Molecular Biology of Erythropoiesis

MOLECULAR BIOLOGY OF ERYTHROPOIESIS

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A PERSONAL TRIBUTE: RICHARD D. LEVERE, M.D.

Writing a personal tribute to my friend Richard Levere is a pleasurable task, though to do so within a constraint of space and without drawing upon most of the laudatory adjectives in the Oxford English Dictionary is a bit unsatisfying.

Richard Levere has been my friend for more than a quarter of a century. I marvel at that simple fact, and in its recalling, treasure it. Unalloyed and enduring friendship is not easy to find, and perhaps may be more difficult to achieve between scientists who work in fields which so overlap each other as do his and mine. One could suppose that we are friends because we are both students of that wonderful man, the late Sam Granick, or because, despite our relative ages, I am really also a student of Richard Levere's. I left steroid endocrinology to enter the field of heme biology on the shaky assumption that because I knew something about the biochemistry of 4-ring steroid structures, I would necessarily know something about the biochemistry of other 4-ring structures such as heme; Richard Levere patiently taught me otherwise. I think perhaps, the most important reason that we are friends is simply that Richard chose, for reasons known only to a man with a good heart, to gift me with his friendship, and happily I was fortunate enough to recognize the value of the gift he was offering me. Those here who have also shared that gift know that the term, friend, is an unconditional one for Richard--a permanent state of grace in which the certainties implicit in friendship can be enjoyed without concern as to their circumstances.

It is inevitable that a man who can bestow such a quality of friendship on others should, when drawn to medicine as a career, become a splendid Physician--a word properly capitalized to affirm its significance as a formal title to which we impute all of the personal and professional characteristics of those who best represent the healing art. Richard Levere is indeed a splendid physician--a patient, compassionate, wise counselor; a diagnostition of the first rank; a therapist of consumate skill. If medicine as a whole had more physicians in its ranks as dedicated, competent and humane as is Richard Levere, many of the ills of our profession would resolve themselves.

It can be no surprise that an academic leader of Richard's calibre and temperament would enrich the scholarly ambience of any institution with which he is associated. That has proved to be the case in each of the two medical schools with which he has been principally affiliated during his career. And that each has benefitted enormously from his dedication to superior medical practice and medical science is evident to all those who are familiar with the academic scene in New York. It is an aspiration common to all of us that we might become superb clinicians, fine teachers, and excellent scientists; but that is a hope more often

felt in the heart than is manifest by our labours. Richard Levere belongs to that very small group of my contemporaries who have managed to make that aspiration a reality throughout their professional lives.

This sort of text is not the place for a detailed review of the scientific achievements of one's friends. But I think it is important to note that Richard Levere's publications recapitulate the man in a remarkable way. Clinical reports in the New England Journal of Medicine, and Blood intermingle with biochemical studies in the Proceedings of the National Academy of Sciences and the Journal of Biological Chemistry; cytochemical observations in the Journal of Cell Biology alternate with molecular genetic investigations in Biochemical and Biophysical Research Communications; work on heme and globin synthesis in the Journal of Experimental Medicine moves on to the intricacies of arachidonic acid metabolism in Science.

I believe I know what this all means. Richard Levere is the medical equivalent of a modern day Jeffersonian man. I do not know what committees of the Continental Congress Thomas Jefferson served on, or if he ever chaired a symposium, as Richard is doing at this meeting. But I am confident that if there has been a occasion for them to meet, there would have been a strong sense of affinity and much animated conversation between them and they would have parted as good friends.

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PREFACE

This volume presents the proceedings of the Fourth Annual Symposium on the Molecular Biology of Hemopoiesis, held in Reno, Nevada, November 1 and 2, 1988. Its focus on erythropoiesis represents an attempt to cover a rapidly expanding field, which has gone from elegant studies of erythropoietin physiology, to molecular biology, to clinical applications and again to physiology. The rapid development has been made possible by cloning of erythropoietin gene and the availability of recombinant hormone.

The regulation of heme and its derivatives has also been aided by techniques of molecular biology; there is now a concerted effort to better understand how these enzymes contribute to proliferation, differentiation and maturation of the erythron. Globin gene derrangements have been targets of recent research in an attempt to correct the defect by genetic engineering. In the chapters of this book, several groups "expressed" their views on this subject. Finally, we analyze various regulators of erythropoiesis, both in vivo and in vitro.

Dr. Richard Levere was a pioneer in many studies of heme metabolism and of erythropoiesis. He has been a generous supporter of research in this field and of our past meetings. It is only fitting that this volume should be dedicated to him.

The Editors

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The organizers are indebted to the following organizations for their partial support of this meeting: Research Section, V.A. Medical Center, Reno, NV.; Department of Medicine, New York Medical College, Valhalla, NY; Division of Hematology-Oncology, Department of Medicine, University of Nevada, Reno, NV.; Amgen, Inc, Thousand Oaks, CA; Ortho-Biotech, Raritan, NJ; Chugai-Upjohn, Inc., Kalamazoo, Mi.; Ms. Sheila Rich provided invaluable assistance and Ms. Melanie Yelity (Plenum Publishing Corp.) was always available.

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ERYTHROPOIETIN: FROM MOUNTAIN TOP TO BEDSIDE

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Erythropoietin is a growth factor for erythroid and myeloid progenitor cells and a differentiation hormone necessary for the blast transformation of CFU-E to proerythroblasts.

The existence of such a regulating factor or hormone was dimly perceived in the nineteenth century in order to explain the maintenance of an "interior milieu" with an optimal number of red cells in the circulation. In the 1850's, Dennis Jourdanet observed that people living at high altitude had an increase in their red cell count and he and his friend, Paul Bert, realized that an increased red cell count would provide a survival advantage for people living at a low oxygen pressure[1]. That the low oxygen pressure actually initiated an increase in red cell production was not, however, realized until much later and it took almost a hundred years to explain how hypoxia affects red cell production. In 1950, Reissmann provided indirect evidence for the existence of a factor released by hypoxia and capable of stimulating red cell production in the bone marrow[2] and in 1953, Erslev showed directly that such a factor is present in plasma of anemic and hypoxic animals[3], and it was named erythropoietin.

A few years later, studies by Jacobsen and co-workers showed that the factor was produced in the kidney and that the kidney actually worked as an endocrine gland[4]. Attempts to localize the site of production in the kidney by crude methods suggested that it was made not by the glomeruli but by tubular or interstitial cells[5]. Recent studies using probes for erythropoietin messenger RNA have pinpointed the site of production to interstitial renal cells, possibly endothelial cells lying in the immediate proximity of the proximal tubules[6,7]. The location here suggests that the tubular cells may in some way provide oxygen sensing and more recent work indicates the existence of a triggering heme protein[8]. Presumably hypoxia will change the confirmation of this heme protein resulting in the release of a short range signal capable of activating the erythropoietin gene.

The human erythropoietin gene has been pinpointed to chromosome 7 and shown to express considerable homology with erythropoietin genes of mice and monkeys[9]. It codes for a polypeptide consisting of 165 amino acids with a molecular weight of 18,000 dalton. This polypeptide backbone is extensively glycosylated in the Golgi apparatus and the final product, erythropoietin, has a molecular weight of about 34,000 dalton. Its half-life is about 6 hours but its site of degradation is still not known. In addition to the interstitial cells in the kidney, hepatocytes and

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possibly Kupffer cells have been shown to contribute 10-15% of total circulating erythropoietin[10]. The hepatic contribution may be a remnant from fetal life during which the liver appears to be the sole producer of erythropoietin with the kidneys first taking over at the time of birth[11].

In the bone marrow, erythropoietin acts primarily as a differentiation hormone and it appears to be necessary for the transformation of the most mature progenitor cell, CFU-E, to a proerythroblast[12]. In the absence of erythropoietin, no such transformation occurs and the CFU-E will die. The more erythropoietin present the more CFU-E's will be transformed, each of them producing a progeny of about 16 red blood cells (see Figure 1). Erythropoietin has also a less well defined capacity to act as a growth factor on all progenitor cells as well as on the most immature of the erythroblasts. The growth of the progenitor cells, however, is more dependent on the presence of locally produced growth factors such as IL-3 or GM-CSF appropriately termed burst promoting factors.

Of these actions only the differentiating function can be considered a true hormonal effect and be incorporated into a feedback system controlling red cell production (Figure 2).

The concentration of erythropoietin in the circulation can be measured by biologic or radioimmune assays[13]. The biologic assay measures the erythropoietic action either in vitro in a suspension of progenitor cells or in vivo using animals in which endogenous erythropoietin has been eliminated by induced polycythemia. The radioimmune assay is based on using pure erythropoietin and a polyclonal antibody responsive to epitopes on the erythropoietin molecule. So far the results of the assay have been interchangeable (Figure 3) and we have yet to identify the production

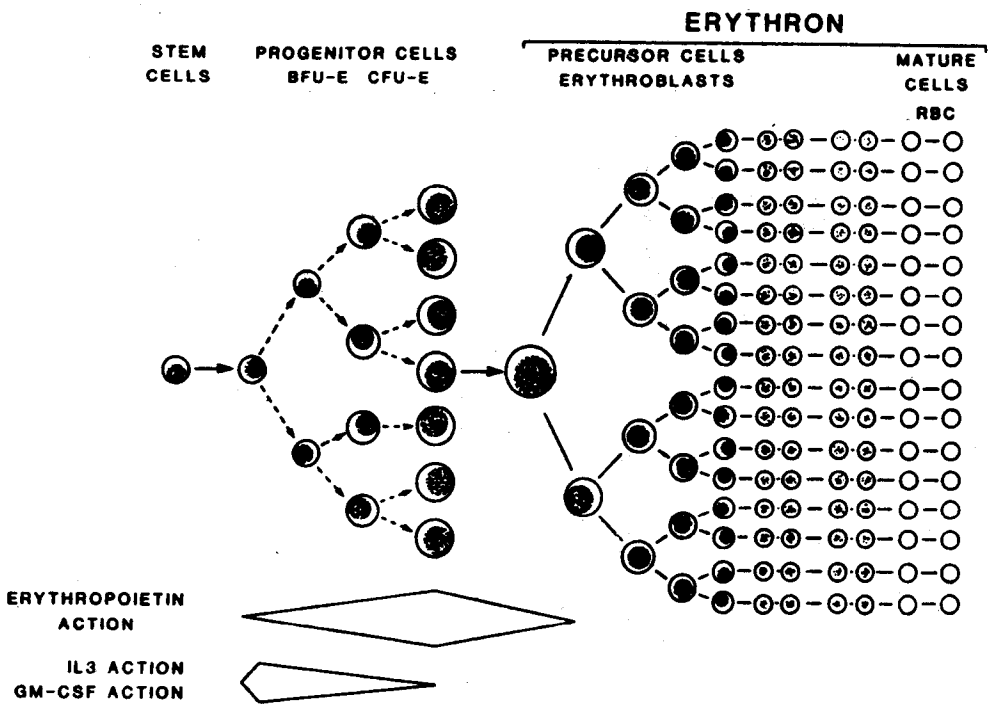

Fig. 1. Erythropoiesis. A schematic overview of the kinetics of red cell production.

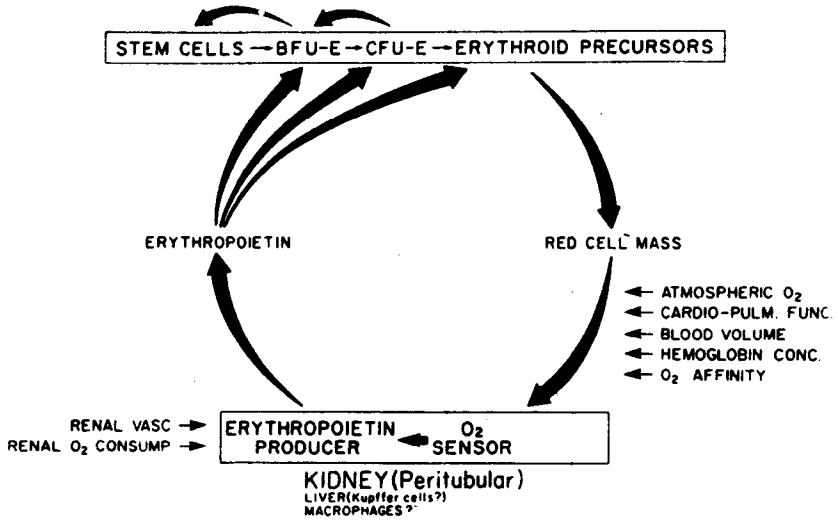

Fig. 2. Bone Marrow. The 1988 version of the feedback system which controls red cell production.

of an immunologically active but biologically inactive hormone.

As can also be seen on Figure 3, there is a steep increase in erythropoietin concentration at low hemoglobin or hematocrit values, much steeper than could be expected from in vitro dose response studies of progenitor cells. It may be that the high concentrations of erythropoietin observed in severe anemias are made primarily to recruit early progenitor cells (BFU-E) rather than merely differentiate late progenitor cells (CFU-E) to erythroblasts.

The measurements of erythropoietin titers can be of diagnostic and therapeutic importance in both polycythemias and anemias.

In primary polycythemia or polycythemia vera, the bone marrow acts autonomously and will overproduce red blood cells causing an increase in hemoglobin and hematocrit. This increase will in turn produce renal hyperoxia and cut off all erythropoietin production. Consequently, one of the most consistent diagnostic features in polycythemia vera is a near absence of erythropoietin production[14]. Since there are many other features which help to provide a diagnosis of polycythemia vera, erythropoietin measurements are rarely needed. However, in difficult cases, the diagnosis should be questioned if erythropoietin titers are higher than 3-5 mU/ml (Figure 4).

In secondary polycythemia, on the other hand, tissue hypoxia will generate erythropoietin which in turn stimulates the marrow to produce more red blood cells. Consequently, erythropoietin titers are usually elevated in contradistinction to those in polycythemia vera.

Polycythemia may also be caused by an inappropriate production of erythropoietin by certain renal or extrarenal tumors or cysts. In the absence of clinical features of either polycythemia vera or secondary polycythemia, it may be of value to measure erythropoietin titers in order to identify an erythropoietin producing lesion[15].

Since erythropoietin is produced primarily in the kidneys, it was

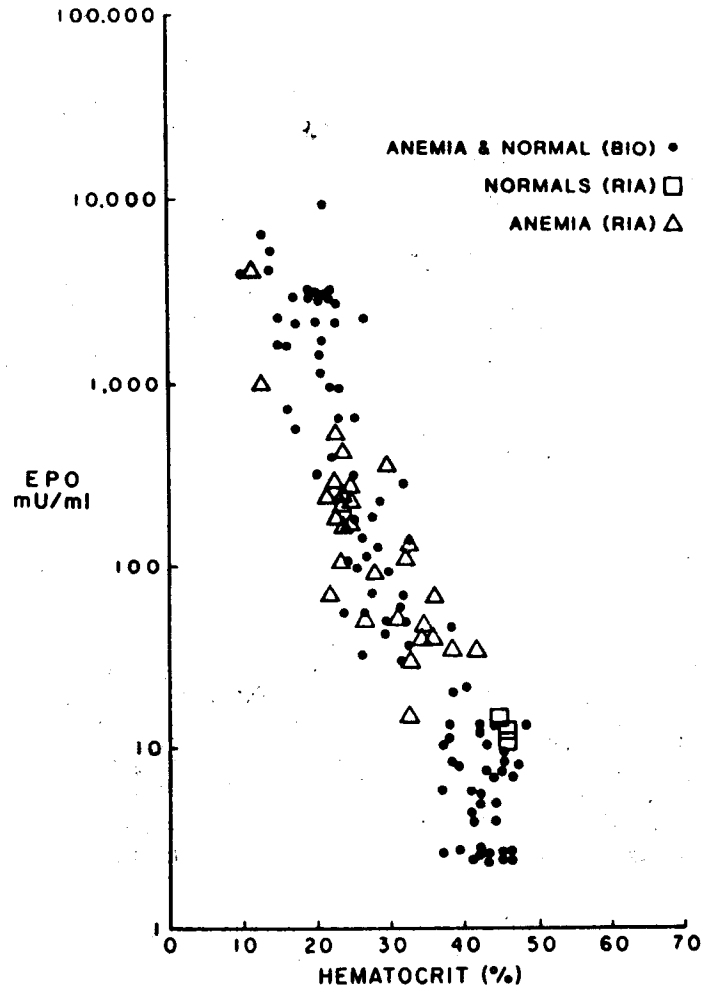

Fig. 3. Erythropoietin titers measured by in vivo bioassay or in vitro radioimmune assay and their relation to the hematocrit of blood.

early realized that patients with chronic renal disease may have a deficient production of erythropoietin. This hypothesis was confirmed by the assay of erythropoietin in patients with chronic renal disease (Figure 5) and it was realized that whenever erythropoietin became available for clinical use, it could be of importance as a replacement agent for patients with anemia of chronic renal disease. In 1985, the gene was isolated through the marvels of molecular technology. It was inserted in hamster cells which have the capacity to glycosylate polypeptides and mass production of human erythropoietin began. The early clinical trials have showed conclusively that erythropoietin will stimulate the bone marrow of patients with chronic renal disease and in fact abolish the anemia[16,17]. Subsequent studies have confirmed these findings and also have shown that an increase in hematocrit may be associated not only with an improvement in the quality of life but also occasionally in aggravation of hypertension and in production of clotted shunts. Except for prematurity[18], no other condition has been associated with a significant and consistent decrease in erythropoietin production and replacement therapy with erythropoietin probably will be restricted primarily to patients with chronic renal disease or without kidneys.

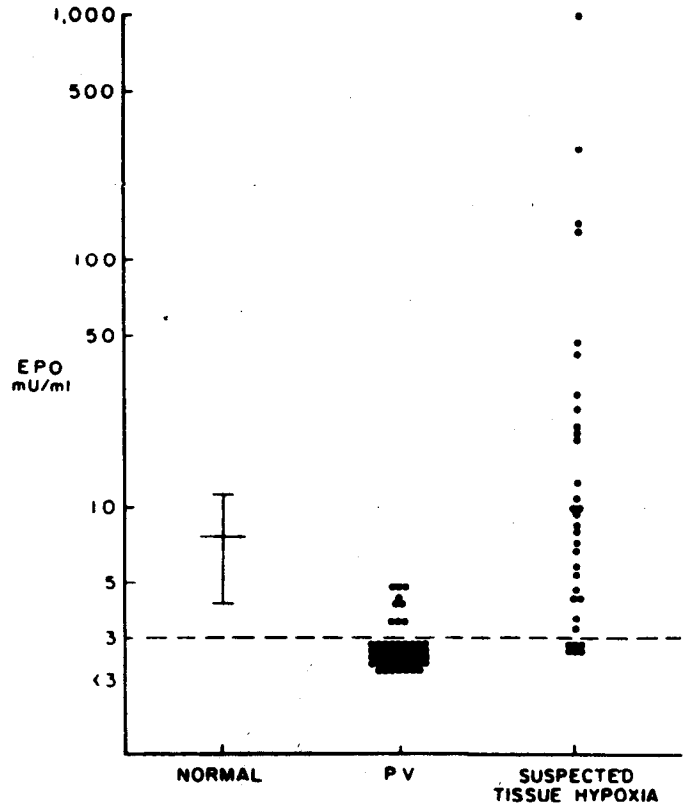

Fig. 4. Erythropoietin titers in blood from normal individuals and patients with primary polycythemia vera or suspected secondary polycythemia.

However, pharmacologic therapy of refractory anemias may have a place when unlimited amount of erythropoietin is available. In other words, even if patients have normal kidneys producing adequate amount of erythropoietin the bone marrow function may be improved by providing additional exogenous erythropoietin. This is especially pertinent for the anemia of chronic disease such as rheumatoid arthritis in which the bone marrow appears to be somewhat resistant to appropriately produced endogenous erythropoietin. So far only a few case reports have been published but in these patients erythropoietin has restored hemoglobin to normal and possibly improved their quality of life[19]. The same may be true for hematologic malignancies with dysfunctioning marrows but large amounts of exogenous erythropoietin will probably be needed in order to make these patients transfusion independent. Clinical trials are in progress but the results are still not known.

Due to its growth enhancing effect on progenitors and early erythroblasts erythropoietin will cause a rapid transit through the progenitor and precursor department with the production of fetal hemoglobin containing cells. This feature could be of importance in patients with sickle cell anemia whose clinical problems would be alleviated by an increase in fetal hemoglobin. Unfortunately, it does not appear that erythropoietin

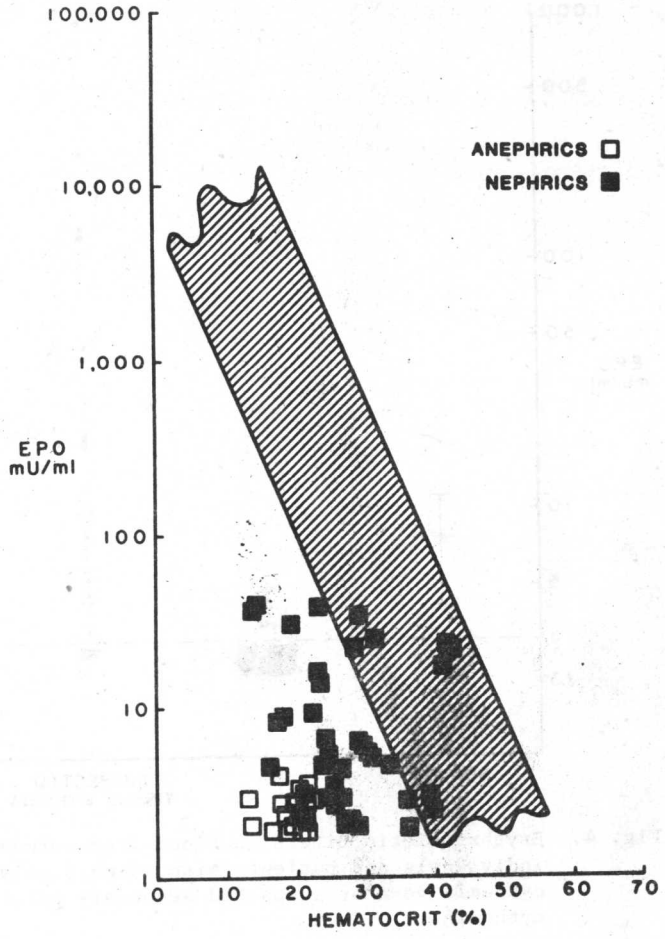

Fig. 5. Erythropoietin titers in blood from anephric patients (hollow squares) and from patients with severe renal failure (solid squares) and their relation to the hematocrit of blood. The cross-hatched area represents erythropoietin titers of anemic, but nonuremic patients (see Fig. 3).

per se will produce that much extra fetal hemoglobin but it may supplement the effect of other fetal hemoglobin producing agents such as hydroxyurea[20].

Because of the inherent dangers of transfusion, more and more people prefer to donate their own blood before an elective operation in order to have their own blood on reserve if need should be. Such donations could be accelerated if the patient simultaneously receive injections of exogenous erythropoietin.

All in all, erythropoietin has come a long way from its discovery as an erythroid growth factor 40 years ago[21]. It is universally active in stimulating the rate of red cell production and in patients with chronic renal disease it has been shown to eliminate their anemia. It seems likely that it also will ameliorate the anemia in many other patients rendering them transfusion independent and improving their quality of life.

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