DISTURBANCES IN HEME SYNTHESIS

Special Considerations of the Sideroachrestic Anemias and Erythropoietic Porphyrias

By

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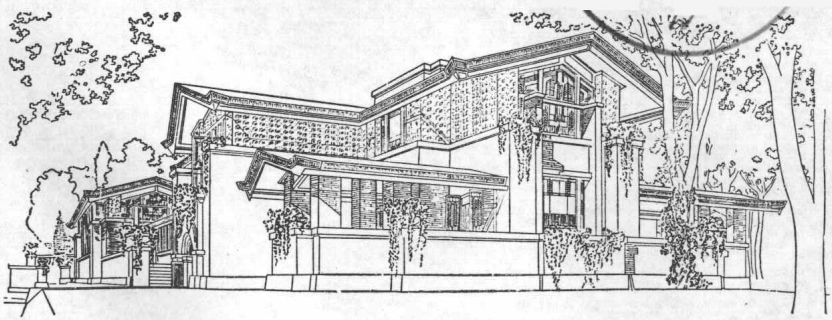

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Preface

THIS MONOGRAPH REPRESENTS an endeavour to establish proof of the presence of a defect in heme synthesis in various anemias and in other disease states. To this end we investigated heme precursor concentrations in bone marrow, peripheral blood and in urine. In addition we studied that part of heme synthesis in peripheral erythrocytes for which the latter contain a complete enzyme complement. This was achieved by incubation experiments on peripheral erythrocytes involving the addition of delta ALA. As is to be expected, the information gained from these experiments is limited as the methods utilized do not permit an evaluation of the total synthetic capacity of the bone marrow. The experimental methods relative to this problem (e.g., incubation with radioactive glycine and delta ALA as well as with Fe^{59}) are still beset with numerous difficulties in their clinical application and as yet cannot be considered routine investigations. Furthermore up to the present time very few investigations in this field have been published. Despite the limitations imposed by our methodology we were able to make a number of interesting observations in the erythropoietic porphyrias and in certain anemias. Although the total heme synthesizing ability could not be determined, the results obtained nevertheless represent a further contribution not only to clinical symptomatology but also are of significance in diagnostic and therapeutic problems.

We beg the reader to direct his attention to the experimental results; the interpretation and conclusions drawn should be considered as speculative, definite answers must wait until further investigative work has been performed.

Should this publication stimulate further investigation and interest in this field, it will have served its purpose well.

Finally I wish to express my deepest gratitude to the editor, Dr. W. H. Seegers for his valuable suggestions and assistance, to Dr. M. Steiner for his excellent translation, and to Charles C Thomas, Publisher for the excellent format of this publication.

LUDWIG HEILMEYER, M.D.

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Chapter I

Introduction

HEME IS THE pigment portion of hemoglobin. On a weight basis, it constitutes about 4 per cent of the total hemoglobin molecule. However, this small portion is of essential importance for the function of hemoglobin. Heme is essential for oxygen uptake and release. Disturbance in the synthesis of heme leads also to disturbed synthesis of hemoglobin. Disturbed heme synthesis, as far as is known, does not lead to an altered molecular structure of heme, but expresses itself only as a quantitative change. It leads to diminished formation of heme and in consequence thereof, to diminished formation of hemoglobin, leading therefore ultimately to diminished hemoglobin concentration in the peripheral blood. Such a state is called ANEMIA. Although anemias have been known for centuries, a disorder in heme synthesis as one of their pathogenetic causes has never been brought into the focus of our consideration. Occasional investigative work is to be found, however. The reason for this lies in the fact that the fundamentals of the biochemistry of heme synthesis have been elucidated only recently, indeed during the last decade. Biochemistry has given us the basis for the clinical investigation of disturbed heme synthesis. The following discussion rests on this basis and derives in major part from investigations that we have conducted over the past five years in cooperation with Dr. Roman Clotten, the Director of my Clinical Laboratory.

Dr. Clotten has improved methods some of which are published here for the first time.

Important references in the literature dealing with these

projects have been used for our discussions and conclusions. However, I have to beg indulgence if some discussions appear incomplete and only a premise to the solution is offered.

Chapter II

The Clinical Picture of Disturbed Heme Synthesis

THE CLINICAL PICTURE of disturbed heme synthesis depends upon whether it exists isolated, or at least predominant, or manifests itself in conjunction with other defects.

A deficient amount of hemoglobin is found in the erythrocyte if this disturbance is isolated or exists in a stronger degree than other disorders of cell maturation and cell growth. This leads to hypochromic anemia. In this case, the hemoglobin content of a single erythrocyte is decreased and is below 30 $\mu\mu$gm. The mean hemoglobin content of a single erythrocyte, the volume and the thickness are also decreased whereas the red cell diameter is variable.

Placing these values in a normogram, according to the method of my co-worker, von Boroviczeny,[23] one obtains a characteristic curve in disordered heme synthesis as is shown in Figure 1.

The number of red cells may be completely normal in spite of a considerably disturbed heme synthesis. One finds even polycythemia with hypochromia caused by a relative iron deficiency. In the majority of cases, however, the cell formation is decreased. The reason for this is not known. Perhaps it lies in a delay of cell formation caused by diminished hemoglobin production.

As the cell is normally released only with a maximal, that is a normal, hemoglobin content, in defective heme or hemoglobin synthesis it will remain for a longer than normal period in the bone marrow. The maturation is delayed. Because of this delayed release from bone marrow the cell can still form a

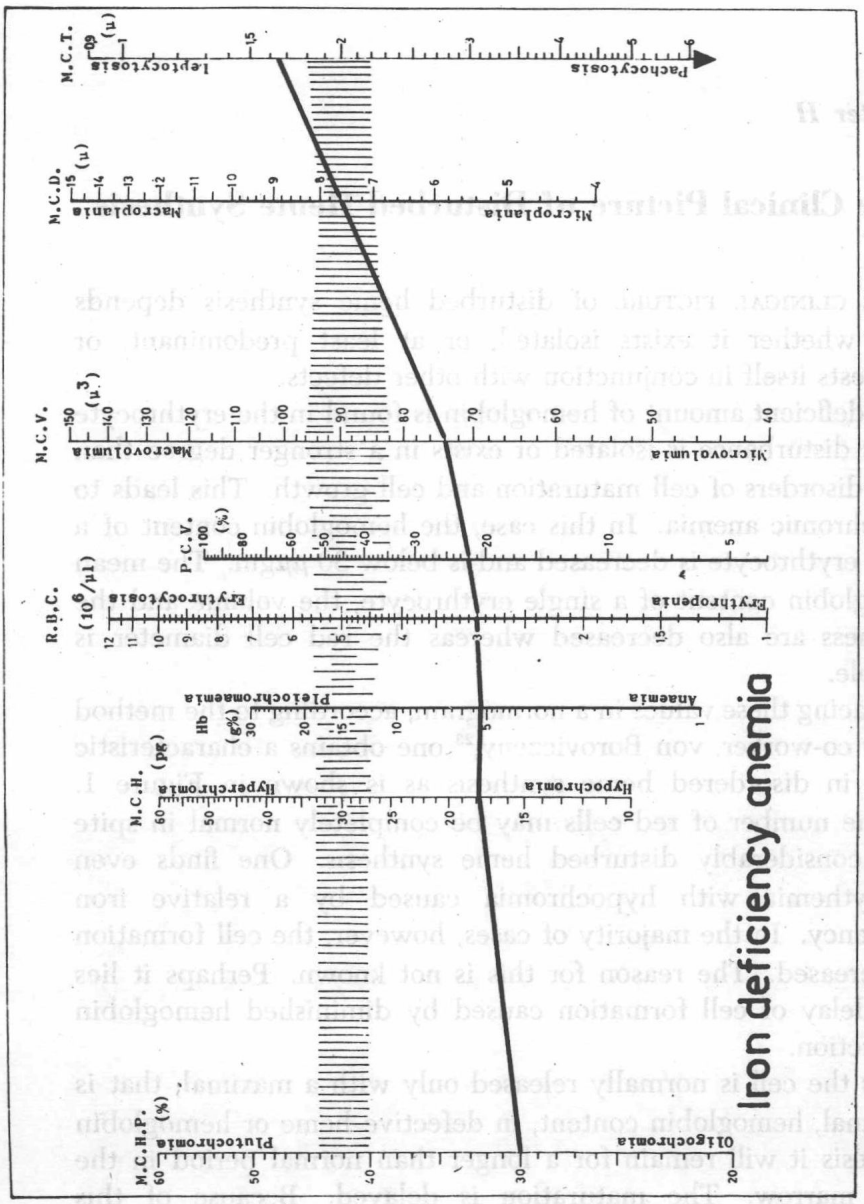

Figure 1. Erythrocytometric values in severely disturbed heme synthesis (iron deficiency anemia) according to von Boroviczeny.[23] M.C.H.P. = mean corpuscular hemoglobin percentage. M.C.H. = mean corpuscular hemoglobin. R.B.C. = red blood cells. P.C.V. = packed cell volume. M.C.V. = mean corpuscular volume. M.C.D. = mean corpuscular diameter. M.C.T. = mean corpuscular thickness.

relatively normal amount of hemoglobin in spite of the deceleration in hemoglobin synthesis.

The prolonged retention in the marrow therefore constitutes a type of compensatory mechanism which is, in the main, insufficient. However, one can find in occasional cases iron deficiency without hypochromia of the red blood cells. In this instance the compensatory mechanism has been fully successful. The prolonged retention of the cells in the bone marrow leads to an increase of early erythropoietic elements in the marrow, i.e., *erythroid hyperplasia.* This, therefore, is a direct consequence of disturbed heme synthesis. I observed cases of severely disordered heme synthesis in which the hyperplasia in the bone marrow was so extensive as to give the picture of Di Guglielmo erythremia. After correction of the heme synthesis disturbance, the hyperplasia disappeared completely and the myeloid: erythroid ratio reverted back to normal. Hypochromia and erythroid hyperplasia are the most important consequences of disturbed heme synthesis. These, of course, are also diagnostically important.

Partial Hypochromia

Diseases exist in which disturbed heme synthesis does not involve all erythroblasts. In these cases, one observes in the peripheral blood, besides normochromic also hyperchromic cells and even severely hypochromic ones. There exist then, various population of cells. Dacie[42] was the first to focus attention on such cases (see page 121).

Nonisolated Disturbance of Heme Synthesis

Disordered heme synthesis is not always associated with hypochromia. It may exist in conjunction with other maturation disturbances of the cell. In these cases, it decreases in importance in the overall hematologic picture and hypochromia is lacking. The anemia in these cases is normochromic or may even be hyperchromic. The important point is how severe the delay in heme synthesis is, as compared to the other disturbances of

maturation of the cell. If the other cell maturation disturbances are more severely delayed than heme synthesis, hyperchromic anemia results, as is the case in pernicious anemia. In the majority of cases of aplastic anemias (panmyelopathies) one finds a disordered heme synthesis which is accompanied by normochromia of the cells.

Chapter III

The Principal Causes of Disturbed Heme Synthesis

HEME CONSISTS OF iron and protoporphyrin 9. Disordered heme synthesis follows if either insufficient iron is supplied or insufficient protoporphyrin 9 is available. A further possibility consists in the inability of iron and protoporphyrin 9 to combine. We know today that this union is catalyzed by a specific enzyme, namely heme synthetase. Any abnormality, either quantitative or qualitative, of this enzyme must lead to a disturbance in heme synthesis. However, other possibilities exist. It could be that, although present, iron is not available in the proper form for heme synthesis. The possibility also exists that structures or mechanisms within the cell responsible for the transfer of iron to protoporphyrin 9 are altered. Bessis and coworkers,[12] investigating sideroachrestic anemias by electron microscopy found that the mitochondrial apparatus was changed in each case. These are the cell elements in which the last step of heme synthesis takes place. Finally the question arises whether free heme is formed at all, or whether the union of iron and protoporphyrin always takes place together with and simultaneous with that of the globin molecule, so that only a complete hemoglobin molecule can be formed. There is much to support the latter supposition because, up to the present, no proof has been found that heme exists in a free form in the cell. If, however, the union of iron and protoporphyrin takes place only in the presence of globin, then disturbance in heme synthesis has to follow if the formation of globin is insufficient per time

unit. Iron and protoporphyrin could then appear non-utilized in greater quantities than normal.

Actually, we have such findings in thalassemia in which, with good reason, a disturbance of globin synthesis can be assumed. I will return to this later.

The prerequisites of normal hemoglobin synthesis are the supply of equivalent amounts of iron, protoporphyrin and globin. In normal circumstances, the supply of iron and protoporphyrin takes place in such a way that a certain excess of both is present in erythroblasts and normoblasts. Erythroblasts and normoblasts always contain free protoporphyrin and a higher percentage of free iron as Prussion blue stains and electron microscopic investigations show (Bilger *et al.*,[14, 15] Bessis and co-workers[11]). There is still a small, fairly constant, amount of free protoporphyrin in the mature red cells (see page 61). Reticulocytes as well still have ferritin (Bessis[11]). Any quantitative deviation of free iron or protoporphyrin in the cell must suggest a disturbance in heme synthesis as we intend to demonstrate below. But first we have to delineate the normal biosynthesis of heme.