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Can Ferritin Provide Iron for Hemoglobin Synthesis?

To the Editor:

We have read with great interest a recent report by Gelvan et al[1] attempting to resurrect an old idea that immature erythroid cells obtain iron (Fe) from ferritin. We wish to comment on one single and extremely important issue in this report, viz, that Fe from internalized ferritin can be used for hemoglobin synthesis. We believe that neither the results of previous studies nor experiments presented by Gelvan et al[1] provide adequate evidence supporting this claim.

In 1957 Bessis and Breton-Gorius[2] presented electron micrographs depicting erythroblastic islands in the bone marrow, in which a central reticulum cell (''nurse cell'') was surrounded by a ring of erythroblasts. In the region of contact between these cells the authors observed ferritin and proposed that it was transferred from the reticulum cell to the erythroblasts by a form of micropinocytosis termed ''rhopheocytosis'' (for review see ref 3). Somewhat later, Jandl, Katz, and coworkers[4,5] showed that immature erythroid cells take up Fe from plasma Fe-binding protein, transferrin (Tf), and suggested the existence of a membrane-bound Tf receptor which may be involved in Fe uptake. The many studies and discussions that followed have led to a general consensus that Tf, not ferritin, is the source of Fe for hemoglobin synthesis, and this view has been supported by the following evidence. First, under normal conditions all plasma Fe is associated with Tf, and ferrokinetic studies have provided clear evidence that all Fe used for hemoglobin synthesis passes through the plasma.[6] Hence, if the reticulum cell ferritin was the source of hemoglobin Fe, these cells would have to acquire it from Tf. However, virtually no Fe enters the reticuloendothelial system from plasma Tf.[7] Second, it should also be pointed out that plasma ferritin has a very low Fe content, and no ferrokinetic evidence supports its role in Fe transport.[7] Third, a specific relationship between Tf and erythroid cells is documented by in vitro studies showing that this molecule is the only physiologically relevant Fe complex capable of providing Fe for hemoglobin synthesis.[8] Fourth, normally developing erythroid cells take up 26 mg Fe/d from plasma Tf,[7] a rate that matches almost perfectly with the daily production of hemoglobin ($\sim$6.2 g). The final and most convincing evidence of an absolute requirement for Tf by erythroid cells comes from observations that both humans and mice with hereditary atransferrinemia have severe hypochromic microcytic anemias.[9-11] Furthermore, when compared with wild-type animals, hypotransferrinemic mice show an extremely restricted uptake of ^{59}Fe by the erythron.[12,13] Collectively, the above studies document the stringent dependency of erythroid cells on Tf-bound Fe. Interestingly, in 1962 Bessis and Breton-Gorius themselves reviewed critically their original hypothesis, and did not exclude the possibility that the phenomenon of ''rhopheocytosis'' takes place in the opposite direction, ie, ''the erythroblast imparts the ferritin to the reticular cell.''[14]

Ferritin is not only an improbable source of Fe for the erythroid cells, but it is also an unlikely mediator involved in intracellular translocation of Fe for heme synthesis. Although ferritin has been postulated to act as an intermediate for heme synthesis in erythroid cells,[15,16] several studies failed to show that ^{59}Fe from ^{59}Fe-ferritin could be incorporated into hemoglobin.[17,18] When heme synthesis was inhibited in erythroid cells incubated with ^{59}Fe-transferrin, very little[19,20] or no[21] ^{59}Fe accumulates in ferritin. More importantly, when heme synthesis is restored in these cells, no ^{59}Fe in ferritin was used for heme synthesis.[20] These findings concur with observations that the intracellular Fe release from ferritin may require its catabolism,[22] and suggests limited availability of ferritin Fe for metabolic purposes.

The evidence from previous studies showing that Tf and not ferritin is the Fe donor for hemoglobin synthesis must now be considered with the recent investigation by Gelvan et al.[1] In this latter study the authors have incubated erythroid precursors with ^{59}Fe-labeled ferritin for 21 hours, at which time they detected some ^{59}Fe in the cells and in heme. Unfortunately, a number of technical flaws prevent the conclusion that ^{59}Fe found in heme comes from ^{59}Fe in the ferritin core. To prepare ^{59}Fe-labeled ferritin, Gelvan and associates have exposed apoferritin to a mixture of 5% ^{59}Fe(III) (as ^{59}FeCl$_3$) and 95% ^{56}Fe(II) in an oxygenated buffer at pH 7.0. It is essential to point out that apoferritin shells can be loaded with Fe(II), whereas attempts to load Fe(III) into the core have failed.[23] In their study, Gelvan et al probably presumed that ^{59}Fe(III), in the presence of a large excess of unlabeled Fe(II), would be converted to ^{59}Fe(II). However, in the absence of Fe-binding ligands such a conversion has been shown to occur only at an extremely low pH ($<$1.0, ref 24). From method description[1] it is not apparent what ligand was present in the MOPS buffer that would stabilize both Fe(II) and Fe(III) in aqueous solution at pH 7.0 to allow electron self-exchange between the two redox states of Fe to occur. MOPS buffers are sometimes supplemented with EDTA (although this is not specified in ref 1) which, however, has almost 11 orders of magnitude higher formation constant for Fe(III) than for Fe(II).[25] Hence, under aerobic conditions the Fe(II)-EDTA complex should be rapidly oxidized to Fe(III)-EDTA complex, but no reduction of ^{59}Fe(III)-EDTA would

occur. On the other hand, in the absence of any ligand, at pH 7.0, [59]Fe(III) would be rapidly hydrolyzed into virtually insoluble ferric hydroxide polymers that could become nonspecifically associated with the external surface of ferritin shells.

The most troubling aspect of this study is that no attempt was made to determine that the ^{59}Fe was actually present within the core of the ferritin molecule in contrast to nonspecifically bound to the surface. Hence, the physiological relevance of this ferritin preparation must be questioned. This poorly defined ^{59}Fe-ferritin was then used for experiments at an Fe concentration of 6.5 mmol/L, ie, about 1,000-fold higher than the concentration of Fe found in the plasma under physiologic conditions. In addition, because the erythroblasts used in this study were not depleted of internalized Tf, it is possible that endocytosed Tf present within the cells could remove ^{59}Fe from the surface of ferritin and donate it to the cells. It is also conceivable that some of the $^{59}Fe(OH)_3$-polymers could be internalized by cells via nonspecific pinocytosis. Moreover, because of the aforementioned problems it is impossible to determine the specific radioactivity of ^{59}Fe available for cellular uptake. Hence, it is impossible to estimate net Fe uptakes in terms of picomoles/10^6 cells, and no reasonable comparisons with Fe uptakes from Tf can be made. Apart from these problems, a number of important control experiments need to be completed before the physiological relevance of Fe uptake from ferritin can be postulated. These include time kinetics of ^{59}Fe-ferritin uptake and saturability of ^{59}Fe-ferritin uptake as a function of ferritin concentration. These latter studies need to be done with ferritin containing ^{59}Fe in its core.

In conclusion, the experiments by Gelvan et al[1] have not been adequately performed to conclude that ferritin plays a role as an Fe donor for hemoglobin synthesis. We strongly believe the investigators should repeat their experiments with ferritin, which has been definitely shown to contain ^{59}Fe in its core. In combination with the additional controls described above, this strategy will then be appropriate to determine the role of ferritin in heme synthesis by erythroid cells.

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Response

We fully agree with Drs Ponka and Richardson that transferrin is most likely the major iron donor for hemoglobin production in erythroid cells, as was also understandable from our report. However, because there is another major iron-protein in biologic systems, namely ferritin, our study dealt with the possibility that this iron-protein, or certain types of it, may be actively involved in iron transfer and utilization and does not serve exclusively for a dead-end storage purpose. Our results, working with early erythroid precursors,[1,2] suggest such a role. This suggestion is backed by numerous other studies. Thus, Blight and Morgan[3] showed that guinea pig reticulocytes take up ferritin by a receptor-mediated process and use its iron for heme synthesis. Moreover, the ferritin iron competed with iron derived from transferrin. Similarly, Nunez et al[4] demonstrated ferritin-iron uptake and utilization for heme synthesis in rabbit reticulocytes. Ulvik and Romslo[5] showed that ferritin iron can be used in the ferrochelatase reaction of isolated rat mitochondria. We have recently shown that iron of ferritin produced by human macrophages was incorporated into hemoglobin when these macrophages were co-incubated with early erythroid cells.[6] Based on our and others studies, we suggest that the transferrin pathway is by no means the only one. The ''consensus'' that transferrin is the only source for heme iron in erythroid cells is unfounded. The fact that atransferrinemic mice and humans still synthesize hemoglobin, albeit at lower levels than healthy individuals, enabling them to survive, supports this view, since in the absence of transferrin ferritin is the only potential iron donor.

Regarding the technical questions raised by Ponka and Richardson, we regret that our attempt to be concise resulted in a misconception. Indeed, the labeling of apoferritin with ^{59}Fe was done in MOPS buffer pH 7.0 with no additional Fe binding ligands. However, equilibration between Fe(II) and Fe(III) was achieved in 0.1N HCl. The iron loading was performed essentially as described by Levi et al,[7] MOPS buffer was chosen over MES buffer because the autoxidation rate of iron in MOPS was slower than in any of several other Good buffers tested, thereby decreasing the tendency for the formation of nonferritin ferric-hydroxypolymers. Small amounts of such polymer and aggregated ferritin, as evident from Treffry et all,[8] were essentially removed by filtration before the use of the ferritin preparation, as shown by polyacrylamide gel electrophoresis under nonreducing conditions followed by autoradiography. The incorporation of the ferritin associated iron into the iron core under the conditions used has been strongly established.[7,8] Our previous study established that free or protein-associated iron-hydroxypolymers, not incorporated in the ferritin core, dissolve during staining with Prussian blue. However, our ferritin-associated iron was unaffected by this stain, strongly indicating iron incorporating into the ferritin core rather

than its accumulation on the protein surface. Thus, we believe that all necessary precautions have been taken to ensure the propriety and purity of the ferritin administered to the cells.

In conclusion, we do not dispute the role of transferrin as the main iron donor to erythroid cells; however, we do not accept the notion that it is the only molecule capable of performing this function.

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Association Between ACP$_1$ and Favism: A Possible Biochemical Mechanism

To the Editor:

An association between hemolytic favism and ACP$_1$ genotype in male subjects from the populations of Rome and Sardinia was reported by our group in 1971,[1,2] but it was not possible at the time to propose a fully satisfactory explanatory mechanism. Since then, a number of important findings regarding the enzymatic properties of ACP$_1$ and the biochemistry of hemolysis have been obtained, suggesting a biochemical mechanism for such association.

Band-3-protein (B3P) may regulate the glycolytic rate and its clustering plays a central role in the chain of events leading to erythrocyte destruction. Phosphofructokinase, aldolase, glyceraldehyde-3-phosphate-dehydrogenase, and catalase have binding sites near the N terminus of the cytoplasmic domain of B3P. A tyrosine residue at position 8 is a substrate for various tyrosine-kinases and its phosphorylation promotes displacement of glycolytic enzymes representing a possible regulatory mechanism for glycolytic activity.[3,4]