B-VITAMINS

For Blood Formation

By

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PREFACE

BIOCHEMICAL progress in the past six years has led to a surge of interest in the megaloblastic anemias and new problems have been posed which are the subject of many discussions in the scientific literature. It is now evident that the processes involved in the production of blood cells have certain chemical relationships to general proliferative changes involving the formation of nuclear material.

In 1945, a new B-complex vitamin, pteroylglutamic acid, was synthesized. This substance was found to be needed for the prevention of deficiency states which had been described as occurring in a variety of animals and micro-organisms. Pteroylglutamic acid was shown to be needed for the cure of certain anemias of dietary origin in human subjects. Its abundant availability in the synthetic form led to a new era in the treatment of sprue and the megaloblastic anemias of infancy, pregnancy and pellagra. Pteroylglutamic acid is a stable, yellow, crystalline substance which is well utilized when given either by mouth or by injection in doses of a few milligrams daily. It is widely distributed in foods of both animal and vegetable origin such as liver, yeast, green leaves and soybeans.

The essential role of pteroylglutamic acid in the formation of blood cells has its counterpart in the activity of various synthetic "antagonists" of this vitamin in depressing cytopoiesis. These new substances were also found to produce an accentuated form of the other manifestations of pteroylglutamic-acid deficiency. The antagonists have been

extensively studied in attempts to manage leukemia and other neoplastic diseases.

Pernicious anemia was first treated successfully with whole liver in 1926, but today there is no certainty as to which of the now-known hemopoietic factors in liver was responsible for the original remissions. Shortly afterwards injectable liver extracts were prepared for the treatment of this disease and it became evident that these extracts had very little effectiveness when given by mouth in contrast to their potency when injected. An apparently unique biological mechanism then came to light; normal gastric secretions were shown to contain an "intrinsic factor" which is needed for the uptake of a dietary "extrinsic factor" from the digestive tract. The extrinsic factor has been identified with a group of substances, the "cobalamins," which are present in many foods of animal origin and are formed by numerous micro-organisms. The mechanism by which the intrinsic factor facilitates the passage of the cobalamins through the intestinal wall is still unknown. The human being who is afflicted with pernicious anemia, a disease peculiar to his species, is deprived of the intrinsic factor by a degenerative process which arrests the formation of normal gastric juice, thus he most literally starves in the midst of plenty. Indeed, injectable and therapeutically active solutions of cobalamins may be prepared from the stools of such patients, whose excreta may actually contain more potency than those of normal subjects.

The isolation of crystalline vitamin B_{12} was described in 1948. This substance is also termed "cyano-cobalamin" and is representative of the cobalamins, which consist of a large and intricate cobalt-containing molecule coordinated with various anions such as cyanide and hydroxyl. The cobalamins are effective in producing remissions in pernicious anemia when injected in quantities as low as 1 to 2

micrograms daily. The use of the crystalline substances in nutritional experiments confirmed earlier evidence that the anti-pernicious-anemia factor of concentrated liver extracts was identical with a vitamin needed by animals. This vitamin was known to be present in such foods of animal origin as fish, lean meat, milk and liver. These discoveries were overshadowed in practical importance by the finding in 1948 that the cobalamins were formed by many bacteria and especially by the *Streptomyces* group of microorganisms. Industrial fermentations were able to provide large quantities of cobalamins for use in animal feeding and in pharmaceutical products.

Studies with lactic acid bacteria led to the recognition of a hitherto unknown "citrovorum factor" which was shown to be a modified form of pteroylglutamic acid with certain biological activities exceeding those of the latter substance. Chemical experiments resulted in the discovery of the structural characteristics of the "citrovorum factor" which evidently is widely distributed in biological materials and which appears to be concerned in the biochemical transfer of "single-carbon" fragments. The factor has been synthesized and has anti-anemic properties resembling those of pteroylglutamic acid.

This short monograph is devoted to a field of study which continues to expand so rapidly that reviews of the subject are soon out-of-date. I am indebted to Miss Josephine Block for her help in the preparation of the manuscript. Grateful acknowledgment is also made to Dr. Byron E. Hall, Dr. Robert P. Parker and to Mr. C. Maresh for furnishing the material which is used in the illustrations.

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CONTENTS

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CHAPTER I

INTRODUCTION —THE MEGALOBLASTIC ANEMIAS

THE PRESENCE of anti-anemic substances in mammalian liver has long been recognized but the purification and isolation of the active principles eluded laboratory investigators for many years. The use of microbiological assay procedures was of great importance in the isolation of these substances, which belong to the group of B-complex vitamins, naturally-occurring biochemical catalysts or coenzymes. During the past few years rapid developments have taken place in knowledge of the chemistry of certain substances which are concerned with the formation of blood cells.

Pteroylglutamic acid was synthesized in 1945 and this compound was widely investigated with respect to its effects in producing a response in anemias which are accompanied by megaloblastic arrest in the bone marrow. Two years later chemical analogs of pteroylglutamic acid were synthesized which are biologically antagonistic to their prototype so that they produce an acute deficiency disease, often accompanied by anemia and leukopenia, in experimental animals.

Vitamin B_{12}, another substance effective against certain megaloblastic anemias, was isolated in 1948. Chemically vitamin B_{12} and pteroylglutamic acid are quite different

but they are closely interrelated in certain biochemical processes. Both substances are involved in the formation of desoxyribonucleic acid as shown by their relation to the biological synthesis of purine and pyrimidine bases and desoxyribosides.

Various schemes have been proposed for classification of the anemias based either on etiology or on cell morphology. The complexity of the situation may be gauged by reference to textbooks of hematology [1] which summarize the years of effort and study which have been devoted to an understanding of the causes and pathology of disorders of the blood and blood-forming organs. The experimental use of drugs continues to throw light on the biochemical nature of the causes of blood dyscrasias. It is the purpose of this monograph to review the chemistry and physiology of some of the newer therapeutic agents in this field. Consideration is given to (a) Pteroylglutamic acid and its chemical relatives; (b) The vitamin B_{12} group; and (c) The "intrinsic factor."

THE MEGALOBLASTIC ANEMIAS

Interest among nutritionists has centered in a group of anemias characterized by megaloblastic erythropoiesis, a similar disturbance in the myeloid series with pathologic macro-myeloid cells and a reduction in number and abnormality in type of thrombocytes.[2] This group comprises the megaloblastic anemias and includes pernicious anemia, sprue and certain other anemias, one of which has been termed "tropical macrocytic anemia." [2, 3] Wills used this name to describe a megaloblastic anemia occurring during pregnancy and not responding to treatment with purified liver extracts known to be effective in pernicious anemia. Tropical macrocytic anemia was alleviated by

the administration of an extract of autolyzed yeast, "Marmite." The existence of two dietary factors which were effective against the megaloblastic anemias was demonstrated by these findings. The first of these, present in concentrated liver extract, was eventually shown to be vitamin B_{12}. The second factor, present in liver and yeast, was found to be folic acid.

Pernicious anemia is accompanied by a failure in the function of gastric mucosa so that the tissue no longer secretes hydrochloric acid even when stimulated by the injection of histamine. The gastric juice in pernicious anemia is further characterized by lacking a specific protein-like substance, the "intrinsic factor," which is present in normal gastric juice and which is evidently needed for the normal uptake of vitamin B_{12} from the digestive tract. A deficiency of vitamin B_{12} is consequently developed by the tissues in this disease and is marked by a macrocytic anemia, specific changes in the bone marrow, and usually by subacute combined degeneration of the spinal cord. Among the physical signs are "lemon yellow" pallor; a clean tongue, either very red with a smooth, swollen shiny tip and lateral portions, or pale, shrunken, smooth and shiny all over; impaired sense of position and vibration and a positive Romberg sign, and retinal pallor with flame-shaped retinal hemorrhages. Important laboratory findings are macrocytic anemia with a mean corpuscular volume greater than 97 cubic microns and color index usually greater than 1; gastric achlorhydria with no free acid in four samples of gastric juice aspirated at intervals of 15 minutes after injection of 0.5 mg. of histamine; increase of nucleated red cells, including more than 2 per cent megaloblasts, in films prepared from fluid aspirated from sternal bone marrow; leucopenia with relative lymphocytosis, hypersegmentation of nuclei of neutrophils and moderate decrease in platelets and increase in

concentration of serum bilirubin which gives an indirect Van den Bergh reaction.[4] The signs and symptoms are relieved by oral administration of a mixture of vitamin B_{12} with the "intrinsic factor." Relief of the cord symptoms appears to be dependent upon the degeneration of the nervous tissue not having progressed to an irreversible state.

Before the isolation of pteroylglutamic acid, which in turn preceded the isolation of vitamin B_{12}, it was evident that folic acid deficiency in animals was not alleviated by concentrated liver extracts, indeed the manufacturing processes employed in the production of such extracts led to the discarding of the folic acid content of crude liver extracts in "side fractions." The absence of significant quantities of folic acid activity from concentrated liver extracts indicated that it was not to be anticipated that pteroylglutamic acid would be effective in pernicious anemia. However, this substance produced a typical hemopoietic response in this disease whether administered parenterally or orally and in the latter case regardless of the absence of the "intrinsic factor." The central nervous symptoms did not respond to pteroylglutamic acid. Pernicious anemia may develop even though "normal" diets are consumed, so these findings led to speculation and experimentation upon the nutritional availability in pernicious anemia of the conjugated forms of pteroylglutamic acid which are present in foods, but definite conclusions cannot be said to have been reached. An interplay between pteroylglutamic acid and vitamin B_{12} has been observed in certain metabolic processes, and it may well be that the riddle of the interchangeable hemopoietic roles of pteroylglutamic acid and vitamin B_{12} in pernicious anemia will be solved only through studies of biochemical reactions.

The therapeutic effect of pteroylglutamic acid upon

the megaloblastic anemias of infancy, pregnancy and pellagra appears to be clear-cut. These anemias are typically associated with a dietary deficiency of folic acid which in the cases of pregnancy and infancy may be aggravated by the high requirement for vitamins during rapid growth. Achylia gastrica is not present so that the uptake of vitamin B_{12} from the digestive tract is not specifically impaired in these anemias as appears to be the case in pernicious anemia. The existence of megaloblastic anemias which do not respond to concentrated liver extracts has drawn the attention of various investigators and the names "refractory megaloblastic anemia" [5, 6] and "achrestic anemia" [6, 7] have been applied to conditions of this type. Davidson [5] has found that "refractory megaloblastic anemia" responds well to folic acid.

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CHAPTER II

B VITAMINS IN THE MEGALOBLASTIC ANEMIAS OF PREGNANCY AND INFANCY

PTEROYLGLUTAMIC ACID AND VITAMIN B_{12} IN THE MEGALOBLASTIC ANEMIA OF PREGNANCY

THE MEGALOBLASTIC anemia of pregnancy is characterized by the presence of macrocytic red blood cells, by megaloblastic changes in the bone marrow,[1] by the presence of hydrochloric acid in the gastric juice and by the absence of neurological signs and symptoms. The disease may occur at any age during the childbearing period in both primigravidas and multigravidas. Most patients have a defective diet. Symptoms usually arise during the third trimester or in the puerperium but occasionally they date from early pregnancy or miscarriage. Excessive vomiting or diarrhea is noted in about half of the cases. The tongue may be sore and slight edema is frequent. A "pearly white" appearance is common and a yellowish pallor is rare. The blood picture is variable; leucopenia is common, and the marrow usually shows a mixed megaloblastic and normoblastic reaction.[1]

The anemia tends to disappear spontaneously after the termination of pregnancy. The early observations by Wills which served to differentiate the curative agent from the anti-pernicious-anemia factor of concentrated liver extract

were reviewed on page 4. Similar findings prior to the availability of pteroylglutamic acid were reported by various clinical groups [2-19] who noted the curative effects of various crude fractions, especially those prepared from liver and yeast.

In 1945 Moore and co-workers [20] described the use of pteroylglutamic acid in the treatment of a patient with the megaloblastic anemia of pregnancy. She had a red blood cell count of 1.1 to 1.2 million per cu. mm. 18 days after parturition. A daily intramuscular dose of 20 mg. was given for 10 days, and a peak value of 48 per cent reticulocytes was reached on the seventh day. The red cell count rose rapidly and the clinical response seems to have been complete. The successful treatment of three patients with 20 to 50 mg. of pteroylglutamic acid daily was described by Spies.[21] The patients showed reticulocyte peaks of 23 to 29 per cent. Three cases which had failed to respond to the injection of concentrated liver extracts were treated with pteroylglutamic acid by Davidson and co-workers and all responded promptly.[22] Analogous results were described in India by Benjamin-Allan.[23]

Shortly after the isolation of vitamin B_{12}, it was reported by Ungley that a case of the megaloblastic anemia of pregnancy failed to respond to 65 micrograms of vitamin B_{12} but the patient subsequently responded to 2.5 mg. of pteroylglutamic acid daily.[24] A failure with vitamin B_{12} was described by Bethell and co-workers,[25] who noted that anemia and leucopenia became more severe and mucous membrane lesions progressed during treatment with vitamin B_{12} but later disappeared when pteroylglutamic acid, 10 mg. daily, was given. A striking case was reported by Day *et al.*[26] The patient had loss of hair, swollen feet, legs and hands and a waxy, pearly-white color of the skin. The stools were watery and numerous, while "the mucous