The Megaloblastic Anemias

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The Megaloblastic Anemias

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Preface

APPROXIMATELY EIGHTY YEARS AGO, Ehrlich [1] described a pathologic type of erythrocyte maturation in pernicious anemia. This abnormal developmental line of erythrocytes was designated as the "megaloblastic," or large germ cell, series to differentiate it from the "normoblastic," or normal sized germ cell, series. Anemia associated with this abnormal morphologic picture has become known as "megaloblastic anemia."

In the eight decades since Ehrlich's first description, much has been learned about the nature and significance of megaloblastic blood cell formation.[2] The overwhelming majority of megaloblastic anemias have been found to be due to a deficiency of either vitamin B_{12} or folic acid, or both vitamins.[3, 4] When a megaloblastic morphologic picture has been observed in conditions other than frank deficiency of these vitamins, the condition has been characterized by inhibition or exhaustion of the bone marrow, with possible local deprivation of vitamin B_{12}, folic acid, or building blocks for nucleoprotein synthesis in which vitamin B_{12} and folic acid serve as co-factors. The accumulated information has made possible a unified concept of the megaloblastic anemias as a single morphologic entity due to defective nucleoprotein synthesis of various causes. This monograph will be concerned with the development of megaloblastic anemia in morphologic and biochemical terms; the causes of megaloblastic anemia as it occurs clinically; application of this knowledge to accurate differential diagnosis of these anemias; and a simple and rational approach to therapy based on the actual clinical problem, as determined by proper differential diagnosis.

This monograph is based in large measure on recent massive and productive research in the field of the megaloblastic anemias, carried out and reported from all parts of the globe. This is supplemented by the author's own research experiences and observations of more than 200 cases of megaloblastic anemia seen

at the Albert Einstein College of Medicine–Bronx Municipal Hospital Center, the Montefiore Hospital, and the Mount Sinai Hospital, all in New York.

The author is indebted to his mentors at each of these three eminent institutions: Dr. Irving M. London, Dr. Theodore H. Spaet, and Dr. Louis R. Wasserman. The freedom of inquiry and wise counsel they gave did much to make this monograph possible. The author is also indebted to Dr. William B. Castle, whose continued interest in the investigative work pursued by the author was a great sustaining force.

Gratitude is also due the New York Heart Association and Heart Fund, Inc., and its Medical Director (now retired), Dr. D. F. Milam, for personal interest in and support of this work from its inception, through four years of Research and Senior Research Fellowships given to the author. Finally, and by no means least, United States Public Health Service Research Grants to Drs. London, Spaet, Wasserman, and the author, as well as a grant from the National Vitamin Foundation and various other grants, provided the equipment and supplies for much of the research related to the megaloblastic anemias with which the author was connected.

The author would like to thank the staff of Grune and Stratton for their patience and editorial help.

VICTOR HERBERT, M.D.

Contents

1 *Incidence*

MEGALOBLASTIC ANEMIA is common all over the world, although the percentage of such anemias due to any specific cause varies widely from one geographic area to another and from one set of circumstances to another in any single area. As examples may be cited the prevalence of nutritional megaloblastic anemia in India,[5–8] Macedonia,[9] and Malaya [10]; the frequency of fish tapeworm megaloblastic anemia in Finland, especially widespread during World War II when particularly harsh conditions prevailed among the Finnish soldiers [11]; and the proneness of persons from the Scandinavian countries, England, Ireland, and Canada to develop classic addisonian pernicious anemia.[12]

The most common causes of megaloblastic anemia in the New York metropolitan area, where the author acquired most of his experience with the disorder, are addisonian pernicious anemia and malabsorption syndrome. The former is most common in the majority of patients; the latter predominates among the inhabitants of the city born in Puerto Rico. In all of the three New York hospitals with which the author has had major affiliation, approximately 10 per cent of the entire hematology department case load consisted of patients with megaloblastic anemia.* It is of interest in this connection that approximately the same percentage of the text of Wintrobe's *Clinical Hematology* [13] is devoted to the subject of the megaloblastic anemias, attesting to the importance of the subject to physicians in general and hematologists in particular.

The hematologic caseload of patients with megaloblastic anemia of the classic addisonian pernicious anemia variety shows every indication of a gradual future increase. This is a result of the fact that addisonian pernicious anemia is primarily a

* Of course, if the many cases of simple chronic anemia (as defined by Wintrobe [13]) and iron deficiency anemia which are never referred to hematology departments were so referred, the percentage of hematology department patients with megaloblastic anemia might be less than 10 per cent of the total caseload.

disease of late adult life,[14] and the average life expectancy of the population is increasing. Addisonian pernicious anemia is very rare in children,[15] rare in adults under 30 years, and seldom seen prior to the age of 40.[14]

It is presently believed that the decrease in serum vitamin B_{12} levels with aging is of dubious significance.[16] However, the increase in gastric atrophy with aging is not in doubt. Gastric atrophy may be a factor in the decrease in serum vitamin B_{12} levels and in the eventual development in some cases of megaloblastic anemia due to vitamin B_{12} deficiency.[17, 18] (See section, "Hereditarily Determined Degenerative Gastric Atrophy," page 21.)

2 *Hematologic Morphology*

IN A WELL DEVELOPED CASE of megaloblastic anemia the picture presented by both the peripheral blood and the bone marrow is usually so characteristic as to be impossible to confuse with the morphologic picture of any other disorder. In the peripheral blood are found many large and frequently oval erythrocytes (macrocytes and macroovalocytes, the latter often referred to as "megalocytes" by European clinicians and morphologists). The nuclei of the neutrophilic polymorphonuclear leukocytes are hypersegmented,[19] [21] as often are the nuclei of the eosinophils; oval neutrophils may be seen [22]; and in addition to frequently being obviously reduced in number, many of the platelets may be giant and bizarre in appearance.[23]

This picture is accompanied by a decrease in the total number of erythrocytes and neutrophilic polymorphonuclear leukocytes, and an increase in the mean corpuscular volume (MCV) of the erythrocytes due to the increased size of the average red blood cell. FIGURE 1 shows the typical appearance of the peripheral blood of a patient with megaloblastic anemia. With respect to the red blood cell morphology, it should be noted that, aside from the characteristic macrocytosis and macroovalocytosis, marked anisocytosis and poikilocytosis are frequently not present when the anemia is not severe.

The reticulocytes in the peripheral blood usually represent about 1 per cent of the total number of circulating erythrocytes. This is probably a consequence of the fact that the asynchronism in development of nucleus and cytoplasm of the erythrocyte precursors in the bone marrow results in frequent disappearance of cytoplasmic reticulum even before the nucleus is extruded. Thus more than half of the erythrocytes delivered to the peripheral blood from the bone marrow may be without reticulum. In megaloblastic anemia, therefore, the reticulocyte count provides an erroneously low figure of the rate of red cell production.[24]

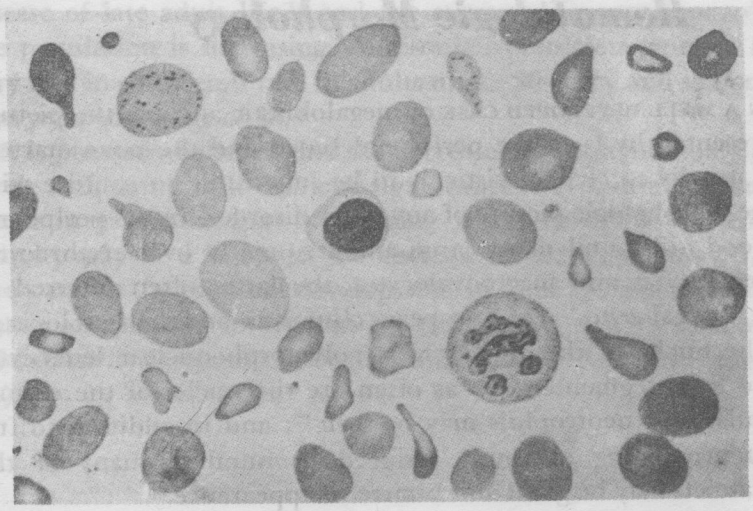

FIG. 1.—The peripheral blood in megaloblastic anemia. (From DALAND, G. A., HAM, T. H., AND PIOTTI, E.: A Color Atlas of Morphologic Hematology. Cambridge, Harvard University Press, 1951).

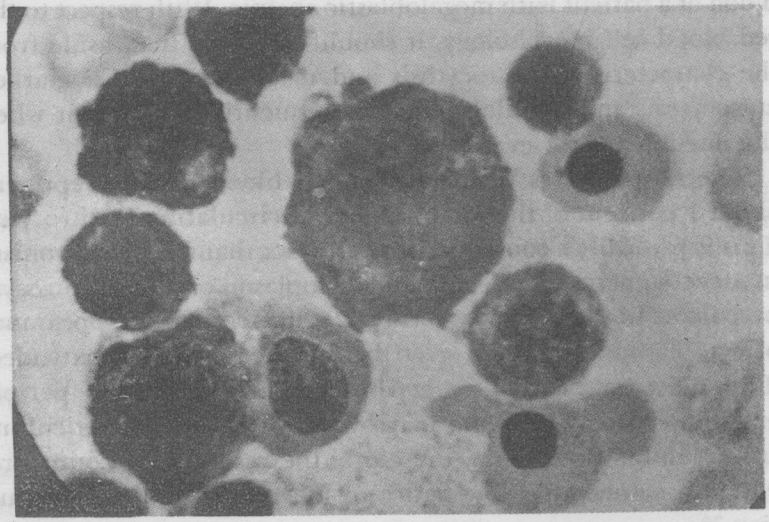

Fig. 2.—Megaloblastic development of the erythrocytes

The bone marrow displays megaloblastic development of the erythrocytes, as shown in FIGURE 2. By comparison with normal (normoblastic) erythrocyte maturation, the megaloblastic series presents a larger size at every stage during development, with a larger amount of cytoplasm in proportion to the size of the nucleus. The nucleus itself is very large compared to the nucleus of a normoblast, and there is a striking disparity between the apparent "maturity" of the nucleus and that of the cytoplasm.[25] This disparity, or nuclear-cytoplasmic dissociation, or asynchronism, as it is variously termed,[25–27] is reflected in the much more particulate nuclear chromatin ("young nucleus," or nucleus with retarded maturation)[25, 28] of the megaloblasts at all stages of their development, even when hemoglobin is clearly visible in the cytoplasm ("old cytoplasm").[2, 29] The megaloblasts with their finely particulate "young" chromatin are easily distinguished from normoblasts with their coarsely clumped "old" nuclear chromatin, especially in those cells where hemoglobinization of the cytoplasm is visibly beginning.

Another striking feature of the bone marrow morphology in megaloblastic anemia is a megaloblastic polymorphonuclear leukopoiesis, manifested most dramatically by the presence of many giant metamyelocytes, as depicted in FIGURE 3.

There is usually a marked increase in the number of mitotic and binucleate cells in the erythroid series.

The degree of overt megaloblastosis of the bone marrow appears generally to be proportional to the severity of the anemia. Study of patients after total gastrectomy[30] demonstrated the evolutionary sequence in the development of megaloblastic anemia to be macrocytosis first, anemia second (after an average delay of one or two years following the appearance of macrocytosis), and megaloblastic marrow third and last.

Note that the first hematologic evidence of vitamin B_{12} deficiency is macrocytosis (and an Arneth count "shift to the right") with the bone marrow presumably remaining normoblastic until two or three years later. Of course, the macrocytes must have come from abnormal precursors, and in every case of

early vitamin B_{12} deficiency disease which the author has seen, careful search of the bone marrow smear has revealed the presence of intermediate megaloblasts and large metamyelocytes. It is probable that these marrow findings are always present when the peripheral blood contains macroovalocytes and neutrophilic

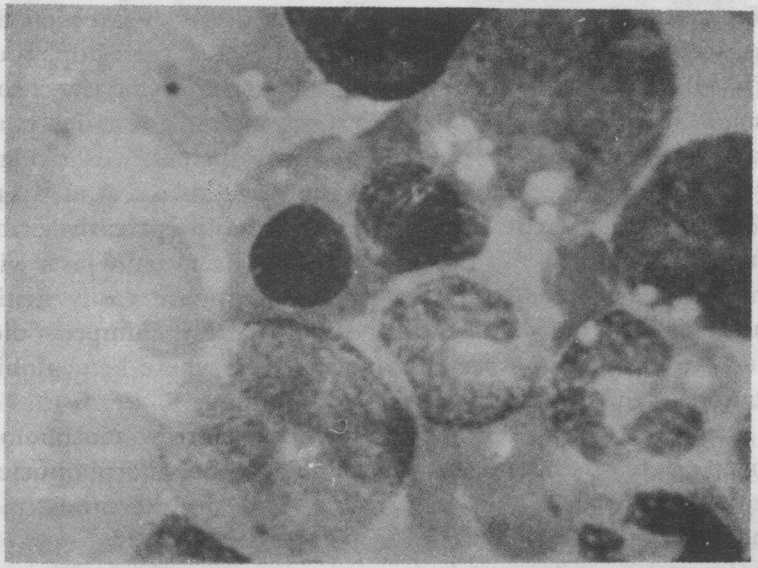

FIG. 3.—Giant metamyelocyte in a megaloblastic bone marrow. (Note also the "pinching" of the nuclei of the smaller and more mature metamyelocytes into multiple segments, resulting in the typical hypersegmented polymorphonuclear leukocytes associated with megaloblastic anemia.)

polymorphonuclear leukocytes whose nuclei show an increased amount of segmentation, even though most workers do not comment in print about abnormalities in the bone marrow until it is frankly megaloblastic. More will be said about the intermediate megaloblast shortly. For the present, suffice it to say that emphasis on the macroovalocyte and the hypersegmented polymorphonuclear leukocyte as the first indicators of a developing megaloblastic anemia is quite proper, since they are usually easily recognized and are found in the peripheral blood. Recognition of intermediate megaloblasts and large metamyelocytes,

however, requires aspiration of the bone marrow and a fair amount of experience in looking at bone marrow morphology. It is wise for the person who does not examine blood and bone marrow preparations daily to compare both the peripheral blood and the bone marrow of the patient with that of a normal control subject. If he does not do this, he will miss many cases of early megaloblastic anemia, since he will not recognize the early hematologic changes.[13]

Darby et al.[31] believe that macrocytosis is the most sensitive indicator of vitamin B_{12} deficiency. Their long term observations of patients with pernicious anemia receiving smaller than minimal maintenance dosages of vitamin B_{12} indicated that the quantity of the vitamin needed for hematopoiesis exceeded that needed for other clinically recognizable physiologic functions. In the author's experience, an Arneth count "shift to the right" invariably accompanies the macrocytosis, and may even precede it.

A single case was reported suggesting that vitamin B_{12} deficiency may be manifested by an increase in the number of giant nuclei in the oral epithelial cells even before macrocytic erythrocytes make their appearance.[32] The patient had overt neurologic damage, a serum vitamin B_{12} level of 60 $\mu\mu$g./ml. (L. leishmanii assay), a serum iron concentration of 140 μg./100 ml., and definitely was not receiving folic acid therapy. It was stated that there was "no hypersegmentation of granulocytes," but an Arneth count was not reported. A photograph of the patient's peripheral blood smear was presented, and interpreted by the authors as normal except for slight anisocytosis, but it appeared suggestive of macrocytosis and at least one of the erythrocytes looked like a macroovalocyte (megalocyte). The hematocrit and red cell count were not obtained, so the MCV could not be calculated.

In the absence of folic acid administration, the author has never seen a patient having a serum vitamin B_{12} level below 100 $\mu\mu$g./ml. without incipient or overt iron deficiency who did not have at least macroovalocytosis and an Arneth count shift

to the right in the peripheral blood, and at least intermediate megaloblasts and large metamyelocytes in the bone marrow.

Dr. Boen generously made the patient's original peripheral blood and bone marrow smears available for examination. The peripheral blood showed only one or two macroovalocytes per oil immersion field, but an Arneth count revealed a definite shift to the right, with 14 per cent of the neutrophilic polymorphonuclear leukocytes having more than 5 lobes. The bone marrow contained a large number of large metamyelocytes and an occasional intermediate megaloblast, but no megaloblasts. A generalized pallor of the erythrocytes suggested a possible concomitant iron deficiency despite the normal serum iron level. (Such a deficiency would explain the lack of a striking macroovalocytosis in the peripheral blood.)

Thus, this patient did have hematologic changes as well as epithelial cell changes, and the case illustrated once again the importance of the Arneth count in helping to ascertain the existence of vitamin B_{12} and/or folic acid deficiency. The question as to whether macrocytic changes due to deficiency of vitamin B_{12} may appear first in the oral epithelial cells or the hematic cells remains open. Theoretically, one would expect macrocytic changes to appear first in those cells which normally proliferate most rapidly. Concomitant iron deficiency might mask the macrocytic changes in the erythrocytes without masking the changes in the oral epithelial cells, whose iron requirement is much lower.

Recognition of the earliest hematologic signs of incipient megaloblastic anemia is particularly important because severe nervous system damage may occur in vitamin B_{12}-deficient patients prior to development of a megaloblastic marrow, and even before anemia appears.[33] The earlier such cases are recognized and proper treatment instituted, the less irreversible damage will be done. (See CHAPTER 6, "Clinical Picture," page 66, for cases in point.)

The process of development of megaloblastic anemia may take years, probably because the body is able to draw on its

liver stores of vitamin B_{12} [34–38] and folinic acid [34, 39] when the exogenous supply is cut off. A simple screening procedure generally applicable to all patients on a routine basis and making it possible to recognize incipient megaloblastic anemia would be of great value. Such a procedure has long been at hand, and has been applied with marked success at the Montefiore Hospital, where in the course of a single year it increased the number of newly discovered cases of vitamin B_{12} deficiency disease from one every six weeks to one every six days.[40] In fully one-third of these cases, no anemia was present and the diagnosis was not even remotely suspected prior to the routine screening; the patients had been admitted for evaluation of unrelated conditions. The positive result of the screening procedure led to confirmation of the diagnosis of early vitamin B_{12} deficiency by determination of the serum vitamin B_{12} level and Schilling type urinary excretion testing.

This screening procedure consists simply in obtaining a routine peripheral blood smear on every new patient and carefully examining that smear for macroovalocytes and hypersegmented polymorphonuclear leukocytes. All of the technicians in the routine clinical hematology laboratory are instructed that the normal average number of lobes of the nuclei of neutrophilic polymorphonuclear leukocytes is 3; that about 40 to 50 per cent normally have 3 lobes, 20 to 40 per cent have 2 lobes, and 15 to 25 per cent have 4 lobes.[41] The finding of more than 3 5-lobed polymorphonuclears per hundred, or even a single polymorphonuclear with more than 5 lobes, or a substantial increase in the percentage with 4 lobes, signals a careful work-up for incipient megaloblastic anemia, even when absolutely no anemia is demonstrable. An increase in the number of polymorphonuclear leukocytes with 4 or more lobes, or Arneth count shift to the right,[20, 21] has preceded the onset of anemia in every case of incipient megaloblastic anemia this author has observed. Others [13] also have observed and reported on this phenomenon.

Frequently in incipient megaloblastic anemia many of the hypersegmented polymorphonuclears look at first glance like

normal young polymorphonuclears with horseshoe- or sausage-shaped nuclei. More careful inspection reveals that what appears to be a single segment is in fact a rosette of overlapping segments, or rather a sausage which is being pinched into a number of small round sausages. These abnormal young polymorphonuclears, when in the bone marrow, are the immediate precursors of the typical hypersegmented polymorphonuclear leukocytes usually seen in the peripheral blood.[42] (See FIGURE 3.)

When macroovalocytes and hypersegmented polymorphonuclear leukocytes are found in the peripheral blood, examination of the bone marrow obtained by aspiration biopsy should be the next step. Even if a megaloblastic marrow is not found in such circumstances, however, work-up of the patient for an incipient megaloblastic anemia will be fruitful in the large majority of cases. It cannot be emphasized too strongly that in cases of incipient megaloblastic anemia, even highly trained hematologists may not find clearly megaloblastic primitive cells in the bone marrow. This happens despite the presence in the peripheral blood of polymorphonuclear leukocytes with hypersegmented nuclei and occasional macroovalocytes, which testify to the fact that some megaloblastic hematopoiesis must be taking place, and despite subsequent confirmation of this diagnosis by determination of the serum vitamin B_{12} level [17] (which may often be in the "low normal" range), and the Schilling test. (See CHAPTER 6, "Clinical Picture," page 10, for cases in point.)

A recent report [43] calls attention to the possible usefulness of discriminating from megaloblasts "lines of red cell development morphologically intermediate between unequivocal megaloblastic erythropoiesis and normoblastic erythropoiesis" (i.e., "intermediate megaloblasts").[26, 44, 45] The authors of that report believe that this modified type of megaloblastic erythropoiesis represents the morphologic result of the combination of a condition that would usually produce a megaloblastic marrow and an associated iron deficiency at the marrow level.

Actually, as eminent authorities [46, 47, 384] have noted, "intermediate megaloblasts," or "macroblasts," [17] may be simply eryth-