THE MOBILISATION OF IRON FROM THE CELL STROMA DURING ERYTHROID MATURATION

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SUMMARY: Adult mammalian erythroblasts from an anaemic rabbit were separated into fractions of cells at different stages of development using the velocity sedimentation technique. The iron content of the stroma and cytoplasm of the cells in each fraction was determined by atomic absorption spectroscopy. A high proportion of the iron in the dividing erythroblasts was found to be associated with the cell stroma. After the final cell division the proportion of stromal iron rapidly declines and it appears that stromal iron is mobilised at this stage and utilised by the non-dividing erythroblasts for haemoglobin synthesis.

INTRODUCTION

Iron incorporation by erythroid cells is not directly related to haemoglobin synthesis. Erythroblasts continue to incorporate iron even when the synthesis of haem is blocked by lead administration (1). In the immature basophilic erythroblasts before active haemoglobin synthesis has commenced iron incorporation has been shown by autoradiography (2) and by ^{59}Fe uptake (3) to be at least as rapid as in the more mature erythroblasts in which haemoglobin synthesis is active. In previous studies we have demonstrated that the intensive phase of haemoglobin synthesis occurs only after the erythroid cell has undergone the final cell division and entered the non-dividing compartment (4). Here we report evidence that a considerable mobilisation of stromal bound iron occurs in the non-dividing compartment coincidentally with the intensive phase of haemoglobin synthesis.

EXPERIMENTAL

Cell Separation

New Zealand white rabbits (2 kg) were made anaemic by five daily injections of neutralised phenylhydrazine HCl (5). Two days after the cessation of

injections the animals were killed and an erythroid bone marrow suspension was prepared. The bone marrow suspension was then subjected to fractionation by the velocity sedimentation technique essentially as previously described (6). A square Perspex sedimentation chamber of cross section 2500 cm^2 was used and 30 fractions of 250 ml each were collected after sedimentation.

The sedimentation velocity technique separates erythroid cells by exploiting the fact that at unit gravity the sedimentation velocity of a cell is proportional to its size, and during erythroid differentiation the cells continually decrease in size from the 15 μm diameter basophilic erythroblasts to the 8 μm diameter reticulocytes. Fractions 1 to 10 consisted mainly of basophilic erythroblasts; fractions 10 to 20 mainly of polychromatic erythroblasts; fractions 20 to 25 of orthochromatic erythroblasts; fractions 25 to 30 mainly of reticulocytes. The cells in fractions 1 to 20 were actively dividing erythroblasts, those in fractions 21 to 30 belonged to the non-dividing compartment. Using this technique complete separation was achieved between cells immediately prior to and immediately following the final cell division because at the final cell division the cell size is halved and during subsequent maturation to the reticulocyte stage the cell size continues to decline gradually.

Iron Determinations

The cells in each fraction collected from the sedimentation chamber were centrifuged and washed three times in phosphate-buffered saline (P.B.S.) (pH 7.4) containing 8 g. NaCl, 0.2 g. KCl, 1.15 g. Na_2HPO_4, 0.2 g. KH_2PO_4, 0.1 g. $CaCl_2$, 0.1 g. $MgCl_2 6H_2O$ per litre of solution. A small aliquot of cells from each fraction was removed, and the number of cells in each fraction determined using a haematocytometer. The cells were then lysed by hypo-osmotic shock using 5 parts double distilled water to 1 part of cells. The lysates were centrifuged at 20000 g. for 10 min. to separate the stroma from the cytoplasm. The stromal pellets were washed twice in distilled water and finally centrifuged into a tight pellet. The supernatant fluid remaining from each wash was added to the corresponding supernatant cytoplasmic fraction. All remaining fluid was carefully

removed from the pellet using a Pasteur pipette. To each pellet was added 150 µl of conc. nitric acid (Aristar grade, British Drug Houses Ltd., Poole, Dorset). After about 6 hr. at 20°C the pellets had been completely digested. The nitric acid solutions were diluted to a volume of 1.0 ml with distilled water. The iron content of each of the diluted digests was determined by atomic absorption using a Perkin Elmer atomic absorption spectrometer Model 303. The concentration of iron in the distilled water and in the P.B.S. was also determined.

The cytoplasmic lysates were concentrated to a volume of 0.75 ml by vacuum desiccation. The iron content of each lysate was then determined by atomic absorption spectroscopy. The absorbance at 415 nm of each lysate was also measured.

RESULTS

The iron content of the distilled water and of the P.B.S. was negligible. The number of cells in each fraction is shown in Fig. 1. The cell types sedimenting in the major regions of the sedimentation gradient are indicated. The total iron content of the developing erythroblast would appear to decrease continuously throughout erythropoiesis from a level of approx. 5 pg. per cell in the basophilic erythroblasts to approx. 0.7 pg. in the marrow reticulocytes (Fig. 1). The iron content of the polychromatic erythroblasts in fractions 15-18 is about

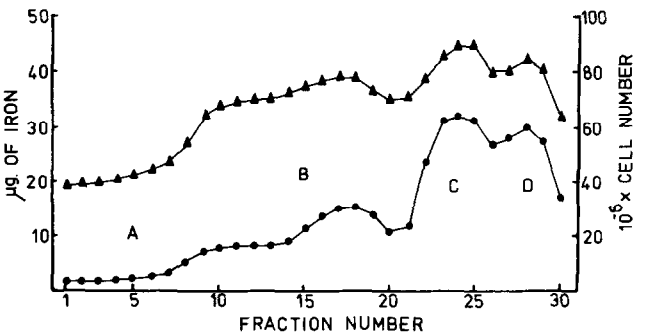

Fig. 1. Cell sedimentation profile and total iron content of cells fractionated by velocity sedimentation.
● cell number in each fraction
▲ iron content of each fraction
A, basophilic cells; B, polychromatic cells; C, orthochromatic cells; D, reticulocytes.

1.3 pg. per cell. These cells undergo the final cell division of erythroid maturation, giving rise to the non-dividing orthochromatic erythroblasts in fractions 21-25 with an iron content of approx. 0.7 pg. per cell, which is about half that of their polychromatic precursors. Subsequently, their iron content remains almost constant during loss of the nucleus and maturation into reticulocytes. The total iron content of the dividing erythroblasts immediately before the final cell division is sufficient to account for the iron content of their daughter cells at the reticulocyte stage without any further uptake of iron.

The iron content of the stroma and cytoplasmic lysate and the absorbance of the cytoplasmic lysate at 415 nm are shown in Fig. 2. The iron content of the cytoplasmic fraction is closely related to the absorbance at 415 nm at all stages of erythropoiesis which suggests that most of the iron in the cytoplasm of erythroid cells is in the form of haemoglobin. A steep increase in the proportion of cytoplasmic iron in haemoglobin occurs in the non-dividing compartment. In the dividing compartment about 85% of the total cell iron is non-haemoglobin associated with stromal material. After the cells have undergone the final cell division and passed into the non-dividing compartment the proportion of iron in the stromal fraction declines quite suddenly to less than 20% of total cell iron.

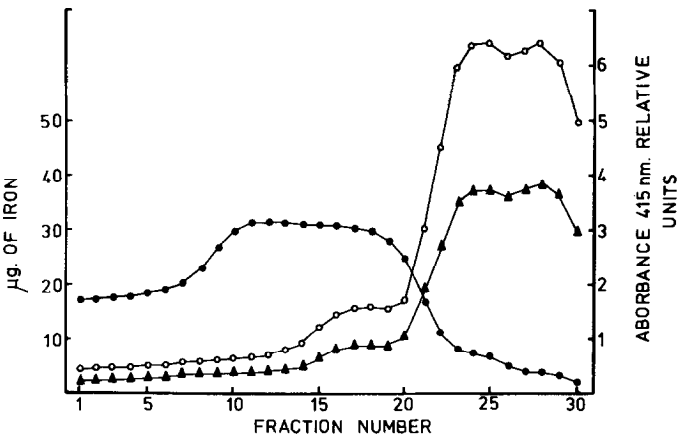

Fig. 2. Iron content of cell stroma and cytoplasm. ● ,iron content of cell stroma in each fraction; ▲ ,iron content of cell cytoplasm in each fraction; ○ ,absorbance at 415 nm of the cytoplasmic lysate from each fraction.

The proportion of stromal iron continues to decline as the orthochromatic cell matures into the reticulocyte.

DISCUSSION

The fact that the iron content of the maturing erythroblast is almost constant at a time when haemoglobin synthesis is intense during the period when the cell manufactures most of its final haemoglobin complement (4) suggests that much of the iron required for haemoglobin synthesis could be provided for by existing intracellular iron without the necessity for further iron incorporation from the bound iron in the plasma transferrin. The additional fact that a massive shift in cell iron from the stroma to cytoplasmic haemoglobin occurs at this time is very strong circumstantial evidence that a major source of iron for haemoglobin synthesis in non-dividing erythroblasts is from a pre-existing iron store in the cell stroma.

The membrane of the red cell has been considered to function as an intermediate between the stage of plasma transport and haem synthesis, but the nature of stroma iron in erythroblasts is not clear (7). A certain proportion could be in the form of transferrin-bound iron on surface receptor sites. However there are only 50000 such sites on the reticulocytes occupying some 2% of the cell surface (1, 7) and although their numbers are probably waning at this stage it is doubtful if anything more than a small percentage of stromal iron could be accounted for in this way. Other membrane iron-binding macromolecular species have been found in reticulocyte stroma (8) and in addition the stroma of immature red cells including reticulocytes is known to contain iron-rich granules which have the appearance on light and electron microscopy of ferritin (1, 9). It is unlikely that much non-haemoglobin iron is haem as the synthesis of haem is closely associated with globin throughout erythropoiesis (10).

The proportion of stromal iron in dividing erythroblasts is surprisingly large and it is tempting to speculate that its function may be to prevent the supply of iron from becoming rate limiting during the most active phase of haemoglobin synthesis in the orthochromatic cells.

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