Editorial

A Picture of Erythropoiesis at the Combined Morphologic and Molecular Levels

By Henry Borsook

Thorell1 in 1947 published his study of the changes in nucleic acid, mixed proteins and hemoglobin in erythroblasts at different stages of maturation. It was the first bridge in erythropoiesis between the morphologic and molecular levels. His conclusion which attracted most attention was that hemoglobin synthesis did not begin until about the beginning of the polychromatic stage. A few years later, with the advent of radioautography it became possible to observe directly, rates of synthesis of DNA, RNA and protein in individual cells in vivo and in vitro. Not long afterwards came the demonstration of the relation of DNA and RNA to protein synthesis as a mechanism of conveying genetic information and of metabolic control. Now, one can make a tentative venture to relate the morphologic and molecular features of erythropoiesis in some detail. Two examples have been selected. One is the abrupt onset of hemoglobinization midway through maturation. The other is the macrocytosis of hemolytic anemia.

Hemoglobin Synthesis

Thorell's conclusion that hemoglobinization does not begin until the polychromatic stage was questioned2,3 but it may now be considered as established that at least the main hemoglobinization begins at this stage.4,8 Whether or not there is some slow hemoglobin synthesis earlier one cannot yet be sure. It may be an important point in understanding the mechanism of the release of the main hemoglobin synthesis.

It has been surmised that the reason why little or no hemoglobin is seen until the polychromatic stage is that it is heme which is missing until then, that the globin is made earlier. This surmise is probably incorrect. There is no accumulation of protein in the earlier stages; the total protein concentration in the erythroblasts before hemoglobinization is less than 10 per cent of what it is after.1,6 Heme and globin are synthesized at equivalent rates both in reticulocytes7 in which hemoglobin is almost the only protein synthesized, and in the mixture of marrow cells.8 where much non-hemoglobin protein is synthesized in basophilic erythroblasts. There is a mutual regulation of heme and globin synthesis under normal circumstances.9 However, the two processes can be dissociated; cobaltous ions depress heme and accelerate globin synthesis;8 X-radiation and starvation disturb the correspondence in variable ways.10,11
ERYTHROPOIESIS AT THE MORPHOLOGIC AND MOLECULAR LEVELS

Four questions come up next. When is the apparatus for synthesizing hemoglobin made? When does it come into operation? When does it stop? Where is it discarded?

All erythroblasts synthesize protein. All but the one key part of the apparatus which is specific for synthesizing globin—an ATP generating system, about twenty amino acid specific activating enzymes with their complementary transfer RNA and ribosomes—are the same whatever protein is synthesized. The intensity of synthesis, i.e. the rate per unit volume, appears to decline a little but not much until the disappearance or degeneration of the nucleus.12,18 The concentration of protein is also nearly constant until hemoglobinization sets in.6 Synthesis and breakdown nearly balance each other up to this point. Therefore, only the key part for hemoglobin synthesis is missing.

The pronormoblast is diploid.14 At each successive mitosis thereafter the erythroblasts, according to Weicker and Terwey,15 become more and more hypodiploid. Among cytologists this is one of those “controversial” conclusions, but it is in accord with Korson’s observations on the DNA content of the nuclei. Hemoglobinization begins in the polychromatic stage which is after the first mitosis in which there is a large loss of DNA. In this stage DNA synthesis comes to a stop in most erythroblast lines,17-19,36,40 and the marked slowing down of RNA synthesis begins.1,18,17 It is reasonable to look for a causal relation in the coincidence of loss of DNA, slowing down of RNA synthesis and the onset of hemoglobin synthesis. The genes for hemoglobin synthesis must be present in the earliest erythroblasts; relief of inhibition must, then, be the event which inaugurates or induces the great acceleration of hemoglobin synthesis. An inhibitor is lost with the lost DNA; it could be an inhibitor Messenger RNA or a protein which at first masks the segment of DNA on which, after unmasking, hemoglobin Messenger RNA is synthesized. All (or nearly all) the RNA synthesis in erythroblasts is DNA-dependent,13 consequently RNA synthesis declines as DNA is lost.

Once the inhibition is relieved hemoglobin Messenger RNA is continually produced so long as there is a functioning nucleus, while the synthesis of Messenger RNA for the synthesis of non-hemoglobin protein declines. All the rest of the apparatus for synthesizing protein is conserved, it is only subdivided by cell division. Accordingly the rate of synthesis of total protein changes little during maturation, but hemoglobin synthesis replaces that of one or more other proteins. Another factor which contributes to the accumulation of hemoglobin is the persistence of breakdown of non-hemoglobin protein. Enzymes disappear during the maturation of reticulocytes;41,42 it is probably a continuation of the catabolic side of non-hemoglobin protein turnover in the erythroblasts. Hemoglobin once synthesized remains until the red cell is lysed and eventually comprises over 95 per cent of the protein of the cell.

The second phase of hemoglobin synthesis is when it slows down to a permanent stop. It occurs after the nucleus is lost12 or degenerates and, since there is no longer any functioning DNA, destroyed Messenger RNA cannot be replaced. The persistence of hemoglobin synthesis in reticulocytes for
hours\textsuperscript{20} testifies to the survival of some of their Messenger RNA, probably for 24 hours or more.

\textit{The Macrocytosis of Hemolytic Anemia}

The mechanism which produces the macrocytosis of hemolytic anemia is a by-passing of the orthochromatic stage.\textsuperscript{12,17,21,28} The resulting reticulocytes are abnormally large,\textsuperscript{12,17,22,28} they mature without significant reduction in volume\textsuperscript{12,28} and the life span of the resulting erythrocytes is shorter than normal.\textsuperscript{12,27,29,30} The immediate stimulus in a number of animals, e.g., man, mouse, rat and rabbit, is an increased production of erythropoietin.\textsuperscript{31} Direct proof is an outpouring of the typical short-lived macrocytes following injection of large doses of erythropoietin into normal\textsuperscript{12,32,33} or even hypertransfused animals.\textsuperscript{27} The primary, or main action of erythropoietin is in the stem cell compartment to increase the rate of generation of erythroblasts; it may also shorten maturation time. Erythroblasts generated under these conditions lose their nuclei at the polychromatic rather than at the orthochromatic stage.\textsuperscript{12} The effect of the whole response is that erythroblasts are generated faster; the reticulocytes to which they give rise appear in the blood sooner; they and the erythrocytes from them are larger and contain more hemoglobin than normal, although in lower concentration,\textsuperscript{28} and when the crisis is past they are quickly disposed of.

In a mutant strain of mice evidence has been found of another stimulus to erythropoiesis than erythropoietin.\textsuperscript{48} This finding may bear on the question whether increased erythropoietin production occurs only in severe hemolysis, i.e., it is only a crisis mechanism.

The by-pass of the orthochromatic stage is responsible for the larger size of the cells. The different classes of erythroblasts have the same mean and modal sizes in the hemolytic as in the normal animal.\textsuperscript{12,13,22} Each successive erythroblast has about half the volume of its predecessor.\textsuperscript{1,15,17,36,37} The volume of the reticulocyte is approximately that of the parent erythroblast minus that of its nucleus.\textsuperscript{12} The only way for abnormally large reticulocytes to be formed is by by-passing one or more of the terminal nucleated stages. When such a reticulocyte matures its volume is not reduced.\textsuperscript{12,28} The short life span of the macrocyte is more likely due to an inborn weakness rather than merely a mechanical effect of its size.

The by-pass also results in a lower hemoglobin concentration\textsuperscript{28} because it reduces the duration of maximum hemoglobin synthesis which depends, as described above, indirectly on a functioning nucleus. When the nucleus is degenerated or lost the rate of hemoglobin synthesis declines.

Without prior DNA synthesis there is no mitosis.\textsuperscript{38,40} It is consistent to associate the by-pass with some failure of DNA synthesis. But what needs to be explained then is that at the height of the response to hemolysis, most (not all) polychromatic cells synthesize DNA and yet most by-pass the next mitosis. The question arises whether a cell can begin to synthesize DNA and have it stop short of enough for mitosis The nucleus would then degenerate, be disintegrated or extruded with the resulting formation of a macrocytic reticulocyte.
When the nucleus degenerates, whether in the polychromat or orthochromatic erythroblast, hemoglobin synthesis declines before the nucleus is lost because losses in Messenger RNA cannot be replaced. Thus the regulation of hemoglobin synthesis is largely a matter of how long the synthesis of Messenger RNA is maintained by a functioning nucleus.

A few erythroblasts by-pass the orthochromatic stage under normal circumstances. Among orthochromatic erythroblasts a few have been observed with active DNA synthesis and also a corresponding incidence of mitosis. One would expect, therefore, in a normal population of erythrocytes some of the corresponding sizes—large and small—with an inverse relation to their hemoglobin concentration. The data reported by Ambs show that this is the case. The mean diameter and hemoglobin concentration of all the cells were 7.6 \( \mu \) and 123 \( \mu \)g. per \( \mu \), respectively; in the group of the largest cells the values were 8.6 and 94, and in the smallest 6.1 and 199. In the population as a whole the inverse correlation between diameter and hemoglobin concentration was very high; \( r = 0.549; d_1 = 89 \).

Three interesting examples fall into accord with the above picture. In the erythroblasts of fetal liver RNA synthesis and hence, probably, also DNA synthesis cease a stage earlier than in normal adult marrow. Hence the last maturation mitosis in adult marrow is by-passed with the production of abnormally large reticulocytes and erythrocytes. Hence the erythrocytes of the newborn, like those of hemolytic anemia, are larger and their life span may be somewhat shorter than in the normal adult.

The picture of erythropoiesis given above predicates adequate nutrition. The picture is different in iron deficiency, it is different again in deficiency of folic acid of B\(_{12}\). When these deficiencies are corrected the response to the microcytic anemia in the one and to the macrocytic anemia in the other is a macrocytic reticulocytosis as in hemolytic anemia.

Maturation in erythropoiesis is a fluid process. It can be made to run faster or slower and to deviate in its course more or less, producing variations in size and hemoglobin content of erythrocytes which are referable to when DNA synthesis ceased and how long its Hemoglobin-Messenger RNA persisted after that.

REFERENCES


5. Ackerman, C. A.: Histochemical study

*The argument rests on the assumption that human is like the rabbit in this respect.


30. Berlin, N. I., and Lotz, C.: Life span of the red blood cell of the rat follow-
ERYTHROPOIESIS AT THE MORPHOLOGIC AND MOLECULAR LEVELS

44. Borsook, H.: Calculated from the data of Amb's.48

Henry Borsook, Ph.D., M.D., Professor of Biochemistry, Division of Biology, California Institute of Technology, and Consultant to the Huntington Memorial Hospital, Pasadena, Calif.
Editorial: A Picture of Erythropoiesis at the Combined Morphologic and Molecular Levels

HENRY BORSOOK