THE POLYCYTHEMIC DISORDERS

BARUCH MODAN, M.D

PREFACE

I WAS FIRST INTRODUCED to the polycythemic disorders as a medical student, in 1956, when the first patient ever presented to our group on the medical ward turned out to be a flushed gentleman, complaining of vertigo and occasional headache. I remember that it seemed puzzling to our inexperienced minds that a mere excess in red blood cells could account for the patient's symptoms. Similarly, the quick improvement which followed phlebotomy seemed quite impressive. It took at least five years before I realized that this patient did not, in fact, have polycythemia vera and that, unfortunately, phlebotomy does not necessarily provide a full solution for a polycythemic patient.

Polycythemia vera is a rare blood disorder, with an estimated incidence of six cases per million per year. In other words, hardly more than one thousand new cases per year are diagnosed in the entire United States, and the yearly death toll is much lower than the harvest of traffic accident deaths on an average holiday weekend. Yet, ever since its first description at the end of the nineteenth century by Vaquez, it has intrigued many a mind in various parts of the globe, primarily because of its close association with a multitude of disorders, most notably leukemia. It might have been just the incorrigible curiosity of the medical profession, and the tendency to prefer the rare and obscure to the prevalent and obvious, that led to overemphasis of this entity; similar in a sense to the eagerness of almost any fresh intern or resident to admit to the ward an LE patient rather than a "plain coronary." On the other hand, it might have been the close relationship of polycythemia with a variety of other conditions that intrigued numerous investigators, with the hope that a better understanding of the nature of this entity could provide clues to the etiology and pathogenesis of other disorders of the hematopoietic system.

Whatever the reasons, these investigators persevered and an extensive amount of material has been accumulated; much has been proposed and then disregarded; much has been accepted and carried forward only to be added to and changed again.

This monograph comprises a review of the clinical and laboratory features of the polycythemic disorders, with emphasis on the relationship to other diseases and on its epidemiological features. It is based primarily on experience gained through a multicenter study performed in the United States between 1961 and 1964, as well as on subsequent experience obtained in the course of a prospective follow-up study of patients with polycythemia

at the Tel Hashomer Government Hospital in Israel. Both studies have been supported by different branches in the U.S. Public Health Service. The generous support of this agency to a nonresident investigator, and especially to a study conducted outside the territorial boundaries of the United States, is gratefully appreciated.

Similarly, I am indebted to the hematologists and other physicians at the seven hospitals where the original research had been undertaken: the Johns Hopkins Hospital, Baltimore, Maryland; The Mount Sinai, Presbyterian and the New York Hospitals in New York City; as well as the University of Rochester (NY), University of Oregon, and the New England Medical Centers. In particular I want to thank Drs. C. Lockard Conley, Ralph L. Engle, Jr., Louis H. Hemplemann, Paul Marks, Robert L. Rosenthal, Joseph E. Synder, Louis R. Wasserman, and the late Drs. William Dameshek and Edwin E. Osgood.

Most helpful comments were given by a number of colleagues who have reviewed certain parts of this monograph at various stages. These include Drs. Meir Djaldetti, Richard M. Goodman, Chaim Hershko, Ernest Lubin, David Rabin, Mordechai Shani, Frederick Stohlman, Jr. and David J. Weatherall, as well as my wife Michaela whose constructive criticism and invaluable assistance in final editing have been of immense help.

Dr. Chaviva Isersky provided aid in the writing, editing, and library search and contributed to the sections of erythropoiesis and anoxemic polycythemia.

Thanks are due to Mrs. Mirjam Merkel, who patiently devoted numerous afternoon and evening hours to retyping draft after draft, and to Mrs. Naomi Rothschild for her assistance in secretarial work.

Finally, a word of appreciation to the four women in my life—wife, daughters and mother, who understandingly shared the family time deficit attributed to polycythemia throughout the past eight years.

Tel Hashomer BARUCH MODAN

CONTENTS

Page

Preface vii

PART I
INTRODUCTION

Chapter

1. Regulation of Erythropoiesis 5
2. Classification of the Polycythemic Disorders 19

PART II
POLYCYTHEMIA VERA

3. Nature of the Disease 23
4. Clinical and Laboratory Manifestations 31
5. Epidemiology and Relation to Other Diseases 45
6. Therapy 64
7. Polycythemia Vera and Leukemia 70
8. Prognosis 86

PART III
SECONDARY POLYCYTHEMIA

9. Anoxemic Polycythemia 103
10. Renal Polycythemia 108
11. Tumor Polycythemia 111
12. Miscellaneous 119

PART IV
OTHER POLYCYTHEMIC ENTITIES

13. Pseudopolycythemia 123
14. Benign Erythrocytosis 124

References 129

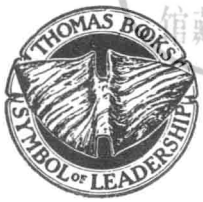
Index 171

The Polycythemic Disorders

By

BARUCH MODAN, M.D., Dr. P.H.

Head, Department of Clinical Epidemiology
Tel Hashomer Government Hospital, Israel
and Tel-Aviv University Medical School



CHARLES C THOMAS • PUBLISHER
Springfield • Illinois • U.S.A.

Published and Distributed Throughout the World by
CHARLES C THOMAS • PUBLISHER
BANNERSTONE HOUSE
301-327 East Lawrence Avenue, Springfield, Illinois, U.S.A.
NATCHEZ PLANTATION HOUSE
735 North Atlantic Boulevard, Fort Lauderdale, Florida, U.S.A.

Library of Congress Catalog Card Number: 72-103582

With THOMAS BOOKS *careful attention is given to all details of manufacturing and design. It is the Publisher's desire to present books that are satisfactory as to their physcial qualities and artistic possibilities and appropriate for their particular use.* THOMAS BOOKS *will be true to those laws of quality that assure a good name and good will.*

Printed in the United States of America
Q-1

PART I

INTRODUCTION

Chapter 1

REGULATION OF ERYTHROPOIESIS

THE POLYCYTHEMIC DISORDERS constitute a group of entities characterized by either a real or spurious elevation of the circulating red blood cells. Therefore, a concise summary of current concepts of the regulation of erythropoiesis seems mandatory.

Erythropoietin

Historical Background

The idea that the circulating blood carries an erythropoietic stimulating factor, hemopoietin, was first proposed by Carnot and Deflandre in 1906.[1, 2] Strangely enough, the concept remained in a dormant stage for almost half a century. It was only in 1948 that Bonsdorf and Jalavisto[3] reviewed the humoral theory, after having produced an increase in red blood cell counts in normal rabbits, injected by plasma from rabbits subjected to low barometric pressure and from patients with a respiratory insufficiency. They were also the ones to introduce the term "erythropoietin" for the humoral factor.

This concept was further substantiated two years later by Reissmann,[4] who demonstrated that chronic hypoxemia, induced in one partner of a pair of parabiotic rats, served as a stimulus for erythropoiesis in the other partner of the pair, who had been kept in normal atmosphere and had normal oxygen saturation values throughout the experiment. Erslev[5] and Hodgson and Toha[6] demonstrated later that plasma[5, 6] and urine[6] from rabbits rendered anemic by bleeding contain a factor capable of stimulating red cell production in normal rabbits.

The first application to human pathology was made by Stohlman *et al.*[7] in 1954, in a patient with polycythemia secondary to patent ductus arteriosus. In this patient, areas deriving their blood supply from the aorta proximal to the ductus had normal oxygen saturation, while areas receiving blood from the distal aorta were hypoxic. Erythroid hyperplasia was present even in bone marrow areas with normal oxygen tension. This seemed to preclude direct effect of the low oxygen tension on the bone marrow, in favor of its stimulation by a humoral factor in response to hypoxia.

Studies on the nature of erythropoietin received impetus from the experiments of Jacobson and his collaborators,[8] who developed a series of sensitive procedures for its bio-assay. These experiments were based on the

fact that rats subjected to hypophysectomy, high oxygen pressure, starvation, or transfusion-induced polycythemia, have a decreased rate of erythropoiesis, but when injected with anemic plasma rich in erythropoietin, an erythropoietic effect may be demonstrated and quantified by the measure of Fe^{59} red cell incorporation or an increase in circulating reticulocytes. Subsequently, erythropoietin has been demonstrated in both normal human urine and plasma.[9-11]

Site and Mode of Action

Erythropoietin most probably functions by causing erythropoietin sensitive primitive stem cells in the hemopoietic tissue to differentiate into erythroid precursors.[12-23]

It is now generally accepted that the production of blood cells in mammals depends on a primitive pluripotential stem cell capable of reproducing itself[24] and of producing megakaryocytes, myeloid and erythroid elements. This notion is supported by the study of colony forming cells. These are cells present in normal mouse hemopoietic tissue, which are capable of forming colonies with hemopoietic activity when this tissue is transplanted into the spleen of heavily irradiated mice.[25] Colonies shown to have been derived from a single donor cell contained cells differentiated along the three blood cell lines.[26] Whang *et al.*[26a] corroborated these findings in humans by cytogenetic studies of patients with chronic myelogenous leukemia.

The conjecture that erythropoietin acts by direct stimulation of the stem cell to differentiate is based on experiments such as the one by Filmanovitz and Gurney.[14] These investigators noted that, upon administration of erythropoietin to mice in which erythropoietic activity was completely suppressed due to transfusion induced polycythemia, erythropoiesis was immediately initiated in the spleen. Within 24 hours after injection early erythroid forms appeared. Up to a certain dose level, the response was directly proportional to the amount of erythropoietin. Studies of colony forming cells in transfusion induced polycythemic mice[20-23] demonstrated a similar effect of erythropoietin. In such conditions, there is a suppression of erythroid in favor of myeloid colonies, which can be reversed by either administration of erythropoietin,[20-21] or by stimulation of its endogenous production.[23]

Further investigations[15-19, 23] suggested that the primitive multipotential stem cells give rise to committed stem cells which serve as the immediate precursors to the recognizable differentiated bone marrow elements. The erythroid committed stem cell, rather than the multipotential one, seems to be the target of erythropoietin stimulation.

Krantz and Goldwasser[27] suggested that erythropoietin promotes the

differentiation of stem cells into erythroblasts by initiating the synthesis of a specific messenger RNA, which, in turn, causes the production of specific proteins such as hemoglobin. These two investigators and others[15, 27-32] demonstrated that erythropoietin induces an increase in hemoglobin, stroma, and RNA synthesis. A correlation between heme and RNA synthesis and stem cell differentiation, in response to erythropoietin, was also observed.[33-35] Orlic *et al.*[36] added that erythropoietin activation is immediately followed by RNA synthesis by erythropoietin sensitive stem cells.

Stohlman *et al.*[15] concluded that erythropoietin induces hemoglobin synthesis, leading to differentiation of the committed immediate precursor cell. It may act as a derepressor, permitting synthesis of messenger RNA which directs hemoglobin formation. Furthermore, either the erythropoietin itself, or information imparted by it to the cell, are intracellularly stored and the subsequent rate of the hemoglobin synthesis is determined by its availability in the individual cell.

Goldwasser[9] suggested that the process of differentiation involves three distinct stages: sensitization, induction, and specialization. Sensitization is the process by which stem cells, potentially capable of being converted to erythroid cells, become inducible. This step could involve the formation of a messenger RNA with a very short life span, concerned with the formation of a specific attachment site on the cell for interaction with erythropoietin. While this attachment site, which also has a short life span, exists, the cell could be "hit" by the hormone and afterwards erythropoietin would have no effect. The induction stage involves the actual mechanism of erythropoietin action, and it occurs once the hormone molecules are within the cell. In the specialization stage, the more evident biochemical and morphological characteristics of the erythroid series arise in the induced cells.

It could be expected that hormones analogous to erythropoietin should regulate the production of myeloid and megakaryocytic elements. Several reports suggested the presence of plasma factors which may, at least under certain circumstances, exert an effect on the rate of production of granulocytes and platelets.[37-41] Further exploration of this problem is necessary.

Bio-Assays

A battery of indices has been used for the assay of erythropoietin, such as a rise in the peripheral red blood cell number, peripheral reticulocytosis, hematocrit level, and radioactive iron incorporation. Most often the erythropoietin response is measured in animals with suppressed erythropoiesis. This has been achieved by a variety of methods; using irradiated, fasted, dehydrated, polycythemic, or hypophysectomized animals among others.[42] Originally, a unit was suggested for measuring comparatively the erythropoietic effect of various preparations[43] designated as the amount of erythro-

poietin that will produce a net increase in incorporation into red cells of 20 percent of injected Fe^{59} in a standardized starved rat assay. In 1961, a research standard (Erythropoietin Standard A) was developed which was prepared from part of a batch of sheep plasma erythropoietin.[44, 45] When the supply of this standard was almost exhausted, it was considered advisable to prepare the second standard from human plasma or urine, since many investigators were interested in a quantitative estimation of erythropoietin in samples of clinical material. Material extracted from human urine was purified, lyophilized, assayed in comparison to the first standard and established as Standard B.[45] This has been established as the International Reference Preparation of Erythropoietin, and the International Unit of Erythropoietin has been defined as the activity contained in 1.48 mg of the International Reference Preparation.

The bio-assays used most frequently are those based on the incorporation of Fe^{59} into circulating erythrocytes, in starved rats[46, 47] or polycythemic mice.[48, 49] The former is advantageous for routine purposes, because of its economy while the latter provides a highly sensitive method for the detection of erythropoietin in small amounts.[50]

Careful standardization of the bioassay is mandatory, since any variation in the procedure might affect the results. The selection of a suitable animal strain and the method of preparation of the test animal have been found to be of great importance.[45, 50a] Injection of purified erythropoietin, together with normal serum, was shown to enhance its activity.[51, 52] Possible hypotheses for the effect were that serum protein might (a) provide a protecting carrier; (b) neutralize an inhibitor; (c) activate an erythropoietin precursor; or (d) cause a change in the rate of release of hormone from site of injections, thus prolonging the action of erythropoietin. A cumulative response to multiple injections was also noted, namely, for a given total dose of erythropoietin, a greater response was produced when this amount was fractionated than when administered as a single dose.[53]

An *in vitro* system of human marrow cell culture capable of responding quantitatively to erythropoietin,[28] is being used in studies of the mechanism of erythropoietin action. The use of this system for the purpose of an assay is limited because of its sensitivity to inhibitors present in crude erythropoietin preparations.[28]

Chemical and Antigenic Characteristics

Erythropoietin can now be obtained from plasma or urine of anemic animals and of humans.[54, 54a] The chemical properties of the hormone from the two sources appear to be different,[9] but these differences may be artifactual, i.e. due to a partial denaturization of biologically inactive part of the hormone's molecule, during passage into and/or storage in urine, or due

to the fact that the hormone might be carried in plasma and urine on different carrier proteins. There is evidence that hormone activity in the plasma is present in more than one form,[55, 56] possibly as complexes with various normally occurring proteins, in both free and bound forms.

Studies of inactivation of erythropoietin[57, 58] suggested the presence of a glycoprotein containing sialic acid. The sialic acid might be required only for transport or to protect the active portion of the molecule from excretion or inactivation.[9] Rosse *et al.*[59] estimated the molecular weight of hormone extracted from the urine of a patient with aplastic anemia to be approximately 27,000. Other investigators[9, 60, 61] found molecular weights of 10,000; 40,000; and 68,000 for plasma erythropoietin. Recently, Goldwasser and Kung[54] summarized that (a) erythropoietin is a sialic acid containing protein; (b) it probably has a molecular weight in the region of 50,000 to 60,000; and (c) phenolic hydroxyls and/or free amino groups may be required for the biological activity while serine and/or theronine hydroxyls, as well as sulfhydryl groups do not seem to be required.[62]

The biologically active part of the molecule seems to be similar in various species, e.g. hormone from human or sheep affects mice. Thus, as expected, a very high degree of antigenic cross-reaction has been shown.[63] Sera obtained from rabbits immunized with human urinary erythropoietin can neutralize the biological effect of human erythropoietin,[64] as well as that of erythropoietin obtained from mice, rats, sheep and rabbits.[63, 65] This neutralization is considered to result from an antigen antibody reaction[64] and occurs most probably by interference with the hormone's activity or its entry into target cells. Similarly, the administration of an antibody to erythropoietin, prepared in the rabbit, into normal mice, resulted in progressive decrease in the relative number of erythroid cells of the bone marrow.[66] Injection of this antiserum into polycythemic mice concurrently with erythropoietin, blocked the stimulation of erythropoiesis. On the other hand, when the antiserum was injected 24 hours or more after erythropoietin administration, erythropoietic response was normal. This excluded cytotoxic effect of the antiserum on the erythroid cells.

Jepson and Lowenstein[67] demonstrated inhibition of erythropoiesis by a factor present in the plasma of patients with erythroblastopenia. Since this plasma exhibited similar behavior to that of antisera to erythropoietin, it was postulated that the inhibitory effect of plasma from these patients may be due to the formation of antibody directed against erythropoietin. Efforts to develop an immunoassay with rabbit antisera,[68] have encountered difficulties because antibodies which neutralize the biological activity are distinct from antibodies which could be used for standard immunoassay techniques, and since the quantitative relationship between the bioassay and the immunoassay is as yet unclear.

Recently, Lange and his associates[848] tried to overcome the poor antigenicity of erythropoietin and produce more effective antisera by employing a variety of immunizing methods. In contrast with several previously published reports, they were not able to obtain specific precipitation with their antisera. On the other hand, passive cutaneous anaphylaxis, complement fixation and hemagglutination could be demonstrated as well as neutralizing antibodies. Of the three above-mentioned *in vitro* techniques, hemagglutination seemed most practicable. The hemagglutinating antibody was distinctly different from the neutralizing antibody. The production of the hemagglutinating and of the neutralizing antibodies seemed independent of each other and also of the method of preparation of the antigen. The question of whether these are antibodies to two different sites on the erythropoietin molecule or to two different molecules has not yet been determined. A hemagglutination inhibition technique which could detect erythropoietin in normal human serum was developed and seems promising for routine purposes. However, before the *in vitro* techniques could replace the bioassay, a more purified standard of erythropoietin and a better understanding of the correlation between the immunological and biological activity are mandatory.

Site of Production

It is now generally accepted that the kidney serves as the main source of erythropoietin.[69-74] This was first suggested in 1957 by Jacobson and his collaborators,[69] when bilaterally nephrectomized rats and rabbits were shown to lose their capacity to respond by erythropoietin elevation to hypoxia and bleeding. Shortly afterwards, Naets[70] demonstrated that bilateral nephrectomy in dogs abolished erythropoiesis. Further evidence for a renal source of erythropoietin has been provided by the detection of erythropoietic stimulating factor in the perfusates of anoxic kidney,[71, 72] its isolation from kidney tissue,[73, 74] and by clinical observations.

Although the consensus of opinion is that the kidney serves as the main source of erythropoietin[69-74] some doubts have been raised as to whether this is the only organ capable of the hormone production.[75] Rambach *et al.*[76] found erythropoiesis stimulating substances in extracts of many tissues. In 1962, Jacobson[77] modified his original hypothesis and suggested that the kidney is the principal erythropoietin producer, but other sites may be responsible for as much as 10 percent of its overall quantity. Reissmann and Nomura[78] believe that the liver serves as an extra site of erythropoietin production while De Franciscis *et al.*[79] suggested that the spleen might constitute a site of production for the hormone. Other investigators maintain that while the kidney produces an erythropoietin-like substance, the erythropoietin itself is produced in the liver.[80] Gallagher *et al.*[81] demonstrated

erythropoietic activity in nephrectomized animals exposed to hypoxia, concluding that the kidney is an important site for erythropoietin production and/or activation but that an extrarenal site does probably exist as well, a concept supported also by Rosse and Waldmann.[82] Using pairs of parabiotic rats in which one partner had been either nephrectomized or ureter ligated, they were able to confirm the fact that the kidney is an important site of production of an erythropoietic stimulating factor but that tissue other than the kidney contributes to the erythropoietic response to hypoxia. Erslev[83] demonstrated that erythroid activity persists in nephrectomized rabbits, indicating the involvement of an extrarenal source for the stimulator.

There is no doubt that the kidney seems to play an important role in erythropoiesis in man; thus, chronic kidney disease is often followed by severe anemia most probably due to lack of erythropoietin.[84, 85] On the other hand, a number of pathological processes such as hypernephroma, hydronephrosis, polycystic kidneys, and renal artery ischemia may often lead to erythrocytosis.[86-88] Furthermore, the same process can be associated in some patients with anemia and in others with polycythemia.

A good model for the investigation of the role of the kidney in human erythropoiesis is provided by patients undergoing kidney transplantation. Nathan *et al.*[89] demonstrated that red cell production can be maintained in anephric patients and that they are able to respond to a hypoxic stimulus. They concluded that man is not totally dependent on a renal source of erythropoietin for red cell production. McMain *et al.*[90] observed a regression of the anemia of chronic renal disease in five patients following transplantation. Increased erythropoietic stimulating activity was noted in one patient, several weeks post-transplantation, when he suffered a severe hemorrhage. They concluded that the normal kidney tissue of the transplant can produce enough erythropoietin to maintain normal erythropoiesis and to respond to the stimulus of a sudden hemorrhage. Denny *et al.*[91] demonstrated significant elevations in erythropoietin activity, post-transplantation in three patients with bilateral nephrectomy.

The exact cellular site of erythropoietin production in the kidney is unknown. Thus far, renal cortex,[92, 93] glomerular tuft,[94] medulla,[78] tubules[95, 95a] or the juxtaglomerular cells[96, 97] have been implicated as possible sites. At present the juxtaglomerular apparatus is the one most frequently designated, primarily due to the relationship between the rate of erythropoietin production and granularity activity of the juxtaglomerular cells,[98, 99] although this is by no means universally accepted.[95a, 100-102]

Mitus and his collaborators[103-106] established that unilateral hydronephrosis, induced in rabbits by ureteral ligation, led to erythrocytosis which subsided once the renal parenchyma became atrophied due to the pressure. Changes in the granularity of juxtaglomerular cells which were associated

with changes in erythropoietic activity were construed as supporting evidence for the relationship of this organ with erythropoietin secretion. In conclusion, it was postulated that decreased vascularity occurring in the hydronephrotic kidney leads to ischemia and hypoxia of renal tissue, which initiate the stimulation of erythropoietin secretion. The fact that kidney anoxia may cause secretion of erythropoietin has been amply demonstrated.[72, 78, 107, 108]

Contrera, Gordon and their associates[75, 109, 110] and Kuratowska,[73] presented evidence that two erythropoietically active factors can be extracted from the rat's kidney. One of these resembles plasma erythropoietin (ESF), both biochemically and biologically, whereas the other lacks erythropoietic activity unless incubated with normal rat serum (REF=Renal Erythropoietic Factor). Originally,[109] two alternate hypotheses were suggested: (a) that REF is a precursor of erythropoietin which must be complexed with a carrier to become physiologically active, or (b) that it is an enzyme which produces erythropoietin by its action on a particular serum protein. Further studies[75, 110] were thought to indicate that REF is an enzyme acting on an erythropoietin precursor, produced in the liver and present in normal serum. This hypothesis is still under debate[111] and needs further study.

Other Regulating Mechanisms

Possible Neurogenic Control

The fact that denervated transplanted kidneys[91] have been shown to induce erythropoietin production immediately following transplantation has been considered to rule out a direct nervous control of renal erythropoietin.[112] On the other hand, there is scattered evidence regarding a neurogenic factor in the causation of polycythemia. Several investigators were able to demonstrate an increase in red cell counts by injecting epinephrine.[113-116] Also, increased red cell count was produced by experimental brain injury[117] or stimulation[118] and by an electric shock therapy,[119] while a reduction of red cell mass and blood volume was noted following sympathectomy.[120]

Seip, Halvorsen and their associates[121] noted an increase in red cell mass and marked reticulocytosis in eight out of twenty-eight rabbits, following hypothalamic stimulation by needle electrodes; in some of the animals an increase in plasma erythropoietin was demonstrated.[122] More recently, Halvorsen,[123, 124] Mirand *et al.*,[125] and Feldman *et al.*,[126] reported observations indicating that the hypothalamus may play a role in the regulation of erythropoiesis. Stimulation of the hypothalamus resulted in an increase of erythropoietin production,[125] while hypothalamic ablation caused a decrease in the normal erythropoietic response to hypoxia. However, the question

whether the hypothalamic influence is mediated via release of pituitary hormones, autonomic nervous impulses, or specific nervous or humoral signals to the kidney has not been answered. Erslev[112] believes that it is premature to conclude that the hypothalamus actually controls red cell production. A similar opinion was expressed by Gilbert and Silverstein,[127] who reported transient erythrocytosis in a twenty-seven-year-old man with occlusion of the middle cerebral artery. They maintain that despite the abundance of reports indicating an association between brain disease and erythrocytosis, the concept of neurogenic polycythemia requires further elaboration.

Hormonal Regulation

The role and mechanism of hormonal control of erythropoiesis are still unsettled, despite numerous studies of such a relationship.[74, 127a-d]

Crafts[128] demonstrated that a moderate anemia follows either thyroidectomy, adrenalectomy, or hypophysectomy. In subsequent experiments[129, 130] thyroxin and cortisone injections were shown to be capable of preventing or eliminating the anemia caused by hypophysectomy, and it was assumed that the latter was secondary to combined hypothyroidism and hypoadrenocorticism. However, further analysis[131] revealed that the type of anemia that followed hypophysectomy was not identical to the one following combined thyroidectomy and adrenalectomy.

Conflicting reports concerning the action of corticosteroids on erythropoiesis appear in the literature. Halvorsen and Lindenman[131a] found that ACTH increased the red cell mass in normal rabbits, and Osnes[97] demonstrated that its administration to hypophysectomized rats produced reticulocytosis. Fisher and Crook[132, 133] noted that the administration of ACTH, corticosterone, dehydrocorticosterone and hydrocortisone to hypophysectomised rats stimulated the incorporation of radioiron and lead to an increase in the rats' blood cell volume. These findings are in line with clinical observations in patients treated with corticosteroids.

On the other hand, several reports have pointed out a suppressive effect of corticosteroids on erythropoiesis in normal mice[133a] and rats.[133b] Mirand and Gordon[133a] found that physiological doses in mice had no effect on erythropoiesis. Low pharmacological doses resulted in a decrease in Fe^{59} incorporation into red blood cells, possibly due to an inhibitory effect on the response of the bone marrow to erythropoietin. High pharmacological doses blocked the response to hypoxia, possibly due to suppression of erythropoietin production. Similar results were noted in rats by Glader *et al.*,[133b] who also demonstrated that when hydrocortisone was administered together with erythropoietin, the result was similar to the one obtained when erythropoietin was administered by itself.