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Preface

Over the past 12 years powerful new technologies have emerged for the study of hematopoietic cells *in vitro*. In 1966 the first reports appeared describing *in vitro* colongenic assay systems for mammalian granulocytemonocyte precursor cells. Subsequently, these systems were improved and extended to include assay systems for erythroid progenitors, megakaryocytic precursors, and T- and B-lymphocytes in semisolid gel culture. The study of hematopoietic cell differentiation and proliferation *in vitro* has stimulated a virtual revolution in hematologic investigation and has led to a major restructuring of concepts relative to the regulation of hematopoiesis. The fields of study approachable by the new *in vitro* technologies have been wide and encompass the basic biology of cell differentiation, viral leukemogenesis, the pathogenesis of human hematologic disease, transplantation biology, and hormonal regulation of cell growth.

The ICN–UCLA Conference on Hematopoietic Cell Differentiation represented the third international meeting of this nature. The first and second were held in Rijswijk, The Netherlands in 1971 and at the Airlie House in Virginia in 1973. The field of *in vitro* hematopoiesis has had its greatest growth since 1973 and it was clear from the enthusiasm and interest shown in the present conference that the meeting was long overdue.

The ICN–UCLA Conference on Hematopoietic Cell Differentiation was a resounding success. All of the participants were enthusiastic, the discussions were animated, and the atmosphere was exciting. The poster sessions were a highlight of the conference. These sessions were of exceedingly high quality and the scientific interchange was both pleasant and efficient. While the altitude may have contributed to the euphoria, this conference confirmed our belief that good science and fun are compatible.

The present volume contains papers summarizing the work presented at the plenary sessions and some of the poster sessions. They reflect the content and hopefully the spirit of the scientific exchange.

The conference organizers were simply participants. Fran Stusser was the real organizer, and she and her staff were in large measure responsible for the success of the conference. They managed to do all of the work and make it look effortless at the same time. We also thank ICN Pharmacenticals, Inc., for its general support of these Symposia, and particularly the National Institutes of Health (Division of Cancer Research Resources & Centers/National Cancer Institute, Immunology, Allergic & Immunologic Disease Program/ National Institute of Allergy and Infectious Diseases, Division of Blood Diseases & Resources/National Heart, Lung, and Blood Institute, National Institute of Arthritis, Metabolism, and Digestive Diseases and Fogarty International Center), Pfizer Inc., Burroghs Wellcome Co., Grand Island Biological Company, Syntex Laboratories Inc., Lilly Research Laboratories, and Miles Laboratories, who provided funds to defray the costs of the conference.

Contents

II. STEM CELLS AND MICROENVIRONMENT

III. LYMPHOCYTE AND MEGAKARYOCYTE GROWTH

IV. LEUKEMIA AND CLINICAL DISORDERS OF HEMATOPOIESIS

V. GRANULOPOIESIS

THE BIOGENESIS AND METABOLISM OF ERYTHROPOIETIN[1]

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ABSTRACT One of the aims of this conference is to discuss the effects of erythropoietin on bone marrow stem cells. In order to translate such effects into a better understanding of physiologic control of red cell production, it is necessary to relate them to the biogenesis and metabolism of both renal and extrarenal erythropoietin. This brief review deals with current concepts of these processes and especially attempts to fit extrarenal erythropoietin into the feedback system which is believed to govern renal erythropoietin production and metabolism.

INTRODUCTION

The red cell mass is a large but fairly unsophisticated organ designed almost exclusively to transport oxygen from the lungs to the tissues. Its size is monitored and maintained by a sensitive yet rugged feedback system capable of responding to both minor and major changes with appropriate compensatory actions. This constancy of the red cell mass has always been taken for granted by our ancestors who readily could observe that blood loss if not fatal would rectify itself with a return to normal of rosy cheeks and physical vigor. However, it was not until about 100 years ago when it was realized that the size of the red cell mass, so carefully preserved, is regulated by its functional interaction with the organs it serves. At that time, a French physician, Dr. Dennis Jourdanet was practicing medicine in the highlands of Mexico. He observed that the blood of his surgical patients was thick, black and viscous and he found that it contained many more red corpuscles than normal blood. Nevertheless, his patients had symptoms resembling those of his old anemic patients in Paris and he proceeded in 1863 to write a book confusingly entitled "The Anemia of

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Altitude" (1). In this book, he coined the word "anoxemia" to express the lack of oxygen in arterial blood and explained that one could be anoxemic either due to lack of oxygen in the air or due to lack of red corpuscles. Although he also described the ease with which plethoric individuals could tolerate exposure to thin air at high altitude, he did not actually suggest that anoxemia could lead to plethora. Dr. Jourdanet was a wealthy patron of the arts and sciences and he provided financial support for further altitude studies carried out by his friend, Paul Bert. Dr. Bert was somewhat of a renaissance man, a lawyer who became a physician and a physiologist and finally a politician and governor of French Indochina. He has been called the Father of Aviation Medicine because he established that survival at high altitude depends on the amount of oxygen which can be made available at the tissue level (2). Although he appreciated that an increase in the number of red corpuscles in the blood would promote the transport of oxygen from a rarefied atmosphere, he did not believe that anoxemia would cause an adaptive increase in the size of the red cell mass, at least not right away. He wrote in his monumental book "La Pression Barometrique" published in Paris in 1878: "But it is very certain that such a change if it takes place, requires a very long time; it is even probable that it can come about only through an inherited disposition, and can come to complete development only at the end of successive generations so that it would explain the acclimatization, not of the individual, but of the race" (3). This conclusion did not stay unchallenged and a few years later, Viault showed conclusively that an ascent from sea level to 15,000 feet is associated with prompt increase in the number of red corpuscles (4). The establishment of an increase in the red cell mass as an adaptable defense against hypoxia led naturally to the question of how this is accomplished. Frederic Miescher, the famous Swiss physician and biochemist who previously had separated and identified DNA, became interested in this question after tuberculosis had confined his activities to an alpine sanatorium. He suggested in 1893 that anoxemia directly stimulates the erythroid tissue in the bone marrow to increased cellular activity in accord with the prevalent belief of those days that a stay in a high altitude spa exerts a stimulating effect on the body (5). This suggestion was generally accepted until the nineteen fifties when it was shown conclusively that the stimulation of the bone marrow by anoxemia is not direct but indirect via the release of a factor or hormone, erythropoietin (6,7). Subsequent studies have shown that this hormone is produced primarily by the

kidney (8) and that it acts by controlling the rate of differentiation of bone marrow stem cells to nucleated red cells (9,10). These findings have led to the realization that the size of the red cell mass is maintained by a seemingly simple feedback system operating between the bone marrow and the kidney and mediated in one direction by oxygen transported by circulating red cells and in the opposite direction by erythropoietin (Figure 1). This system in

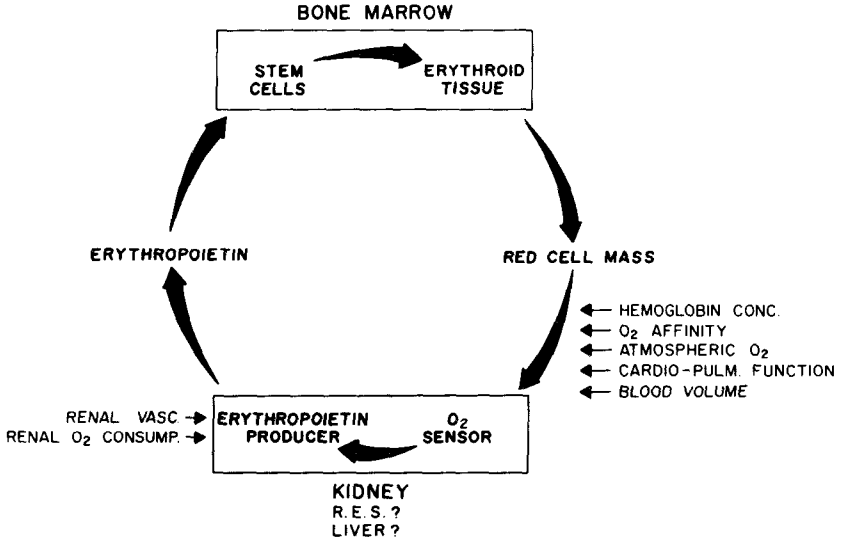

FIGURE 1. Erythropoietin feedback control system hinging on bone marrow-produced red cells transporting oxygen to oxygen sensors in the kidney and in the liver. These sensors regulate the production of renal and extrarenal erythropoietin which in turn affect the rate of differentiation of bone marrow stem cells to red cell-producing erythroid precursors.

normal individuals at sea level is set at maintaining a red cell mass of about 30 ml/kg of body weight. The specific setting depends on a complex interaction among the many components involved in the supply and demand for oxygen at the cellular level. The setting can be displaced downwards as in patients with certain hemoglobinopathies (i.e., Hgb.

Kansas) in whom the oxygen affinity of hemoglobin is low (11), or in patients with a decreased tissue demand for oxygen (i.e., myxedema) (12). However, the setting is more often displaced upwards as in conditions with an inadequate supply or transport of oxygen to the tissues. Although a displacement upwards will result in a self-defeating increase in whole blood viscosity and a decrease in blood flow, an increase in red cell mass will also cause an increase in blood volume and the dilated vessels will reduce viscous drag and actually improve oxygen transport (13,14). Consequently, there is considerable room for a compensatory adjustment of the red cell mass upwards, and secondary polycythemia is the normal adaptive response for people living at high altitude or patients with chronic anoxemia.

With the gross outline of the feedback circuit firmly established, recent studies have been aimed at unravelling its finer functional structure. The participants in this conference will discuss the action of erythropoietin on the proliferation, maturation and differentiation of stem cells and I would merely like, as a background, to review the metabolism and production of erythropoietin.

ERYTHROPOIETIN METABOLISM

Erythropoietin is an acidic glycoprotein with an apparent molecular weight of 39,000 and, as is true for many glycoproteins, is a fairly poor antigen (15). This fact as well as difficulties in purification has impeded the development of a sensitive radioimmunoassay and we still have to rely on a crude bioassay for the quantitation of erythropoietin. This assay has a lower limit of sensitivity of about 50 mU/ml, a level higher than that needed to maintain a normal rate of red cell production (Figure 2). A normal level however, can be detected with our bioassay if the plasma is first heat treated and then concentrated. Such a treatment will remove most of the plasma proteins but only some of the erythropoietin and assay of this concentrate will permit us to measure concentrations down to 5 mU/ml (Figure 3). Normal plasma is found to have titers ranging from 3 to 18 mU/ml with a mean of 7.8 mU (Figure 4). Plasma from patients with polycythemia vera and hematocrits between 53 % and 69 % all have erythropoietin levels of less than 5 mU/ml presumably the level which would have been attained if normal individuals were hypertransfused.

It is of interest that an erythropoietin titer of about 10 mU/ml can maintain a normal rate of red cell production while it takes about 2-5,000 mU of erythropoietin

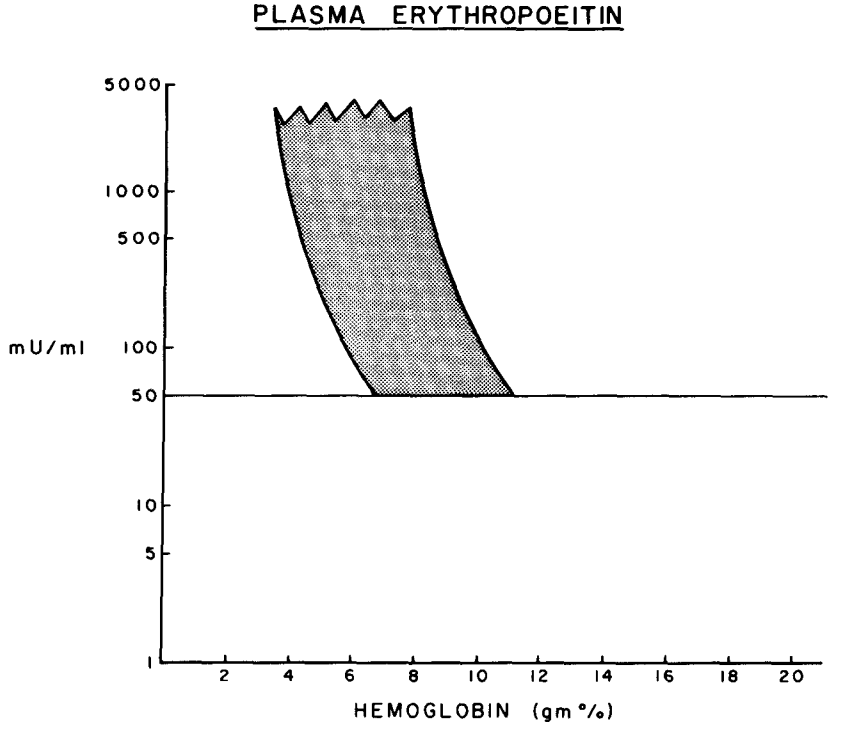

FIGURE 2. Plasma concentrations of erythropoietin as a function of hemoglobin concentrations. Only concentrations of 50 mU/ml of plasma can be measured accurately with routine bioassay techniques, and the erythropoietin content in normal or polycythemic plasma is not measurable.

per ml to maintain a ten times increase in this rate as observed in severe bleeding or hemolytic anemia. The reason for this steep increase in requirement for erythropoietin by the erythroid tissue is unknown.

The glycoprotein nature of erythropoietin may also affect its life span. Erythropoietin with its sialic acid component removed has been shown to remain active in vitro, but not to have any activity in vivo because of rapid removal from the circulation (16). Normal sialic acid-containing erythropoietin has a half-life in rats and

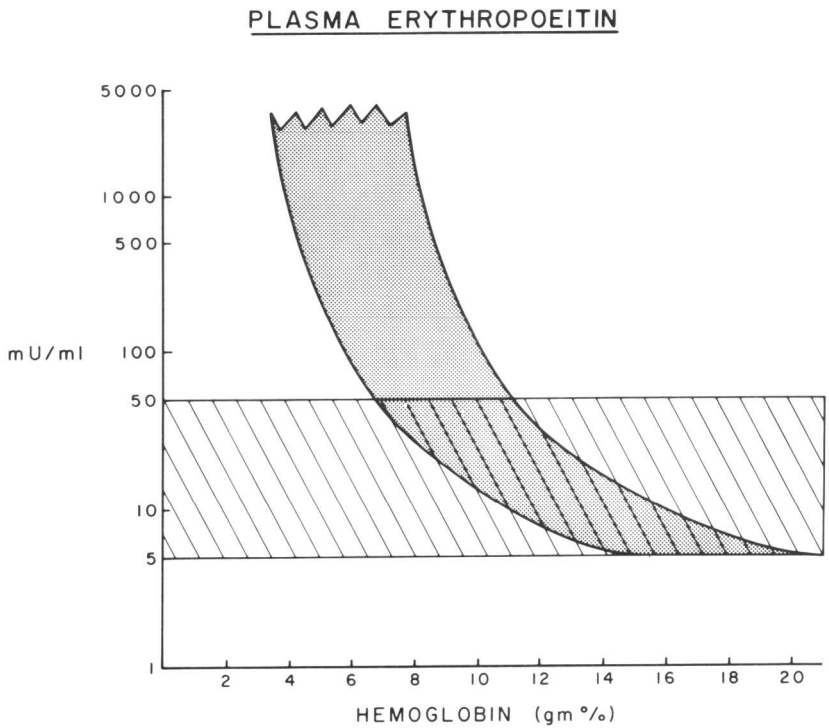

FIGURE 3. Extension of the erythropoietin curve to normal and polycythemic hemoglobin values by means of assaying plasma concentrates. With a 40 times concentration the limit of sensitivity is about 5 mU/ml plasma.

rabbits of about 1-3 hours (17). In man, its life span has not been determined accurately but the few measurements made suggest that it is considerably longer than in small rodents (18). It is cleared slowly by the kidneys with a clearance rate of about 0.5 ml per minute. Initially, it was believed that the life span was determined by the amount of erythropoietin-consuming erythroid tissue in the bone marrow. Although it still appears that patients with aplastic anemia have a higher erythropoietin titer than patients with bleeding or hemolytic anemia, the difference is not as impressive as first thought (19). Furthermore,

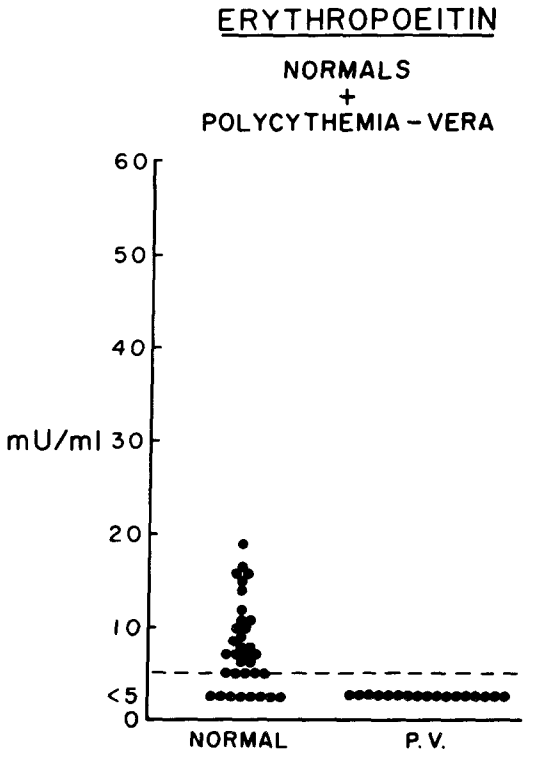

FIGURE 4. Erythropoietin concentrates in the plasma of 34 normal individuals and 17 patients with polycythemia vera.

the observation that after bleeding or hypoxia erythropoietin titers increase rapidly before the bone marrow erythroid tissue has become active and then fall at the same time as erythropoietic activity becomes noticeable may not be related to a consumption of erythropoietin by erythroid tissue (Figure 5). It has been shown that this early erythropoietin peak probably is due to an early and temporary alkalosis which increases oxygen affinity of hemoglobin and adds a further hypoxic stimulus to arterial anoxemia (20,21).

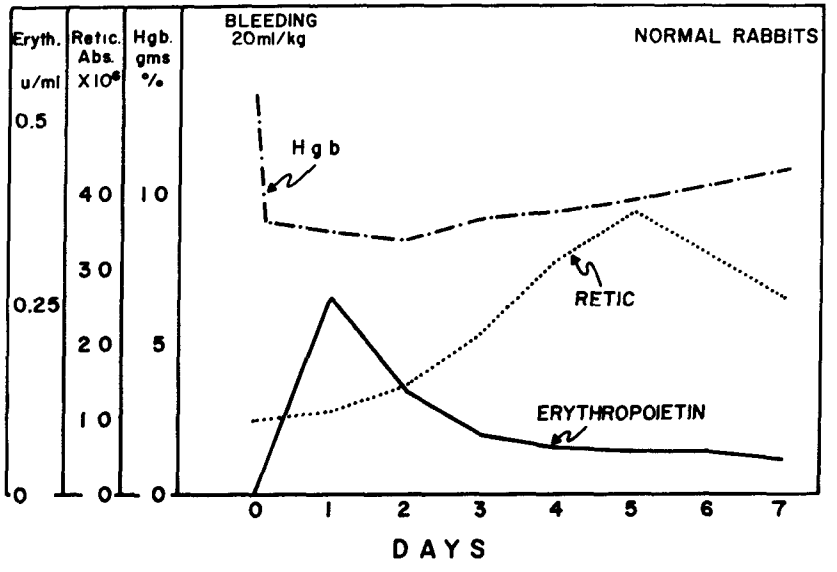

FIGURE 5. Mean hemoglobin concentrations, reticulocyte counts and erythropoietin concentrations of blood from normal rabbits bled 20 ml/kg body weight.

Currently, it seems more likely that the major part of erythropoietin is destroyed either by the liver or the kidneys (17).

ERYTHROPOIETIN PRODUCTION

Renal: The production of erythropoietin is still believed to occur primarily in the kidneys. Many indirect studies suggest strongly that the kidneys both sense oxygen and produce erythropoietin (22). Direct confirmation has been more difficult to achieve and it has been proposed that although the kidneys sense oxygen, they merely respond by releasing an enzyme which in circulating blood activates an inactive erythropoietin precursor presumably synthesized by the liver (23). Recently, however, it has been shown that a kidney removed from an anemic rabbit will continue to synthesize erythropoietin in vitro, whether perfused