulocyte count. A short first cycle of thrice-weekly i.v. administration of between 48 and 166 U/kg of rHuEPO led to maximal increases in the number of reticulocytes by the eight week followed by an increase in the hemoglobin values at the twelfth week. At the same time, in vitro studies showed a recovery of the normal development of erythroid precursors such as CFU-E, while BFU-E development which is less sensitive to erythropoietin [17, 18], increased but without reaching normal levels.

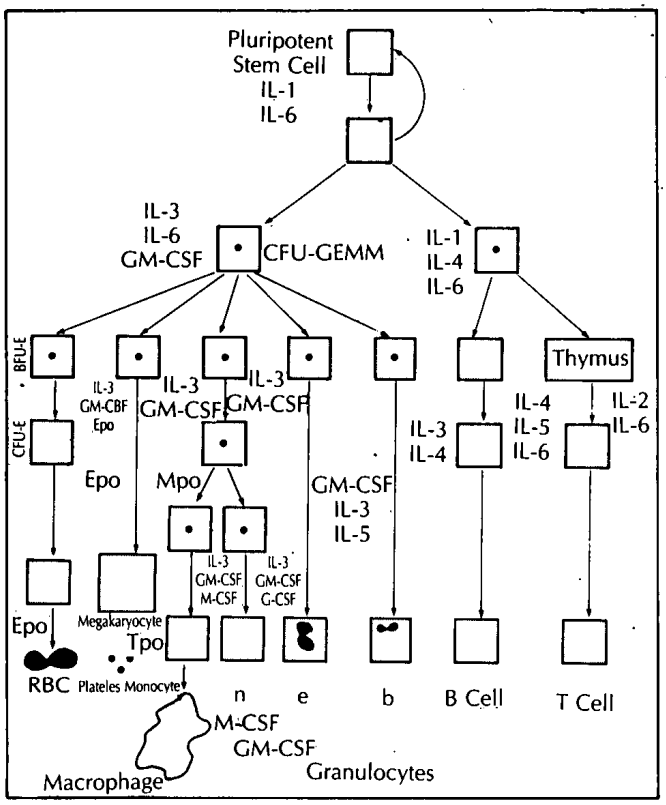

Fig. 2.—The humoral regulation of human hematopoiesis.

IL-1: Interleukin-1.
IL-6: Interleukin-6.
IL-3: Interleukin-3.
GM-CSF: Granulocyte Megakaryocyte Colony Stimulating Factor.
GFU-GEMM: Colony Forming Unit Granulocyte Erythroid Monocyte Megacaryocyte.
IL-4: Interleukin-4.
IL-5: Interleukin-5.
BFU-E: Burst Forming Unit Erythroid.
CFU-E: Colony Forming Unit Erythroid.
EPO: Erythropoietin.
IL-2: Interleukin-2.
M-CSF: Megacaryocyte Stimulating Factor.
G-CSF: Granulocyte Stimulating Factor.
RBC: Red Blood Cells.
n: Neutrophils.
e: Eosinophils.
b: Basophils.

There have been reports that under these experimental conditions rHuEPO stimulates megakaryoblastic development and has no effect on the other series [19]; our research has confirmed this. In the patients thus treated, blood transfusion was no longer necessary. No side effects were observed, though iron supplementation was necessary in the majority, thus confirming the recovery of bone marrow activity [20].

This supplementation was not required in the patients who had previously been transfused, who for the most part had high values of serum ferritin, often at the level of hemosiderosis; this abnormality was nevertheless corrected by rHuEPO therapy.

If during the first cycle of treatment, the rHuEPO dose was reduced, there was a sudden worsening of the

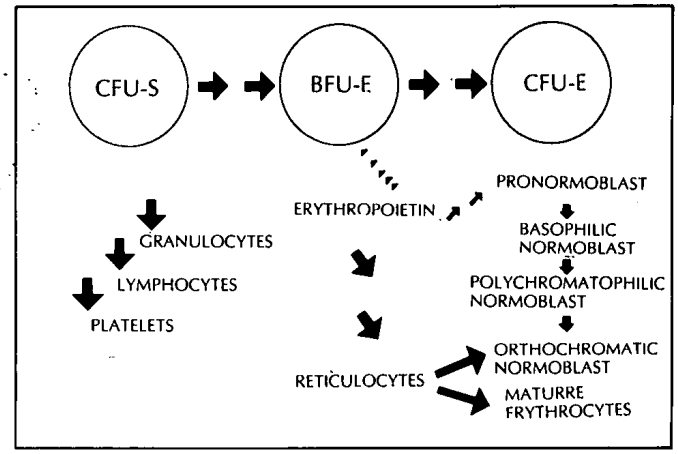

Fig. 3.—The proliferation and the maturation of human erythropoietic cells.

hematological parameters, which subsequently improved following the return to higher doses.

In a second cycle in which treatment continued until the 32nd week it was found that to maintain the hemoglobin levels which had previously been reached (10-12 g/dl) it was necessary to continue the administration of erythropoietin with constant doses of, on the average, 400 U/kg per week. Notwithstanding this, there was a modest decrease in the reticulocyte count and in the CFU-E development in vitro. which stabilized at the upper limits of normal.

Studies so far done have shown that in the first phase of therapy the effects of rHuEPO are dose-dependent, in that increasing the dosage is followed by an increase in the hematocrit [21]. It was been seen, however, that the sensitivity to the same rHuEPO dose may vary greatly from patient to patient and between groups of patients.

When the sensitivity is below a certain point it is necessary to use elevated doses of rHuEPO to reach the maximum increases in hematocrit levels and

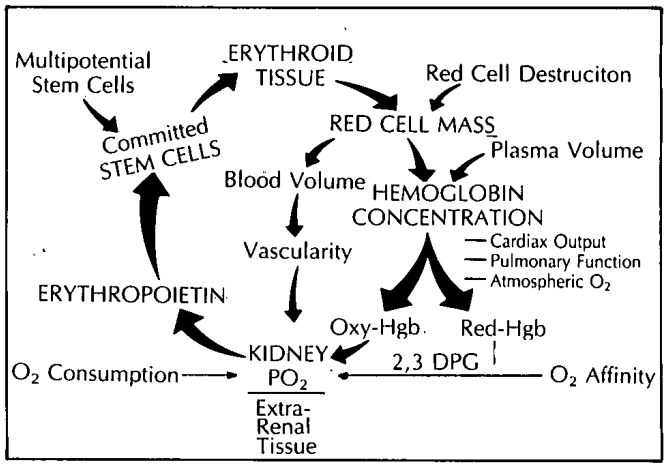

Fig. 4.—The O_2-dependent regulation of erythropoietin synthesis in humans.

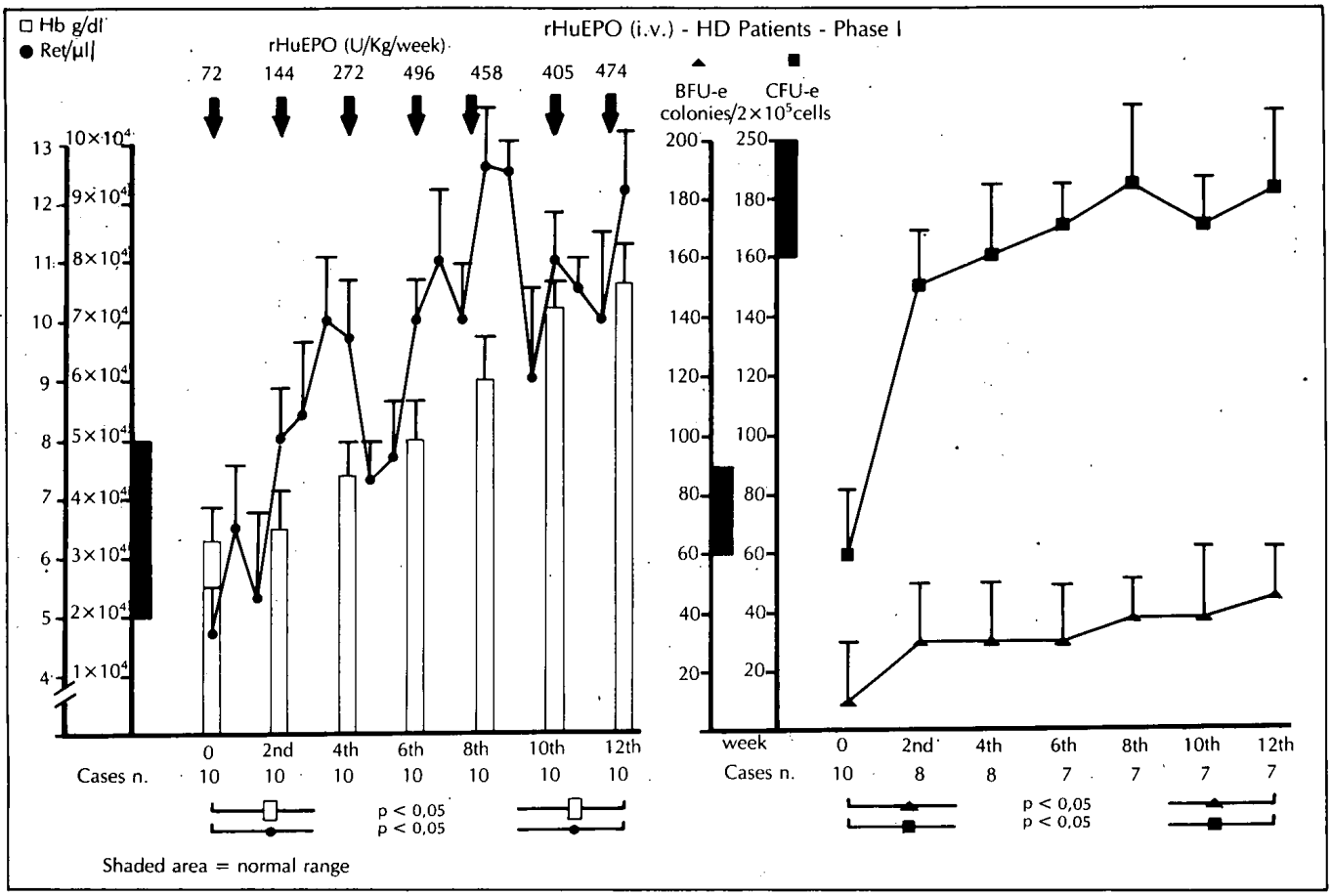

Fig. 5.—Effects of 12 weeks of i.v. recombinant human erythropoietin (rHuEPO) therapy in some anemic hemodialysis (HD) patients on hemoglobin (Hb) levels, reticulocyte (Ret.) count and on bone marrow Burst Forming Unit Erythroid (BFU-E) and Colony Forming Unit Erythroid (CFU-E) development.

reticulocyte count, which are nevertheless delayed. This resistence to therapy occurs frequently and its cause is sometimes clear, other times not. Possible factors which maydetermine this resistence are: occult blood loss, hyperhemolysis, iron deficiency, iron overload, altered metabolism of certain trace elements, aluminum intoxication, secondary hyperparathyroidism, ineffective dialysis, dyserythropoiesis, and other unknown factors[22]. While the actions of the first of these factors are clear, those which affect erythropoiesis more-or-less directly are more difficult to classify. Resistence to erythropoietin has not been found with secondary hyperparathyroidim, thus exclusing the possibility of a negative effect of parathormone at the level of the bone marrow [23].

On the other hand, an inverse relationship between plasma aluminum concentration and response to rHuEPO treatment has been found, thus implying bone marrow inhibition by aluminum.

The last factors must also be associated with abnormal bone marrow response to rHuEPO and this may be due to difficulties in the first phase of erythropoiesis with a decreased number of erythropoietin target cells for both exogenous and endogenous reasons. This hypothesis is confirmed by the delay in reaching the maximum reticulocyte levels and, in vitro, the normalization of CFU-E development, and by the fact that patients in CAPD, a therapy which usually improves the interaction between the immune system and erythroid precursors, require lower doses of rHuEPO than do hemodialysis patients to obtain the same results [24]. Due to the variations in individual sensitivity, determining the optimal hormone dose may be difficult. Test doses of rHuEPO are therefore used initially so as to give an idea of the response based on the entity of the results and the time needed to reach them [25].

This is a rather complicated operation since the use of insufficient doses impedes the attainment of optimal results, and excessive doses may cause important complications. Besides those directly due to rHuEPO which are hypersensitivity reactions such as for any drug, the most important ones are those related to the organic modifications caused by the therapy. These include hypertension, thrombosis at various levels, detoxification defects, etc.

Nearly all research excludes the possibility that

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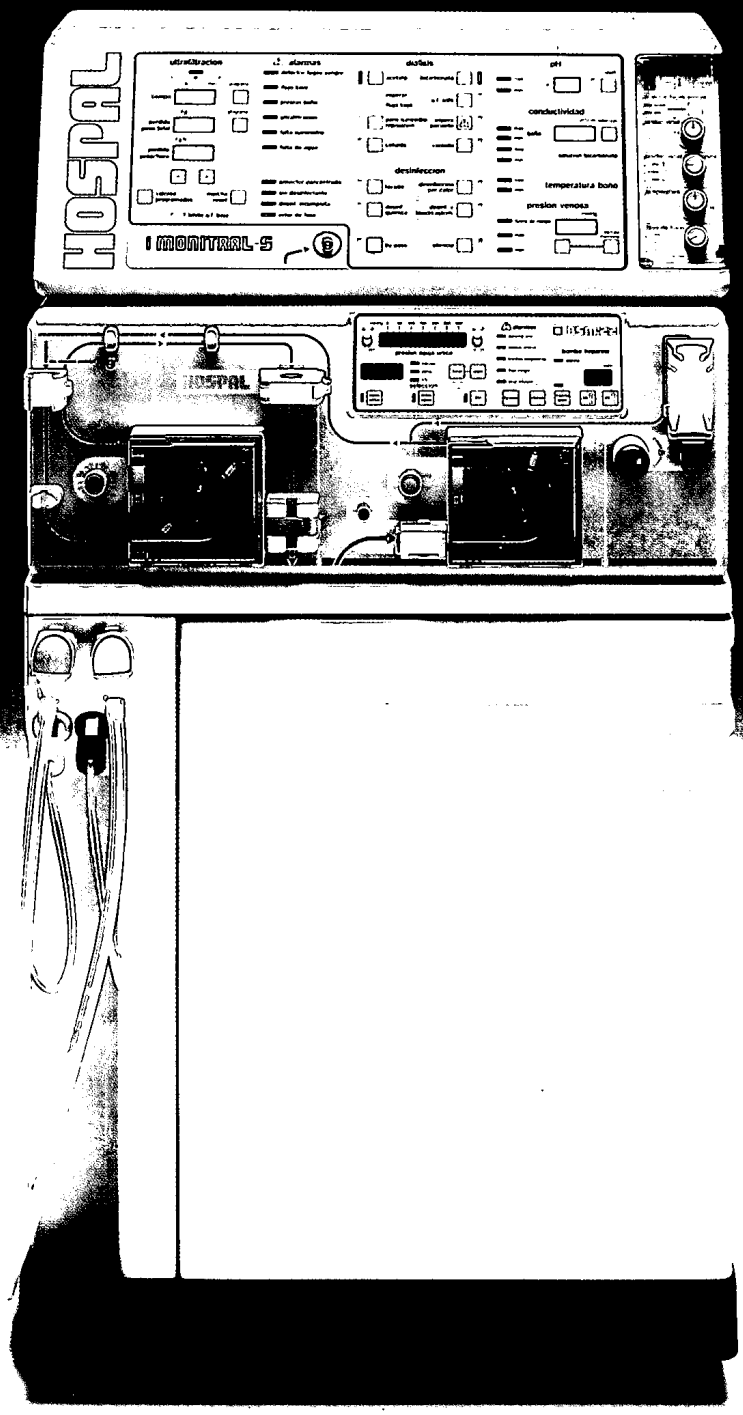

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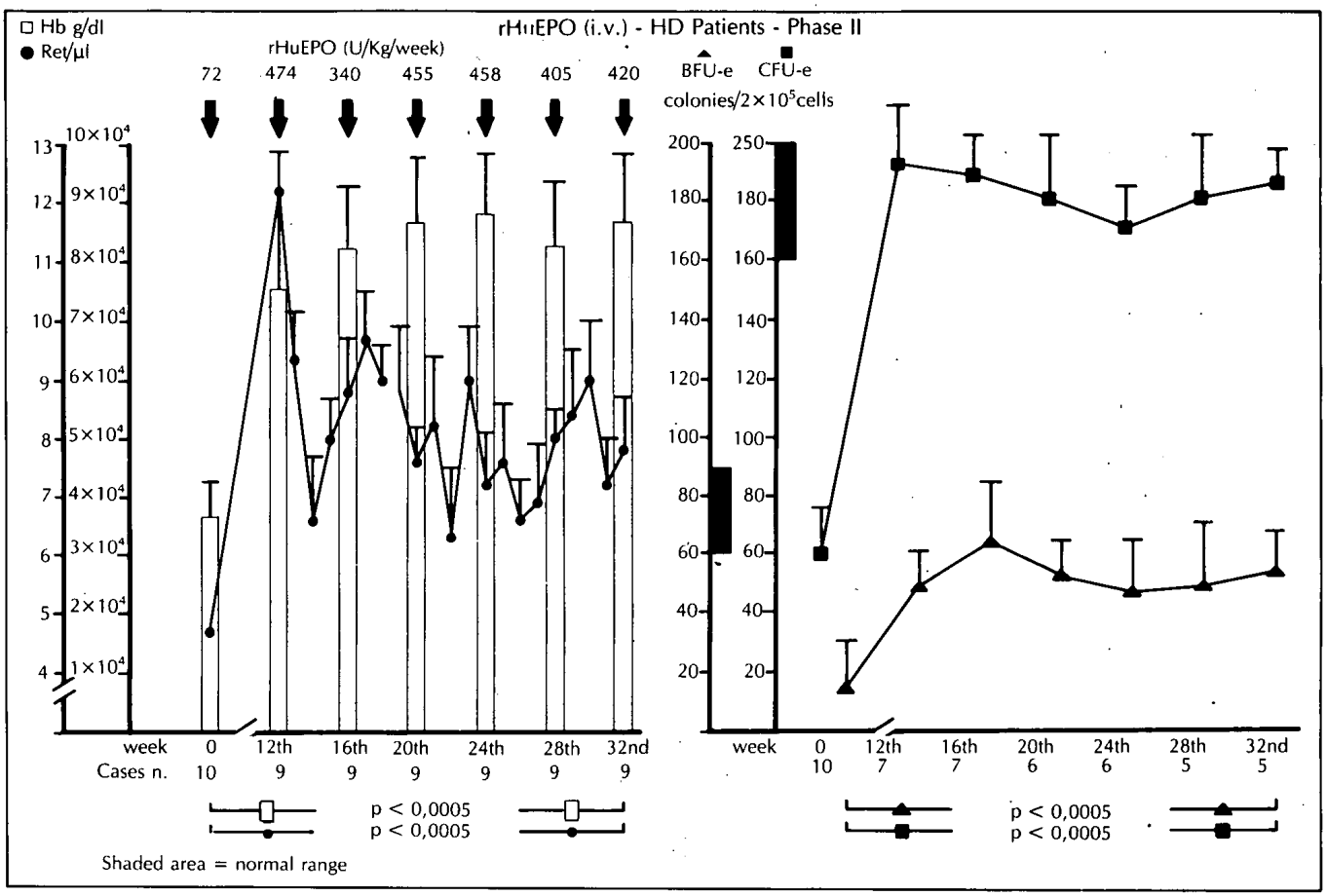

Fig. 6.—Effects of 32 weeks of i.v. recombinant human erythropoietin (rHuEPO) therapy in some anemic hemodialysis (HD) patients on hemoglobin (Hb) levels, reticulocyte (Ret.) count and on one marrow Burst Forming Unit Erythroid (BFU-E) and Colony Forming Unit Erythroid (CFU-E) development.

rHuEPO has a direct hypertensive effect, but indicates that increased pressure may occur in patients with a history of hypertension. This may be due to greater blood viscosity subsequent to the rise in hematocrit, increased peripheral resistence due to the disappearance of the chemical mediators which in response to the hypoxia of anemia cause vasodi- latation, 2,3-DPG modifications within the erythrocytes and a reduction in the nitric oxide concentration due to binding with the hemoglobin in excess [26-28].

These changes together with an increased platelet count, which may exist due to the ability of rHuEPO to stimulate megakaryocyte proliferation, may be the cause of hypertensive encephalopathy and may facilitate the formation of thromboses within blood vessels as well as in the dialysis circuit [29].

Excessive doses of rHuEPO with consequent over-correction of the anemia may cause a reduction in the depurative efficiency of the dialyzer and structural abnormalities of erythroblasts.

The former is due to a reduction of the plasma/erythrocyte ratio which leads to a decreased clearance of catabolites, like creatinine, and of some electrolytes such as potassium and phosphorus. It may therefore be necessary to use dialyzers with a larger surface, a greater number of fibers, etc. It is also possible that the feeling of well-being with increased appetite induced by rHuEPO may play a role in these hematochemical alterations and, thus, it may be necessary to institute dietary changes.

The structural abnormalities of erythroblasts are probably related to a persistent excessive hormone dosage which hyperstimulates the erythroid cells. Erythroblastic structural alterations, a sign of cell damage, have been observed in other conditions in which the feedback between tissue oxygenation and EPO production is no longer sufficient to moderate this production, thus leading to excessive plasma concentrations [30, 31].

We have found identical abnormalities, though to a more modest degree, in the erythroblasts of uremics treated for a long period with elevated doses of rHuEPO.

Electron microscope evaluation of these cells showed a double nucleus with nuclear extroflexions in the cytoplasm, anomalies of nuclear development with a tendency toward macrocytosis and signs of damage to the endoplasmic reticulum [32].

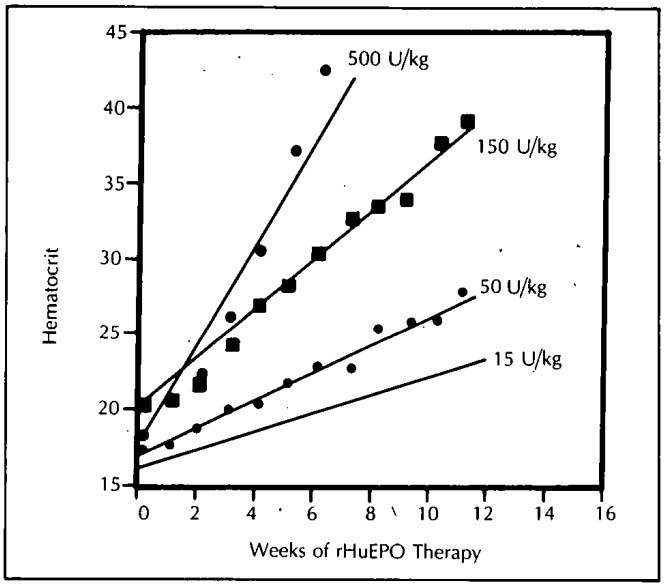

Fig. 7.—Relationship between the i.v. dose of recombinant human erythropoietin (rHuEPO) and the hematocrit values in 4 anemic hemodialysis patients.

Prevention of these complications depends on finding the rHuEPO dosage which best corresponds to the needs and the tollerance of the patient. This means avoiding excesses in the degree and the rapidity of the anemia correction. Useful, even if modest, results are preferable to violent changes which may cause dangerous complications. As rHuEPO therapy must continue indefinitely, even small errors in dosage may limit or even annull its effects.

The most recent European trials, which lasted for a period of 60 weeks, suggest that it is best to begin with relatively low doses of rHuEPO (24 U/kg i.v. thrice weekly) for a minimum of two weeks. The dose is then doubled if the hemoglobin increase is less than 10 % of the initial value. Further increases in dosage are made only if necessary, up to a maximum of 192 U/kg thrice weekly, to stabilize the highest hemoglobin level reached which usually occurs in the 12th week and is about 10-12 g/dl. At this point the dosage and frecuency of administration are reduced to a maintenance dose of, on average, 200 U/kg/week. The dosage and time necessary to reach the maximum effect are dependent on the initial hemoglobin levels: the lower the hemoglobin, the greater the dose required.

With this therapeutic regimen there is a great reduction in the number and intensity of complications. It can certainly be said that treatment with rHuEPO has greatly improved the quality of life in uremic patients.

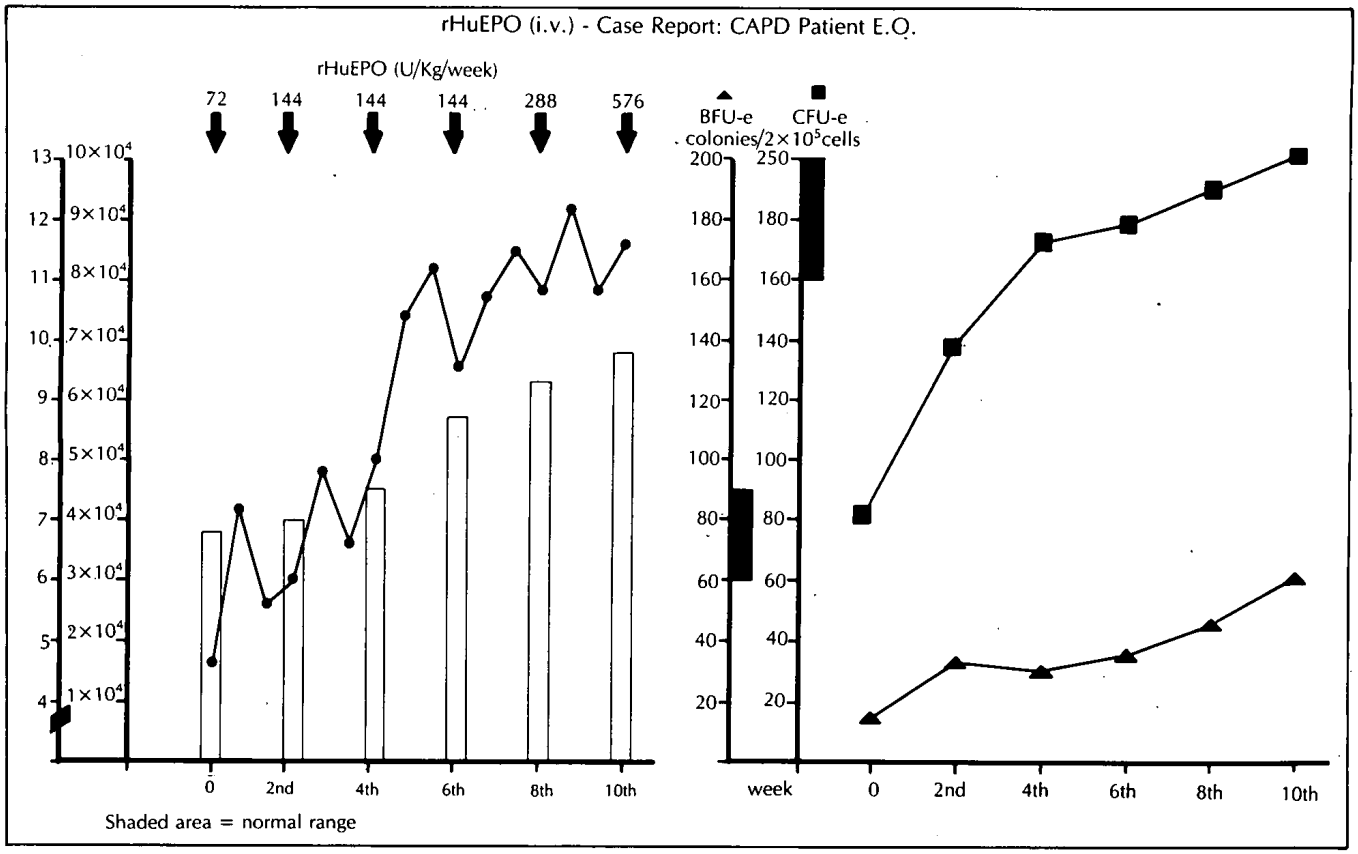

Fig. 8.—Effects of 10 weeks of i.v. recombinant human erythropoietin therapy in an anemic CAPD patient (E.O.) on hemoglobin (Hb) levels, reticulocyte (Ret.) count and on Burst Forming Unit Erythroid (BFU-E) and Colony Forming Unit Erythroid (CFU-E) development.

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