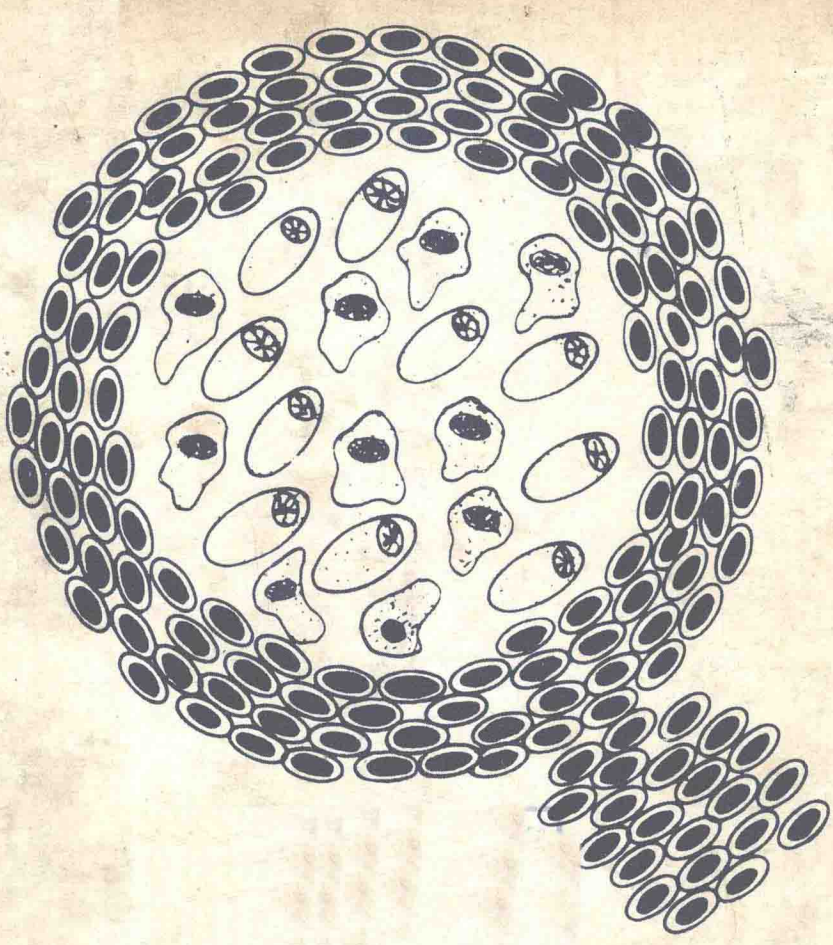

edited by
W. L. Holmes

blood cells as a tissue

Blood Cells as a Tissue

Proceedings of a Conference held at The Lankenau Hospital
October 30-31, 1969

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PLENUM PRESS · NEW YORK–LONDON · 1970

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Library of Congress Catalog Card Number 78-136210

SBN 306-30510-0

A Division of Plenum Publishing Corporation
227 West 17th Street, New York, N.Y. 10011

United Kingdom edition published by Plenum Press, London
A Division of Plenum Publishing Company, Ltd.
Donington House, 30 Norfolk Street, London W.C. 2, England

Printed in the United States of America

Blood Cells as a Tissue

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PREFACE

This volume is a collection of the presentations given at the sixth international research conference, Blood Cells as a Tissue, at The Lankenau Hospital, October 30 and 31, 1969.

The conference was supported in part by the following pharmaceutical industries: Abbott Laboratories, North Chicago, Illinois; Baxter Laboratories, Inc., Morton Grove, Illinois; Bristol Laboratories, Syracuse, New York; Burroughs Wellcome and Company, Tuckahoe, New York; Eli Lilly and Company, Indianapolis, Indiana; Geigy Pharmaceuticals, Ardsley, New York; Merck Sharp & Dohme Post Graduate Program, West Point, Pennsylvania; Riker Laboratory, Northridge, California; Schering Corporation, Bloomfield, New Jersey; Squibb Research Institute, New Brunswick, New Jersey; and Smith Kline and French Laboratories, Philadelphia, Pennsylvania. This valuable support is gratefully acknowledged.

The Conference Committee is especially indebted to the four chairmen, Dr. Allan J. Erslev, Dr. William J. Williams, Dr. R. Philip Custer and Dr. Sol Sherry, for their advice and guidance in the formulation of the program. We are also grateful to the speakers for their splendid cooperation, and to all participants for their stimulating interest. The continuing support of the members of the Board of Trustees, of the Hospital Administration, and the Women's Volunteer Service played a major role in the success of the conference.

We wish to express our sincere gratitude to the many members of the Hospital staff who contributed so willingly of their time to make the conference possible. A special vote of thanks goes to Mr. Ralph Hollerorth, Mrs. Carolyn Hyatt and Mrs. Marjorie Palmer for their untiring efforts in administering the many details involved in the organization of this meeting.

The Conference Committee
The Lankenau Hospital

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INTRODUCTION

In the short span of two decades, the process by which red cell production is regulated has, to a large degree, been elucidated.

Although a humoral stimulator of erythropoiesis was described in 1906 (17), it was the classical experiment of Reissmann in 1950 which established the existence of a plasma erythropoietic factor (146). Reissmann reported erythroid hyperplasia in the marrow of both rodents joined in parabiosis when one of them breathed gas of reduced oxygen content. The most likely explanation - a humoral stimulator produced in the hypoxic animal and transferred across the union - undoubtedly is the correct one. Erslev demonstrated convincingly a factor in the plasma of anemic rabbits which produced a reticulocytosis in normal animals (36), and after Jacobson and his co-workers developed bioassays based upon sound physiological principles, brilliant insights, and the use of radioiron (143, 54, 55, 93), the stage was set for a host of investigators to ask questions and seek the answers which constitute the material to be reviewed here.

THE ASSAY OF ERYTHROPOIETIN

Although reticulocytosis can be observed in the rat following injection of biological fluids rich in

erythropoietin, such a procedure does not lend itself to quantitative assay. The recent production of an anti-erythropoietin antibody (158,115) and subsequent efforts to develop an immuno-assay (11, 116) indicate a substantial advance - the perfection of a sensitive and reproducible immuno-assay - is in the offing. The impurity of the best preparation of erythropoietin used as an antigen, and the diverse nature of the antibodies produced (117,159) present obstacles yet to be overcome, and the bio-assays developed by Leon Jacobson and his colleagues, so valuable in the past, will undoubtedly continue to be employed for sometime.

Radioactive iron can be utilized more simply than reticulocytes can be counted, and erythropoietin can be detected by an increase in the rate at which the isotope is incorporated into erythrocytes of normal rodents after injection of the hormone (143). When the anemia of hypophysectomized rodents was used as evidence to incriminate the pituitary as the source of erythropoietin, Jacobson and his colleagues substituted these animals for normal rats in the bio-assay (54). The retention of sensitivity to erythropoietin in hypophysectomized animals, whose basal, pre-stimulation rate was reduced strikingly, constituted a great advance in the development of an effective assay procedure. After the concept of the dynamic equilibrium of erythropoiesis was proposed, equating erythropoietin production directly to oxygen demand and inversely to oxygen supply (94), it became apparent that a plethoric rodent warranted attention as a bio-assay animal. Mice can be rendered plethoric by transfusion (25), several weeks exposure to a hypoxic environment (20,118), or prolonged exposure to low concentrations of carbon monoxide (52). Bio-assay results are less variable and control values are more nearly basal when animals are prepared for bio-assay by transfusion than when plethora is induced by hypoxia. Nevertheless, one should constantly keep in mind several pitfalls when using this bioassay procedure. The activation of endogenous erythropoietin in the assay animals, as by androgens and triiodothyronine, can give a false positive result. Substances which alter ferrokinetics in the assay animal might theoretically also lead to mis-interpretation.

A second major advance, after the perfection of simple bioassay procedures, was the establishment of a

reference standard of erythropoietin by Cotes and Bangham who defined an international unit and made available to interested investigators throughout the world reference samples, to which locally prepared batches could be related (21).

A by-product of investigations of the effects of erythropoietin on tissue culture, undertaken primarily to study the mechanism of action of erythropoietin, has been in the development of an *in vitro* bioassay (106,178). The acceleration of radioiron uptake and incorporation into heme by cultured marrow cells under the influence of erythropoietin has not yet gained wide employment as a bioassay, perhaps in part because one or more plasma inhibitors of erythropoiesis may at times alter the results in unpredictable ways.

For all its limitations, and there are many, the stimulation of erythropoiesis in the plethoric mouse and the quantitation of the response by determination of incorporation of radioactive iron into newly formed erythrocytes is currently the most widely employed assay system for the detection and measurement of erythropoietin.

THE CHEMISTRY OF ERYTHROPOIETIN

The major problem encountered by the chemist attempting to define the chemical nature of erythropoietin is the difficulty associated with obtaining adequate amounts of purified material. As little as .4 units of erythropoietin probably approximates the normal daily production in the mouse (4). Since small samples with more than 7,000 units of activity per milligram of protein have been produced (69), it is apparent that man, like a mouse in so many ways, might be sustained by one or two hundred micrograms of active material per day.

When in plasma, erythropoietin is resistant to short periods of boiling (10). Purified preparations are destroyed by trypsin, chymotrypsin (11), pepsin (166), and neuraminidase (122,182). These results indicate the active material is probably a sialic acid containing protein. Limited studies to date have indicated the biochemical properties of erythropoietically active concentrates obtained from renal tumors, renal cystic fluid, or cerebellar cyst fluid of patients with secondare polycythemia are quite similar to the properties of

erythropoietin obtained from plasma and urine of anemic mammals.

As greater degrees of concentration and purity are achieved, especially with urinary erythropoietin, instability becomes a further problem. It has long been suspected that a plasma factor acting as a carrier protein may stabilize and protect erythropoietin. This suspicion may be strengthened by the observation that neuraminidase-treated erythropoietin, inactive in vivo, retains its activity in tissue culture (69). It is therefore not clear which of the characteristics attributed to erythropoietin, including molecular weight estimates varying between 27,000 (152) and 70,000 (69), might in reality be properties of one or more carrier proteins or other extraneous substances. Clearly much work remains before the chemical characteristics of erythropoietin can be defined with assurance.

SITE OF PRODUCTION OF ERYTHROPOIETIN

The liver, spleen, bone marrow, and many other organs have been removed or destroyed in the rat with at most equivocal reduction of erythropoietin production in response to bleeding or the cobaltous ion. However rats no longer respond to cobalt or bleeding if these treatments are preceeded by nephrectomy (95). Ureter-ligated rats serve as control animals, since comparable levels of nitrogenous retention are observed in them, but their ability to produce erythropoietin remains intact. The importance of the kidney in erythropoietin production was substantiated by the finding that isolated kidneys perfused with hypoxic blood yielded erythropoietin to the perfusing medium (113,147). Erythropoiesis is depressed in dogs after nephrectomy, and amelioration of the azotemia by peritoneal dialysis does not restore red cell production. These animals do not respond to bleeding by demonstrable production of erythropoietin (133). The role of the kidney in the production of erythropoietin was further supported by the frequency with which plasma obtained from patients whose anemia was secondary to renal disease was found on bioassay to be devoid of erythropoietic stimulating properties (78,119). The restoration of normal red cell counts, and at times the development of polycythemia after successful renal transplantation (92) are consistent with the thesis that kidneys are somehow implicated in erythropoietin production. Erythropoietic activity was obtained when the nuclear fraction of homogenized cells

of anemic rabbit kidneys were incubated with alpha globulins of plasma (114). Kuratowska has proposed a renal erythropoietic factor is labile and is stabilized by union with plasma globulins, the resultant product being erythropoietin (114).

The kidney is clearly not the sole source of erythropoietin, however. Anephric man sustains red cell production (139a) and, if sufficiently anemic, his plasma contains demonstrable titers of erythropoietin. (134) Nephrectomized rats produce small amounts of erythropoietin after a few hours of hypoxia (128), and nephrectomized rabbits continued to produce reticulocytes and retain the ability to produce small amounts of erythropoietin (37). The hormone is also found in the plasma of baboons between 2 and 10 days after nephrectomy (129). Following a combination of erythropoietic stimuli applied simultaneously,substantial amounts of erythropoietin can be elaborated in nephrectomized rats (56). Finally, small amounts of erythropoietin have been detected in blood after perfusion of the liver (147).

In the last few years, evidence has been presented to substantiate the claim that the kidney participates in the regulation of erythropoiesis not by producing erythropoietin, but rather by making an enzyme, Renal Erythropoietic Factor (R.E.F.), which converts an inactive plasma globulin into an active erythropoietin (72). Generation of REF has been localized to the ribosomal complex of the endoplasmic reticulum of the microsomal fraction of renal cells, and is thought to migrate from there to the peroxisomes of the light mitochondiral fraction within the same cells (15). Although several investigators have reproduced some of these results, a word of caution is indicated, since Erslev has neither been able to produce REF nor demonstrate an erythropoietic effect in polycythemic mice by REF supplied by others (38).

Virtually every area of the kidney has been staked out by one or more explorers who have established their claim to the site of erythropoietin production. The renal cortex (167), the juxtaglomerular cells (141,90, 27), the glomerular tuft (48) and the renal medulla (132) have all been reported to be the site of erythropoietin. Since REF was found in the light mitochondrial fraction of all areas of the kidney (183), it is difficult at this time to evaluate the validity or importance of these observations.