The Nature and Significance of Megaloblastic Blood Formation

By Edward H. Reisner, Jr.

Following his introduction of the use of stains to study dried smears of blood, Ehrlich described a type of red blood cell precursor peculiar to pernicious anemia which he called a “megaloblast.” Megaloblasts are seen in the marrow of patients with pernicious anemia and related anemias due to deficiency of vitamin B₁₂ and folic acid in relapse; and following the administration of specific therapy they rapidly disappear. Blood cells of closely similar appearance are seen in the early stages of intrauterine life and in a variety of clinical conditions. There has been considerable debate as to whether the latter cells are truly megaloblasts and whether megaloblasts represent a stage in the normal line of red blood cell development or constitute a separate line of abnormal erythropoiesis.

Earlier discussions of the megaloblast-normoblast problem are based almost entirely on the interpretation of stained smears. It is the purpose of this paper to reexamine the subject with a view to correlating the observable morphologic differences between megaloblastic and normoblastic marrows with our current knowledge of the chemistry and physiology of developing red blood cells, and evolving from such an approach a hypothesis which will fit the known clinical characteristics of the diseases in question.

Ehrlich¹ applied the term megaloblast to the entire developmental line of erythrocyte precursors seen in pernicious anemia. He regarded the earliest embryonal erythroblasts as being similar in nature, and apparently considered pernicious anemia as being in the nature of a reversion to an embryonal type of blood formation. He felt that at all stages of maturation megaloblasts and normoblasts constituted separate lines of development. Ehrlich’s original observations were widely accepted in Europe and expanded by Naegli,² Pappenheim,³ Ferrata,⁴ and Ellerman⁵ among others.

Maximow,⁶ studying hematopoiesis in the mammalian embryo, applied the term megaloblast to the earliest cells appearing in the embryonic circulation, and considered them to mature into normoblasts. Later⁷ he replaced

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the term megaloblast with "primitive erythroblast" (erythroblast being a term that had been used until then in the generic sense). Danchakoff also used the word megaloblast to apply to the earliest blastodermal erythropoietic elements of the chick embryo, an example which was followed by Sabin in this country. Unfortunately, Doan, Sabin, and Cunningham expanded the term to include all the most primitive blood cells of both normal and pathological blood states in man. According to their theory the megaloblast was the earliest definite erythroid element, developing into the erythroblast, which became hemoglobinated to form a normoblast. The megaloblast was not seen in normal adult marrows, but would appear under conditions of strain placed on the blood-forming organ. Later, when the erythrocyte maturation factor in liver was discovered, it was hypothesized that the megaloblast matured into the erythroblast under the influence of E.M.F. Megaloblastosis was regarded as a simple maturation arrest. Despite the objection that this concept took no account of the observable morphologic differences between the more mature, hemoglobinated megaloblasts and normoblasts, it gained wide acceptance in this country, due, in part, to the simplicity of its dynamics, as well as to the prestige of its proponents.

With the increasing study of marrow brought about by the introduction of marrow aspiration technics, the fallacies in the Doan-Sabin concept were repeatedly pointed out by many investigators, including Jones, Dameshek, and Limarzi in this country, and Rohr, Storti, and Fieschi abroad. It is now accepted by most hematologists that megaloblasts are a separate developmental line of abnormal red cell precursors, arising under pathologic conditions, of which pernicious anemia is the classical example.

I. Characteristics of Megaloblasts

(1) Morphology

Many authors have described these cells, and the reader who wishes a detailed discussion of the subject is referred to the presentation in Wintrobe's text or the articles by Jones, Dameshek and Valentine or Downey. Megaloblasts are chiefly distinguished from normoblasts by the fact that the nuclear chromatin at all stages of their development is more particulate.* The earliest forms with basophilic cytoplasm (with or without nucleoli) are, for the most part, similar in size to cells of comparable maturity in the normoblast series, although "giant" forms may often be encountered. As the megaloblasts mature and the nucleus becomes smaller and more pyknotic, the cells tend to remain larger and become somewhat oval until they develop into the oval macrocytes characteristic of pernicious anemia. The nomenclature followed by the

*Many different descriptive terms have been employed to describe this property of megaloblasts. The nuclear chromatin has been referred to as comma-like, with accentuated, delicate "scrollwork," particulate, reticular, or with increase in the parachromatin or the interchromatin spaces. Feulgen stains of the nuclei illustrated in reference 59, show that the nuclear chromatin in these cells is present in much finer particles, which fact the author believes is largely accountable for their appearance when stained by Romanowsky stains.
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authors referred to above is based on purely morphologic considerations. The promegagloblast and the basophilic megaloblast are distinguished mainly by the presence of nucleoli in the former group. This separation is based on the assumption that the forms without nucleoli are more mature than those with them. (This is only true in broad relative terms, since the nucleolus disappears as the cell enters mitosis, and reappears with the reconstitution of the newly divided cells in which it plays an important role in the elaboration of cytoplasmic nucleoprotein required for the further development of the cell.) The maturing forms have been designated "hypochromatic" or "polychromatic" in the stage of decreasing basophilia, and "orthochromatic" in the final nucleated stage where hemoglobinization is complete.

During the latter phase of red blood cell development there is a decrease in the size and nucleic acid content of the nucleus and a decrease in the nucleic acid content of the cytoplasm. It seems unlikely that normal mitosis could occur after the nucleus has begun to involute, since under such circumstances nuclei with less than the normal complement of chromatin would result from the ordinary diploid division. It is, therefore, logical to think of the life span of the developing cell in two parts: the phase in which the cell is capable of dividing into two cells with a normal amount of chromatin, for which I have coined the term "mitotable" (i.e., capable of mitosis) and the postmitotic or maturation phase in which this property has been lost. One occasionally sees binucleated hemoglobinated forms that appear to be dividing, but these probably represent abnormal exceptions to the usual pattern of maturation that have failed to complete division at an earlier stage.

The normal ratio of mitotable to maturing forms is roughly 1:10 to 1:5. In contrast, a distinguishing feature of megaloblastic marrows is the relative predominance of younger forms. In the normal marrow only a small proportion of the erythrocyte precursors are in the deeply basophilic stage, with or without nucleolus. These earlier forms represent mitotable phases of the cells and, following mitosis, the process of cell maturation with accompanying pyknosis of the nucleus, loss of cytoplasmic basophilia, and formation of hemoglobin produces the picture seen in normal marrows. In megaloblastic marrows, on the other hand, the earlier (mitotable) forms comprise a portion of the total red cell population which is inversely proportional to the height of the peripheral red blood cell count. In severe cases it is not unusual to see marrows in which 60 per cent or more of all nucleated cells are megaloblasts, of which over half are mitotable forms.

Mitotic figures are more easily demonstrable in megaloblastic than in normoblastic marrows. However, the proportion of cells actually dividing to cells at a level of immaturity capable of doing so is low. The picture is characteristic of a state of blockade in the premitotic resting phase (interphase) of the cells. Following treatment, this premitotic blockade is relieved, and the proportion of mitotable forms is restored to normal figures in a few days.

Abnormal mitotic figures with scattered chromosomes, chromosomes left over after completion of mitosis, and abnormal numbers of chromosomes may occur. These aberrations may be interpreted as individual instances of cells
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that have circumvented the blockade rather than as evidence of a general increase in mitotic activity.

Other morphologic characteristics of megaloblastic marrows affecting the white blood cells and platelet precursors have been described, but they are usually overshadowed by the tremendous change favoring the red blood cell precursors in the myeloid:erythroid ratio, which is often reversed. The giant metamyelocyte is, however, quite characteristic of such marrows and some authorities regard it as being of great diagnostic significance, particularly in marrows from patients with mild pernicious anemia where the number of early megaloblasts is few.

Following specific therapy the changes in marrow morphology consist of a decrease in the mitotable cells referred to above, an increase of mitotic figures according to most observers, and an increase in maturing forms. The nuclear chromatin pattern becomes coarser and the formation of hemoglobin more rapid, and in a surprisingly short time the normal proportion of mitotable to maturing red cells is restored. There is a simultaneous increase in myelopoietic activity, which leads to a leukocytosis in the peripheral blood at the time of the reticulocyte increase, and a restoration of more normal myeloid:erythroid ratios.

(2) Occurrence

Although the overwhelming majority of instances of megaloblastosis occur in patients with deficiency of folic acid or vitamin B₁₂, the phenomenon may occur under other circumstances. For categorical purposes one may distinguish between megaloblastic marrows exhibiting the features described in the preceding section of this paper; megaloblastoid marrows in which the cells approach the megaloblastic appearance but may be regarded as intermediate between normoblastic and megaloblastic (atypical megaloblasts [Jones] or pseudomegaloblasts); and marrows in which varying numbers of typical megaloblasts may be seen although most of the red cell precursors present are normoblasts.

Megaloblastic marrows are the result of deficiency of vitamin B₁₂ (i.e. pernicious anemia, sprue, fish tapeworm anemia, postgastrectomy) or folic acid (megaloblastic anemia of infancy, pregnancy, nutritional macrocytic anemias). Megaloblastic anemia responding to folic acid has been reported to occur as a result of ingestion of diphenylhydantoin (Dilantin).¹⁷

Megaloblastoid marrows occur most commonly in the developing fetus and in severe liver disease. In the embryo the early red blood cells show the same type of particulate chromatin associated with megaloblasts. Wintrobe¹⁹ has pointed out that the blood picture of the fetus in the last trimester of pregnancy simulates the picture of pernicious anemia under therapy in that

*The sprue syndrome may be caused by deficiency of folic acid, and some observers regard the condition as primarily due to this cause. Our Co"B₁₂ absorption studies, however, have shown that many of these patients have a complete failure to absorb vitamin B₁₂ even in the presence of intrinsic factor."
TABLE 1.—Summary of the Principle Differences Between Megaloblastic and Normoblastic Hematopoiesis.

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<th>Normoblastic</th>
<th>Megaloblastic</th>
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<tr>
<td>Nuclear Chromatin Particles</td>
<td>more homogenous</td>
<td>diffuse, particulate and meshlike</td>
</tr>
<tr>
<td>Ratio of mitotable to maturing cells:</td>
<td>Usually 1 : 10 to 1 : 5</td>
<td>Much increased, and even inverted in severe anemias.</td>
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<tr>
<td>Cells develop into</td>
<td>Normal erythrocytes</td>
<td>Oval macrocytes</td>
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<tr>
<td>Cytoplasmic RNA</td>
<td>almost gone by time hemoglobin appears</td>
<td>persists well into phase of hemoglobinization.</td>
</tr>
<tr>
<td>Nuclear DNA</td>
<td>Quantitatively the same in resting nuclei of both series but in smaller particles in megaloblast.</td>
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a low, macrocytic count is replaced by a normal, normocytic count. The macrocytes of the six-month fetus are oval and similar in appearance to those of pernicious anemia. The similarity in appearance of megaloblasts and the earliest embryonal blood elements was readily perceived by the early investigators from Ehrlich onward, and was, in fact, the basis for the Doan-Sabin hypothesis of erythropoiesis to which reference has already been made. Jones has made meticulous comparisons of developing red cells from the yolk sacs of animal species and human megaloblasts, and concludes that there are some differences in histologic details. He regards these cells as not being true megaloblasts and calls them “macronormoblasts.” In general, however, erythropoiesis, as it first appears in the embryo, is of a type closely similar to megaloblastic, which is replaced by a normoblastic type as gestation approaches completion.

The other condition in which the marrow may simulate pernicious anemia is severe chronic liver disease. In general, the more severe liver damage and the more profound the anemia the more it tends to become macrocytic. While in most cases the marrow is midway between normoblastic and megaloblastic in appearance, cases can be seen which are indistinguishable from pernicious anemia, even to and including the characteristic giant metamyelocytes (fig. 2F). Such cases usually do not respond to treatment with folic acid or vitamin B12.

Occasional megaloblasts occur in varying numbers (sometimes to a degree approaching a real megaloblastic marrow) in a variety of disorders associated with anemia. Perhaps the most frequently encountered is acute leukemia, usually of the aleukemic variety with a refractory macrocytic anemia as the presenting sign of the disorder. In some of these cases the myeloblasts have an appearance very similar to megaloblasts (fig. 1A), but in most cases they are easily distinguishable from the latter (fig. 1B, C, D). Conley has

*Although the belief that the megaloblast is derived from the myeloblast may be disputed, this type of marrow certainly suggests the possibility under these conditions. It is this author’s opinion, however, that the same factors producing megaloblastosis of the red cell precursors could account for a similar morphologic alteration in the primitive white cells.
Fig. 1.—Megaloblasts in cases of leukemia.

A. Acute leukemia in which the myeloblasts resembled megaloblasts very closely. True megaloblasts were also present designated "m."

B. C. and D. Three cases of acute aleukemic leukemia, preceded by refractory macrocytic anemia.

E. Subacute myelogenous leukemia with associated macrocytic anemia.

F. Megaloblastic anemia following sulfonamide administration and responding to folic acid but not to vitamin B₁₂. Before remission was complete patient developed a fulminating myeloblastic leukemia. When this was treated with aminopterin the marrow once more became megaloblastic.

G. Subacute myelogenous leukemia.

H. Myelofibrosis with splenomegaly and megaloblastic anemia, the latter responding to massive doses of vitamin B₁₂. (Kindness of Dr. C. L. Conley.)

reported an interesting case of myelofibrosis with megaloblastic marrow which is illustrated in figure 1H. In figure 2A is shown a marrow from a patient with plasma cell myeloma with numerous megaloblasts present. A similar picture in the marrow of a patient with metastatic breast carcinoma has been observed by the writer, and its occurrence in other types of cancer has been reported. Many of the red cell precursors shown in cases of the so-called erythremic myelosis of De Guglielmo have a typical megaloblastic appearance (fig. 2E).

Megaloblasts and frank megaloblastic marrows may be encountered in severe anemias, particularly in those due to hemolysis. In addition, aplastic states of the marrow such as agranulocytosis, myelofibrosis, tuberculosis, leishmaniasis, sepsis, hemochromatosis, and those following radiation
A. Multiple myeloma. For five years this patient had a refractory, macrocytic anemia. The marrow contained both megaloblasts and myeloma cells that closely resembled megaloblasts. (Megaloblasts in the picture designated by “m.”)

B. Hemolytic anemia.

C. Aplastic anemia, idiopathic.

D. Chronic blood loss with superimposed acute hemorrhage.

E. Erythremic myelosis (De Guglielmo).

F. Advanced cirrhosis of the Liver.

G. Acute leukemia treated with 6-Mercaptopurine. No megaloblasts were present in the marrow prior to treatment.

H. Chronic blood loss with superimposed acute hemorrhage.

I. Chronic, refractory hemolytic anemia of unknown cause.

<table>
<thead>
<tr>
<th>Method</th>
<th>CONTROL</th>
<th>FOLINIC AC.</th>
<th>B-12</th>
<th>B-12 + I.F.</th>
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<tbody>
<tr>
<td>SUSPENSION</td>
<td>0</td>
<td>++++</td>
<td>++</td>
<td>±</td>
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<tr>
<td>ON GLASS</td>
<td>0</td>
<td>++++</td>
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<tr>
<td>ON CLOTS</td>
<td>±</td>
<td>++++</td>
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Fig. 3.—Conversion of megaloblastic to normoblastic hematopoiesis in marrow cultured in P.A. serum alone, and with added metabolites.*
injury\textsuperscript{12,43} have all been reported to show typical megaloblasts. Figures 1 and 2 contain some examples taken from the author's slide collection. Of particular interest is figure 1E showing megaloblasts in the marrow of a 26-year old man who developed a macrocytic anemia after a month of therapy with sulfapyridine. The marrow was completely megaloblastic. The anemia, like that described in rats following sulfanilamide administration,\textsuperscript{44} did not respond to vitamin B\textsubscript{12}, but folic acid caused a good reticulocyte response, conversion of the marrow to normoblastic blood formation, and a rise in the peripheral red blood cell level. After a month, however, the patient developed an acute myeloblastic leukemia which caused his death six weeks later. Treatment with aminopterin caused the reappearance of megaloblasts in the marrow. Others have reported megaloblastosis following therapy with folic acid antagonists\textsuperscript{45,46} and it also occurs (though less often) in the marrow of patients treated with 6-mercaptopurine.\textsuperscript{47}

It will be noted that when megaloblasts occur in conditions other than primary deficiency of vitamin B\textsubscript{12} or folic acid, one or both of two conditions are present: there is either a severe strain on the blood-forming organ (hemolysis, hemorrhage, leukemia) or some influence inhibiting cell division (antimetabolites, x-ray, agranulocytosis), or both. Hematologists have long known that restoration of the blood level to normal in a patient with pernicious anemia will cause the disappearance of the early megaloblasts in the marrow\textsuperscript{48} or a marked decrease in their numbers.\textsuperscript{49} This is unassociated with reticulocytosis or other evidence of reestablishment of normal marrow function, and the later forms of megaloblasts persist. The amounts of vitamin B\textsubscript{12} contained in transfusions are not more than 0.25 \( \mu \text{g.} / 500 \text{ ml. of blood} \) (assuming the transfused blood has a serum vitamin B\textsubscript{12} level of 500 \( \mu \text{g.} / \text{ml.} \)), an amount shown to be ineffective when given parenterally daily to a patient with pernicious anemia in relapse.\textsuperscript{50} This minute amount of vitamin B\textsubscript{12} may, however, be sufficient to permit mitosis of some of the mitotable megaloblasts, and the depressive effect of transfusion on erythropoiesis demonstrated by Smith\textsuperscript{51} may delay the reappearance of the mitotable forms, thus accounting for the rapid disappearance of the early megaloblasts under such circumstances. The most obvious effect of transfusion, however, is to alleviate the demand on the marrow for new blood formation. The degree of megaloblastosis of the marrow in pernicious anemia is inversely proportional to the red blood cell level, and not directly correlated with the level of vitamin B\textsubscript{12}, since severe combined system disease may exist without any anemia or evidence of marrow dysfunction. The relationship of demand for blood formation to megaloblastosis was strikingly demonstrated by one of our pernicious anemia patients who developed a fulminating hemolytic anemia associated with a kidney infection. When the patient was admitted to the hospital he had been maintained in remission as an out-patient for several years with bimonthly injections of 50 \( \mu \text{g.} \) of vitamin B\textsubscript{12}, and his blood contained 4,000,000 erythrocytes/mm\textsuperscript{3} and 12 Gm. of hemoglobin/100 ml. Because he was so sick it was anticipated that he might benefit by additional protection and he was given 50 \( \mu \text{g.} \) of vitamin B\textsubscript{12} every day. One week after admission his blood level had fallen to 1,500,000 erythrocytes/mm\textsuperscript{3} and 4.5
Gm. of hemoglobin/100 ml., and his marrow was completely megaloblastic despite the large amounts of vitamin B12 he had been receiving.

Mollin’s group have indicated a correlation between serum levels of vitamin B12 and the appearance of megaloblasts in the marrow of partially treated patients with pernicious anemia who are allowed to relapse. However, the amount of this substance required is so small that very low blood levels of vitamin B12 may exist in patients in complete hematologic remission. It would appear from such cases and those referred to in the preceding paragraph that the available concentration of vitamin B12 relative to the demands for blood formation is of greater importance than the actual quantity present in the blood, and that anything that reduces this demand, such as transfusion, will permit normoblastic hematopoiesis to proceed with the supplies available. In animal experimentation in which extreme degrees of deficiency of vitamin B12 or folic acid are developed, megaloblastosis may appear before the animal becomes anemic, a circumstance which is understandable when one considers that this occurs after only a few weeks of the deficient diet while the animal still has the bulk of its circulating erythrocytes that were formed before the development of the depleted state.

The megaloblastosis of leukemic marrows may be attributed to the increased demands for co-enzymes used in nucleoprotein synthesis of the leukemic cells; perhaps it is due to folic acid deficiency since it is established that patients with leukemia have high levels of vitamin B12. The megaloblastoid marrows of liver disease and intrauterine life might be the result of defective synthesis of less well-defined constituents of nucleoproteins due to the damaged liver or inadequate development of fetal enzyme systems such as those involved in the formation of folic acid derivatives essential for hematopoiesis. In summary, occurrence of megaloblastic blood formation appears to require that a strain be placed on the blood-forming organ leading to at least a relative deficiency of agents vital for the synthesis of substances essential for erythropoietic cell division.

(3) Chemistry and Physiology

Probably because of their phosphoric acid radicals, nucleic acids stain basophilically with aniline dyes. With Romanowsky technics the cytoplasm of the early megaloblast stains deep blue, while the chromatin of the nucleus is usually somewhat more purplish. By the use of nuclease enzymes it has been shown that the blue staining component of the cytoplasm is ribonucleic acid (RNA) and the stained nucleus is deoxyribonucleoprotein (DNA). The latter fact is confirmed because the Feulgen reaction stains only the nucleus. The nucleolus contains RNA and no DNA.

Caspersson introduced the technic of microspectrophotometry for the quantitative measurement of chemical constituents of individual cells. With this technic it is possible to determine the concentration of any substance with a characteristic absorption spectrum by measuring the amount of light of the appropriate wave length which passes through a measured microscopic field of the cell in question. In unstained cells, nucleoproteins of cytoplasm or nucleus, as well as hemoglobin, can be measured by the use of ultraviolet
light. In stained smears, light of appropriate wave lengths can also be used to measure substances stained by special technics such as the Feulgen stain for DNA, or methyl green for depolymerized DNA.

Using ultraviolet light, Thorell\textsuperscript{57,58} has shown that during normal red cell maturation the concentration of cytoplasmic RNA rapidly diminishes between the basophilic and hypochromatic stage and has almost disappeared from the cell at the time that hemoglobin begins to appear. In contrast to this, in pernicious anemia the RNA disappears much more slowly and persists in significant concentrations even in the late stage when hemoglobinization is well advanced. Reisner and Korson\textsuperscript{59} studied nuclei of red cells in pernicious anemia marrows before and after treatment and normal marrows stained by the Feulgen technic. They found no difference in the nuclear content of deoxyribose (presumably DNA) in megaloblasts and normoblasts at all stages of maturation, although the morphologic differences between the two types of cells described earlier in this paper were readily apparent in the Feulgen stained smears, in which the chromatin of the megaloblasts was present in smaller particles. In both series, as the diameter of the nucleus decreased there was a gradual disappearance of DNA.

Chemical methods based on the disintegration of cell-containing tissue such as marrow and its digestion by nuclease enzymes have also been used to determine the amounts of nucleoproteins present in megaloblastic marrows. These methods unfortunately yield the nucleoproteins contained in both erythroid and myeloid precursors. Such studies indicate an increase in the total amount of DNA present in pernicious anemia marrow in relapse, but a much greater increase in the amounts of RNA.\textsuperscript{60,61} This result is probably indicative of the large number of early cells present in such marrows.

Methyl green is a dye which is believed to be specific for depolymerized DNA\textsuperscript{62} and the amount of this material present can be determined by the microspectrophotometric technic. By this method it has been shown\textsuperscript{60} that there is no difference in the amount of depolymerized DNA present in the nuclei of megaloblasts before and after treatment of pernicious anemia and normoblasts from normal marrow.

DNA differs from RNA in containing deoxyribose instead of ribose and the pyrimidine base, thymine, instead of uracil. Mueller et al.\textsuperscript{63} have shown that the ratio of uracil to thymine in marrows of pernicious anemia in relapse is increased, and that it decreases following specific therapy.

In addition to the measurements made directly on individual megaloblasts, a great deal of information is available about the chemistry and physiology of the marrow as a whole and the megalocytes of the peripheral blood in pernicious anemia. Studies of the oxygen consumption of megaloblastic marrow are conflicting.\textsuperscript{64} one group reporting values below and another values in excess of those obtained in normoblastic marrow. There is considerable evidence, however, that the megaloblastic marrow, far from being in a state of arrest, is the scene of more than normal metabolic activity; this would lead one to expect an increased consumption of oxygen. The studies of Watson\textsuperscript{65} and Dobriner and Rhoads\textsuperscript{66} indicated that patients with pernicious anemia in relapse showed an increased excretion of coproporphyrin I, a bile pigment.
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not derived from the breakdown of blood cells, but related to states of increased synthesis of hemoglobin. London’s group found increased levels of N-labeled stercobilin in the stools of pernicious anemia patients in relapse, of which at least 40 percent appeared before it could have been derived from the breakdown of circulating red blood cells. Studies by Finch, using labeled iron, have confirmed the fact that there is a marked increase in the rate of iron turnover in the marrow of pernicious anemia patients in relapse.

The megalocytes of pernicious anemia show an oxygen affinity below normal. They contain increased amounts of magnesium, zinc and carbonic anhydrase, normal levels of potassium, and decreased cholinesterase. They show increased permeability to dextrose, malonamide and thiourea and greater than normal susceptibility to the hemolyzing activity of lipemic serum. Their life span is considerably shorter than that of normal erythrocytes, and although their osmotic fragility is not significantly altered they show increased susceptibility to hemolysis by saponin.

Normoblasts are much less susceptible to the action of arsenic than megaloblasts. Years ago arsenic was sometimes used in the treatment of pernicious anemia, with evidence of marrow stimulation in enough cases to arouse interest in its effect on the marrow. It is known to be a general protoplasmic poison and Dustin believed that it exerted its influence on nuclear chromatin prior to chromosomal or prepyknotic condensation. More recently, Skipper demonstrated that potassium arsenite blocked the incorporation of C-labeled formate into nuclear purines. Limarzi showed that following treatment with arsenic there was a dissolution (karyorexis) of early megaloblasts and an increase of maturing megaloblasts in pernicious anemia marrows, and that this sensitivity to arsenic was not exhibited by the early normoblasts of comparable maturity.

(4) Biology

The biologic behavior of megaloblasts has been studied in vivo by serial observations on the marrow of patients with pernicious anemia before and after treatment, and in vitro by observations on cells grown by tissue culture methods. Most of the in vivo studies have concerned themselves with the question of whether the megaloblasts develop into normoblasts or whether they disappear by maturation along megaloblastic lines and are replaced by newly generated normoblasts. Serial observations of pernicious anemia marrow 24 to 48 hours following therapy reveal a rapid disappearance of early (mitotable) megaloblasts and a more gradual disappearance of maturing forms, with an accompanying marked increase in early normoblasts. The observation of Limarzi that preliminary arsenic therapy did not inhibit the response to liver therapy, although it did destroy the earliest megaloblasts, supports the belief that the normoblasts represent a new generation of blood cells.

Horrigan demonstrated that the bone marrow taken from one ilium of a patient with pernicious anemia in relapse, at the site of a previous injection of a minute amount of vitamin B, was normoblastic in contrast to the still
megaloblastic marrow simultaneously removed from the opposite ilium. From this he postulated that the action of vitamin B₁₂ was directly on the developing red blood cells. The success of such an experiment depends upon the deposition of vitamin B₁₂ in the marrow itself, and not in a sinusoid in which it will be diluted and swept into the circulation, and upon the ability of the investigator on the second aspiration to hit the exact spot where the vitamin was first injected. Thus, positive results from such an experiment, supported by convincing photographic evidence as submitted by Horrigan, are difficult to reject, but negative results are not necessarily contradictory. To this author's knowledge other investigators have succeeded in duplicating this experiment after several attempts. Horrigan's conclusion that folic acid had a general rather than local action was based on the finding of normoblastic hemopoiesis on the opposite side following its injection. The amount of folic acid employed, however, was large enough so that some of it could easily have gained access to the general circulation. Vitamin B₁₂ is known to be adsorbed by cells and their constituents and has been shown by Swendseid et al. to be localized in the mitochondria, whereas folic acid in the form of folinic acid was distributed throughout the cell in both the particulate and supernatant fraction of ultracentrifugates. Accordingly, Horrigan's conclusions with respect to folic acid may be open to question.

Conclusions from observations made in tissue cultures often have been contradictory. In surveying the literature, it is apparent that some of these disagreements are attributable to differences in interpretation based on fundamentally different concepts of erythropoiesis by the various authors. Because of these difficulties the author has devoted about two years to the study of various tissue culture technics and their applicability to the megaloblast problem. We found that what happened to megaloblasts in vitro depended on the culture technic, as well as the medium employed. Before one can assess the merits of contradictory reports, it is essential to know the conditions and especially the variables in the experiments.

The identification of megaloblasts depends on their morphologic appearance in air-dried smears stained with Romanowsky stains. Suspension cultures of the type first described by Osgood and Brownlee are excellent in this respect and yield well preserved cells for several days, all that is necessary for the study of megaloblast-normoblast maturation. They also lend themselves to quantitative evaluation by direct cell-counting, an important advantage, since it is only by a quantitative technic (free from the influence of subjective evaluation involved in differential counting of stained cells) that such experiments can be submitted to impartial statistical analysis.

Most observers are in agreement that in cultures of this type, megaloblasts suspended in pernicious anemia serum (diluted with varying proportions of balanced salt solution) will mature slowly along megaloblastic lines and will be replaced by normoblastic cells upon the addition of liver extract or folic or folinic acids. Lajtha, Thompson, Nieweg, Franco and Arku, and Astalidi found no effect of vitamin B₁₂ on megaloblasts in suspension cultures, in contrast to ourselves and Sachetti. In our studies we found that vitamin B₁₂ was active in this respect, but to a lesser degree than folinic acid. Recently,
Thomas has studied the rate of DNA synthesis by measuring the incorporation of $^\text{N}^5$-labeled glycine into megaloblasts suspended in pernicious anemia serum. He showed a significant increase upon addition of vitamin $B_{12}$ and a less constant increase when folic acid or folinic acid were used. His experiments only covered the first eight hours following the addition of vitamin $B_{12}$ or folic acid to the culture. (When megaloblastic marrow was grown on glass or chick plasma clots in a medium containing embryo extract, the effect of vitamin $B_{12}$ was just as marked as that of folinic acid.) Lajtha claimed that when vitamin $B_{12}$ was first incubated with normal gastric juice, it became active in the culture. In our experiments we found no support for this conclusion since vitamin $B_{12}$ was less active in the presence of gastric juice or intrinsic factor concentrate, as judged by both the degree of normoblastic conversion observed on stained smears and the actual counts of cells produced in the cultures.

Of greater interest to the theme of this review is the production of megaloblasts in normoblastic marrows grown in pernicious anemia serum. Lajtha and Thompson observed fewer megaloblasts produced from normoblastic marrows grown in progressive dilutions of pernicious anemia serum and postulated an “inhibitory” factor in the serum responsible for megaloblast formation. Feinman found no evidence to support such a concept in his experiments. Thomas found no inhibition of DNA synthesis in normal marrow cultured in pernicious anemia serum. We found that megaloblasts could be produced in normoblastic marrows grown in pernicious anemia serum or in normal serum with the addition of a folic acid antagonist. Progressive dilution of pernicious anemia serum resulted in the appearance of increasing numbers of megaloblasts which could be prevented by the addition of minute quantities of vitamin $B_{12}$ to the diluted sera. From these observations we concluded that there was no evidence of a megaloblast-producing factor in pernicious anemia serum, and that the decreasing availability of vitamin $B_{12}$ in such sera was one important factor in megaloblast formation. What was, to us, most significant in these studies was the observation that the numbers of megaloblasts that could be produced from normoblastic marrows by any technic was always small and related to the numbers of mitotable normoblasts present in the original marrow. Normal marrows in which only a few of the normoblasts were in the mitotable stage were unsatisfactory for the production of megaloblasts.

II. The Relationship of Megaloblastosis to Nucleoprotein Synthesis

From the characteristics of megaloblasts just discussed, it is apparent that these cells possess morphologic and chemical abnormalities of the cytoplasmic and chromosomal nucleoproteins, that they arise in the marrow under circumstances in which there is a demand for increased blood formation, and that the megaloblastic marrow shows an apparent blockade of cells in the predivisional phase. Further consideration of nucleoprotein metabolism will help to clarify this relationship.

When a somatic cell divides by mitosis, each daughter cell, under normal conditions, ends up with the same number of chromosomes that were present
in the parent cell before division occurred. During interphase and prophase, when the chromatin is diffused throughout the nucleus, it can be measured microspectrophotometrically. Swift’s studies, as well as those of others, have shown that for the cells of any species the concentration of DNA per cell is constant, except during polyploidy, and that, then, the increase is proportional to the nature of the division; e.g., diploid division has twice as much DNA, etc. This increase probably takes place in the early prophase.

In the sea-urchin egg, Brachet demonstrated that the DNA content underwent a marked increase during cleavage, while the total nucleoprotein content was unchanged. This could only mean that the DNA was derived from the RNA of the cytoplasm. While this is true for the sea urchin it is not so well established in more complex forms in which both RNA and DNA increase. During actual mitosis, there is a marked pallor of the cytoplasm due to the disappearance of RNA which reappears after mitosis is completed. This is apparent in cells of many different tissues, including blood cells. Mitchell has observed that when mitosis is inhibited by x-ray irradiation of cells there is an ensuing increase in intracellular RNA and a cessation of DNA synthesis.

Chambers has shown that while under normal conditions the cytoplasm and nuclear constituents are sharply demarcated from each other, prior to mitosis there is a softening of the nuclear membrane which allows the diffusion of cytoplasm into the nucleus. According to Duryee, mitosis is accompanied by an increase in the nucleolar substance which breaks up into small nucleoli in close proximity to each chromosome strand during early metaphase. One might infer that the production of the extra amounts of DNA required for the multiplication of chromosomes was regulated in some fashion by the nucleolus.

After mitosis the cell enters another resting phase during which time the chromosomes disarrange themselves, and their chromatin becomes once more diffusely distributed throughout the nucleus. The nucleolus now reappears, and the cell may undergo another division in due time or enter the phase of maturation. During the latter stage, the RNA of the cytoplasm gradually decreases as hemoglobin increases and probably plays a contributory part in hemoglobin synthesis. The nucleolus has been observed to be quite prominent in such cells as neurones with a high RNA content and thought to play an important regulatory role in its synthesis. In the maturation phase there is a gradual decrease in the amount of DNA in the nucleus, and by the time the pyknotic nucleus is ready for extrusion it retains only a fraction of its original DNA content.

The fact that the functions of mitosis and maturation can occur in vitro indicates that nucleoprotein and hemoglobin synthesis is an intracellular phenomenon, and the apparent reciprocal relation of DNA and RNA to each other during mitosis, on the one hand, and RNA and hemoglobin during maturation, on the other, has led many investigators to postulate that RNA is a parent substance. However, the marked dissimilarity of their chemical nature is such that there is little support for the direct conversion of RNA to DNA or hemoglobin. It is known, however, that “pieces” of RNA
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are convertible to "pieces" of DNA (e.g. ribosides to deoxyribosides). It has been suggested that the cytoplasmic granules are the site of protein synthesis. Proponents of this theory point to the fact that these bodies are ideal organelles for such a function in that they are in close proximity to abundant sources of RNA, contain proteases capable of synthesizing peptide linkages and respiratory enzymes that can break down ATP to produce energy, and, in fact, contain small quantities of the protein to be synthesized, such as hemoglobin in the case of red blood cells. For a more detailed discussion of this point, Brachet's book should be consulted.8

Because of the biologic evidence suggesting a reciprocal relationship between DNA and RNA, chemists have given a great deal of attention to their respective origins. The difference between the two nucleic acids is one methyl group in a pyrimidine base (thymine is 5-methyl uracil) and the absence of an hydroxyl group on carbon-2 of the ribose moiety.

With the widespread utilization of isotopic-labeling technics it has been learned that the pathways of nucleoprotein synthesis are multiple, one pathway or another being used preferentially in different species or under different conditions. Purines are synthesized from glycine which is coupled with 5-phosphoribosylamine to form a nucleotide precursor for which the additional carbon atoms are derived from formate and CO₂ to make the finished purine.104 Pyrimidines, on the other hand, are derived from CO₂ (largely from aspartic acid and its metabolites) with the exception of the methyl group of thymine which can be derived from many sources, e.g. formaldehyde, the alpha carbon of glycine, beta carbon of serine, and other precursors of active "one-carbon units" such as choline and purines.105 Of particular interest is the biosynthesis of thymidine, because this is a reaction that must be involved in any interconversion between RNA and DNA. Many investigators had indicated that such uracil derivatives as oxaloacetic acid, ureidosuccinic acid, and orotic acid may all be used as thymidine precursors in microorganisms,105 but it remained for Friedkin107 to show that in chick embryos and rabbit marrow cells, labeled uridine is incorporated into thymidine, whereas labeled uracil is not. This reaction proceeded much more slowly in folic acid-deficient chicks, was enhanced by citrovorum factor (folinic acid), and could be blocked by aminopterin. It now appears likely that methylation occurs at the deoxyuridylic acid stage forming thymidylic acid.

It is easy to conceive of an intracellular metabolic pool to which the nucleoproteins themselves can contribute the ribosides, ribotides, pyrimidine and purine bases, amino acids and labile carbon atoms essential for nucleoprotein synthesis. Prior to mitosis the reactions move in the direction of

\[
\text{RNA} \rightleftharpoons \text{POOL} \rightleftharpoons \text{DNA}
\]

Cell protein (Hgb) \quad \text{Chromosomes}

During mitosis, reactions move in direction of DNA synthesis; in direction of RNA synthesis during maturation.

Fig. 4.—The dynamic concept of the intracellular metabolic pool.

Metabolic pool of nucleic acid components, e.g. (nucleosides and tides, purines, pyrimidines, amino acids, etc.) B-12 and Folinic acid act here.
DNA synthesis for chromosome formation. During maturation the reactions move in the opposite direction toward RNA synthesis for building cell protein, e.g. hemoglobin (fig. 3). In this metabolic pool, vitamin B₁₂ and coenzymes derived from folic acid play a vital role in promoting the chemical reactions involved.

III. Vitamin B₁₂ and Folic Acid in Nucleoprotein Synthesis

It has already been mentioned that the methyl group of thymine can be derived from formate, the alpha carbon of glycine, beta carbon of serine, all the carbons of choline, and carbons 2 and 8 of purines. These compounds are collectively referred to as "one carbon donors" which, to some extent, are metabolically interchangeable with one another. The transfer of the one-carbon units involves co-enzymes derived from folic acid, and, in some less clearly defined way, vitamin B₁₂ also. The literature on the subject is voluminous and excellently reviewed by Shive, Jukes and Stokstad, and Welch and Nichol. It is apparent that both vitamin B₁₂ and folic acid are essential for a large variety of metabolic conversions, all of which involve the addition of labile carbon atoms or methyl groups. Some of the syntheses that one or both of these substances can be shown to participate in are the formation of serine from glycine, methionine from choline, and thymidine-5'-phosphate from deoxyuridine-5'-phosphate. The exact manner in which they accomplish this is unknown. It has been shown that a common building block from which one-carbon additions are made is formate, and the formyl radical is carried by tetrahydrofolic acid (see below).

Folic acid is converted in the body into several physiologically active forms. The first of these to be recognized was folinic acid (citrovorum factor) which is N⁵-formyl-tetrahydropteroylglutamic acid. In addition, N¹⁰-formyl-PGA, tetrahydro-PGA, and N⁵-hydroxymethyl-tetrahydro-PGA, as well as a compound of as yet undetermined structure have all been shown to be physiologically active. The nature of this activity is the transport of single carbon atoms, carried as formyl, hydroxymethyl, or formimino groups, in several different syntheses, including that of derivatives of thymine. It has been suggested that vitamin B₁₂ plays a role in the synthesis of folinic acid from folic acid, but Jukes has shown that in the rat the addition of vitamin B₁₂ has no effect on the urinary excretion of folinic acid following the injection of folic acid. In nucleic acid synthesis, folic acid seems to be more concerned in the synthesis of derivatives of purines and thymine, while vitamin B₁₂ may be related to deoxyribose formation. There is also some evidence linking vitamin B₁₂ to formation of the pyrimidine ribosides of RNA. In the methylation of nicotinamide to N¹-methyl nicotinamide, while both folic acid and vitamin B₁₂ promote the reaction, they appear to do so by different mechanisms. Whether vitamin B₁₂ functions as an enzyme to catalyze reactions utilizing the carbon carried as formate by folic acid derivatives can only be conjectured. It is apparent, however, that both substances act closely together, and there is convincing experimental evidence that if one is not present in at least a minimal concentration, the other will not be effective in reinaugurating nucleoprotein synthesis. Such a hypothesis
explains the failure of the so-called pernicious anemia of pregnancy\textsuperscript{117} and certain refractory megaloblastic anemias\textsuperscript{118} to respond to vitamin B\textsubscript{12}, when they respond readily to folic acid, on the ground that they are pure folic acid deficiencies. On the other hand, when one substance is given it apparently speeds up reactions which consume the other, and so depletes it by a sort of mass action. This is best exemplified by the aggravation of dorsolateral spinal cord disease and the development of refractoriness to folic acid therapy in patients with pernicious anemia who were treated with folic acid and who were initially responsive to it,\textsuperscript{119,120} the decrease in serum vitamin B\textsubscript{12} levels in pernicious anemia patients given folic acid,\textsuperscript{121} and the greater ease with which folic acid deficiency can be produced in experimental animals if vitamin B\textsubscript{12} is simultaneously given.\textsuperscript{122,123} The situation in which administration of vitamin B\textsubscript{12} aggravated symptoms of folic acid deficiency in man was described in two cases by Wintrobe.\textsuperscript{124} In vitro studies have indicated that the two substances exert a mutually sparing (or enhancing) effect on the growth of bacteria that can utilize both of them,\textsuperscript{126} and there is some evidence that this is also true clinically.\textsuperscript{127} Nieweg et al.\textsuperscript{125} have proposed that the reason the neurologic lesions of pernicious anemia respond only to vitamin B\textsubscript{12} is because folic acid is concerned only in DNA formation (thymine methylation) while vitamin B\textsubscript{12} is involved in the closure of the pyrimidine rings for both DNA and the RNA essential for maintaining the integrity of the neuronal cytoplasm.

IV. The Significance of Megaloblasts

The two main features that distinguish megaloblasts are the particulate character of the chromatin and the delayed disappearance of ribonucleic acid from the cytoplasm. The decreased thymine/uracil ratio is probably a consequence of the latter. Microspectrophotometric studies have shown no quantitative difference between megaloblasts and normoblasts with regard to the total nuclear DNA content or the proportion of depolymerized DNA present. Yet the differences in size of the Feulgen-stained particles is readily apparent, and the megaloblasts in such preparations can be easily distinguished from normoblasts of the same size. The DNA in megaloblasts must, therefore, be more thoroughly diffused through the nucleus during the resting phase between mitoses. Biologists assume that during the resting phase there is a “realignment of forces” preparatory to another division. One of the requisites for mitotic division is a doubling of the amount of DNA. It seems reasonable to anticipate that the more slowly this extra amount of DNA can be synthesized the longer the interphase will last, and the more thorough will be the dispersion of chromatin through the nucleus. This results in the formation of smaller particles of DNA containing chromatin, a circumstance which gives the nucleus of the megaloblast its typical appearance. (The greater susceptibility of the megaloblast to the toxic effect of arsenic is perhaps a manifestation of decreased resistance of the nuclear DNA resulting from the prolongation of interphase.)

The principle source of the extra DNA required for mitosis is probably the constituents of the cytoplasmic RNA. In favor of such an idea is the
natural proximity of RNA both in the cytoplasm and, more particularly, in the nucleolus. The increased nucleolar activity during mitosis, described by Duryee, is particularly suggestive of a direct relationship. The existence of this biochemical pathway has been convincingly demonstrated by Friedkin,\textsuperscript{167} as well as the fact that folic acid plays an important role in the reactions involved.

The persistence of RNA in the cytoplasm of megaloblasts and high proportion of uracil to thymine in such marrows is readily explained if we assume that there is a block in the normal conversion of these substances to DNA and thymine, respectively, such as would occur with a defect in the synthesis of the methyl group of DNA-thymine. When supplies of vitamin B\textsubscript{12} or folic acid are inadequate, DNA synthesis proceeds only slowly, permitting an occasional mitosis, but causing the marrow to become crowded with cells waiting to synthesize enough DNA to divide. This accounts for the predominance of mitotable forms in megaloblastic marrows, and these early forms assume the nuclear characteristics of megaloblasts due to greater dispersion of chromatin throughout the nucleus during the wait.

Under these circumstances three things may happen. A few cells may synthesize enough DNA to undergo normal mitosis. These cells then become normoblasts unless they enter another prolonged interphase. Some cells may divide with an abnormal amount of DNA leading to the formation of the bizarre mitotic figures so often seen in pernicious anemia marrows. Most of the cells, after waiting awhile, go on and mature without dividing. We know that RNA is closely related to the structural protein of cells, and it is an old axiom of tissue culture that the slower the rate of cell division, the larger the cells grow.\textsuperscript{128} Since these cells have not utilized their cytoplasmic RNA for DNA synthesis they now have more RNA available for the formation of structural cell protein, in the case of red blood cells, hemoglobin. Therefore they grow into the macrocytes characteristic of this type of blood formation.

As soon as the missing enzyme is supplied the block is removed with an immediate increase in mitotic activity, and the mitotable megaloblasts are rapidly replaced by normoblasts of comparable maturity. The megaloblasts that have begun to mature complete their maturation and, therefore, can still be seen for a few days, their numbers steadily decreasing as they are replaced by maturing normoblasts. One of the big mysteries about megaloblastic marrows has been how to explain the rapid disappearance of megaloblasts after treatment, but this is readily accounted for by the above hypothesis.

If megaloblasts are simply red cell precursors with a retarded rate of division, it should follow that any condition such as leukemia or hemolytic anemia that depletes supplies of vitamin B\textsubscript{12} or folic acid faster than they can be replenished, or a marrow aplasia that retards or inhibits mitosis sufficiently could cause a similar ultradispersion of chromatin that would give a megaloblastic appearance to the early red cells in the marrow. In some instances megaloblasts may arise due to nonspecific factors retarding blood formation. In liver disease there is probably a breakdown of basic metabolic functions having to do with the formation of the simpler building blocks of nucleoproteins such as amino acids. In intrauterine life the enzyme sys-
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Mitosis Maturation

MEGALOBLASTIC TYPE

Hypochromatic Orthochromatic

NORMOBLASTIC TYPE

Fig. 5.—Schematic representation of megaloblast formation. At the conclusion of mitosis the resulting basophilic normoblasts enter another resting phase from which they may either mature or undergo mitosis. If there is a delay in the synthesis of chromosomal DNA the dispersion of chromatin begun in telophase continues until the chromatin is in fine particles characteristic of the basophilic megaloblast. Should mitosis occur now, the resulting cells would have the appearance of normoblasts until they had undergone another prolonged interphase. Most of the cells at this stage mature into hypochromatic and orthochromatic megaloblasts and eventually into oval macrocytes. The particulate character of the chromatin persists into the end stages of maturation because there has been no opportunity for recondensation.

tems essential for such syntheses are probably not yet developed. But the conditions above all others that meet the requirements for the formation of megaloblasts are the absence of the co-enzymes (or their precursors) particularly concerned with nucleoprotein synthesis, vitamin B₁₂ and folic acid. If either of these substances is lacking, the result is sooner or later a typical megaloblastic marrow which can only be restored to normal by supplying the missing vitamin.

An important factor influencing megaloblastosis is the demand for blood formation. Relieving the anemia of pernicious anemia by transfusion does not correct the basic metabolic deficiency but causes a disappearance of megaloblasts from the marrow. This explains the presence of megaloblasts occasionally seen in marrows of patients with acute blood loss or hemolysis on the basis that because of diet, or deficient absorption, the patient has levels of vitamin B₁₂ or folic acid inadequate for the increased demands for hematopoiesis with resulting exhaustion of the enzyme leading to megaloblast formation.

SUMMARY

Evidence is presented for a hypothesis that megaloblasts are red blood cell precursors with a prolonged resting phase between mitoses, allowing a longer time for dispersion of the chromatin throughout the nucleus. Anything that retards the rate of cell division can produce cells of similar ap-
pearance, but the conditions most conducive to megaloblastic blood formation are states in which vitamin B₁₂ or folic acid are deficient. These two substances function in the synthesis of nucleoproteins as co-enzymes or their precursors concerned with the transfer of one-carbon units. In their absence the synthesis of the extra amounts of deoxyribonucleic acid essential for mitosis can go on but slowly, and the marrow becomes crowded with cells waiting to divide.

The principle source of the extra DNA is cytoplasmic RNA which appears to supply the ribonucleotide precursors of several deoxyribose containing compounds. Most important among these is deoxyuridylic acid which is methylated to form thymidylic acid. The failure of these reactions to occur in the absence of the essential co-enzymes leads to a persistence of RNA in the megaloblastic cytoplasm during the maturation phase, and this is probably responsible for their development into macrocytes.

This hypothesis accounts for the occasional presence of megaloblasts in conditions other than pernicious and related deficiency anemias and the occurrence of "macronormoblastic" or "megaloblastoid" marrows in intrauterine life and liver disease. The hypothesis also explains the rapid replacement of megaloblasts by normoblasts in the marrow following specific therapy.

**SUMMARIO IN INTERLINGUA**

Es presentate datos in supporto del hypothese que megaloblastos es precursores erythrocytic in que un prolongate phase de reposo intermitotic permitte le dispersion de chromatina in omne partes del nucleo. Omne rellation del division cellular, sin reguardo al natura de su causa, pote resultar in le production de cellulas de un simile apparentia, sed le conditiones que es le plus favorabile al formation megaloblastic es statos in que il existe un carentia de vitamina B₁₂ o de acido folic. Iste duo substantias participa in le synthese de nucleoproteinas como co-enzymes o precursores de tales que es concernite con le transferimento de unitates a carbon unic. In lor absentia le synthese del quantitates supplementari de acido disoxyrihonucleic (que es indispensabile pro le mitose) progredite lentissimemente, e le medulla abunda in celular preste a divider se.

Le fonte principal de quantitates supplementari de acido disoxyrihonucleic es cytoplasmic acido ribonucleic que apparentemente provide le precursores ribonucleotidic de plure composito continente disoxyribose. Le plus importante inter istos es acido disoxyuridylic que es methylate e se transforma assi in acido thymidylic. Le non-occurrentia de iste reactiones—in consequentia del absentia del requisire co-enzymas—resulta in le persistentia de acido ribonucleic in le cytoplasma megaloblastic durante le phase de maturation, e isto es probablemente responsabile pro le disveloppamento de megaloblastos in macrocytos.

Iste hypothese explica le non infrequente presentia de megaloblastos in conditiones altere que anemia perniciose e relationate anemias carential. Illo etiam explica le occurrentia de medulla "macronormoblastic" e "megaloblastoid" durante le vita intrauterin e in patientes con morbo hepatic. Le mismo
es ver pro le rapide reimplacimento de megaloblastos in le medulla per normoblastos post le initiation de un therapia specific.

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The Nature and Significance of Megaloblastic Blood Formation

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