

# Iron biominerals in medicine and the environment

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Dedicated by J.W. to the memory of Professor Luigi Sacconi, whose work on 5-coordinate complexes did much to stimulate my undergraduate interests in coordination chemistry and whose generous advice diverted me from postgraduate studies in Florence to bioinorganic chemistry at CalTech.

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## Abstract

The nature and function of iron biominerals, particularly ferrihydrite ( $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ ) and goethite ( $\alpha\text{-FeOOH}$ ), in medicine and the environment are reviewed through three case

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studies: the tissue iron deposits formed in the iron overload associated with the genetic disease of thalassemia, a medical condition of global significance; the tissue iron deposits formed in the liver of the endangered tropical marine mammal, the dugong *Dugong dugon*; the granules formed in the tissue of the freshwater mussel *Velutunio angasi*. © 1999 Elsevier Science S.A. All rights reserved.

**Keywords:** Ferrihydrite; Goethite; Thalassemia; Dugong; Mussel; Mössbauer spectroscopy

## 1. Introduction to biominerals

Iron oxides and oxyhydroxides occur as several phases that have been reported in geological deposits (often of immense magnitude), industrial processes, environmental water sediments and, for some phases, as components of biological systems [1–5]. These phases are listed in Table 1, where those found in biology are specifically indicated. Biominerals are the inorganic phases occurring in biological systems, from microorganisms to the largest of mammals, as can be seen in the surveys reported in several monographs [3–5]. Many mineralogically distinct inorganic phases are now known to occur in the biodiversity of the natural world, with the biominerals of calcium being predominant. Biominerals of barium, strontium and iron are also well known. An illustrative though not exhaustive list of biominerals is shown in Table 2 [6]. Generally, biominerals are found as oxides, hydroxides, phosphates and carbonates but some sulfides such as  $\text{Fe}_3\text{S}_4$  have been reported from reducing sulfidic environments.

Biominerals often occur as key components of larger functional structures. In the case of iron biominerals [7] in the molluscs, chitons and limpets, the teeth are constructed as composite materials with biominerals embedded within a proteinaceous and polysaccharide matrix. In the case of the chiton, the dominant iron biomineral is magnetite  $\text{Fe}_3\text{O}_4$ . This gives a distinctive black coloration to the teeth of the tongue-like radula used for feeding on crustose coralline algae growing on the rocky substrate on which the animals live [8–15]. In the case of the limpet, the dominant iron biomineral is goethite ( $\alpha\text{-FeOOH}$ ), whose acicular crystals are brown in colour [16–18]. These functional structures are shown in Fig. 1 for the chiton

Table 1  
The major iron oxides and oxyhydroxides

| Oxyhydroxides         |                            | Oxides                                             |                           |
|-----------------------|----------------------------|----------------------------------------------------|---------------------------|
| Formula               | Mineral                    | Formula                                            | Mineral                   |
| $\alpha\text{-FeOOH}$ | Goethite <sup>a</sup>      | $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ | Ferrihydrite <sup>a</sup> |
| $\beta\text{-FeOOH}$  | Akaganeite                 | $\alpha\text{-Fe}_2\text{O}_3$                     | Hematite                  |
| $\gamma\text{-FeOOH}$ | Lepidocrocite <sup>a</sup> | $\gamma\text{-Fe}_2\text{O}_3$                     | Maghemite <sup>a</sup>    |
| $\delta\text{-FeOOH}$ | Feroxyhte                  | $\text{Fe}_3\text{O}_4$                            | Magnetite <sup>a</sup>    |

<sup>a</sup> Found in biological systems.

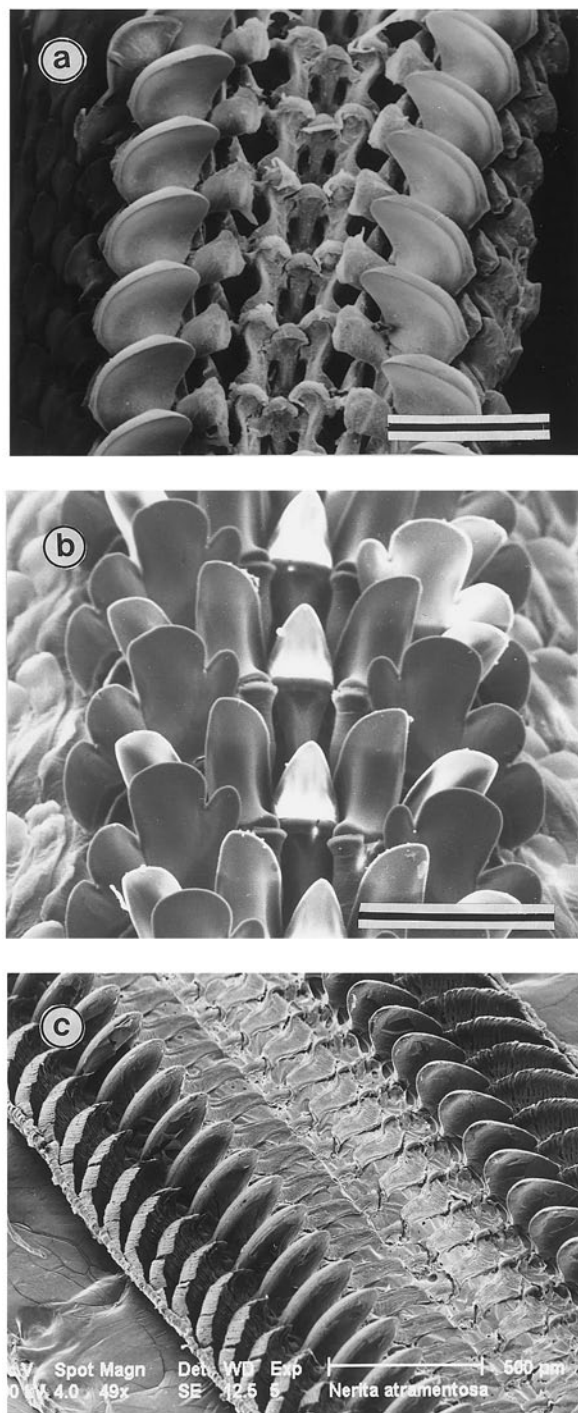


Fig. 1. Scanning electron micrograph of the radula (near the mature end) of (a) the chiton *Acanthopleura hirtosa* (b) the limpet *Patella laticostata* and (c) the gastropod *Nerita atramentosa*. Scale bar = 500  $\mu\text{m}$  for (a) and (c) but 250  $\mu\text{m}$  for (b).

Table 2

Illustrative list of biominerals, their origins and functions (adapted from Refs. [3–6])

| Mineral         | Formula                                            | Organism/Function           |
|-----------------|----------------------------------------------------|-----------------------------|
| Calcite         | $\text{CaCO}_3$                                    | Algae/exoskeleton           |
| Aragonite       | $\text{CaCO}_3$                                    | Molluscs/exoskeleton        |
| Vaterite        | $\text{CaCO}_3$                                    | Ascidians/spicules          |
| Hydroxyapatite  | $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$       | Vertebrates/endoskeleton    |
| Weddelite       | $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ | Plants/Ca store             |
| Gypsum          | $\text{CaSO}_4$                                    | Jellyfish larvae/gravity    |
| Barite          | $\text{BaSO}_4$                                    | Algae/gravity device        |
| Celestite       | $\text{SrSO}_4$                                    | Acantharia/cellular support |
| Silica(opaline) | $\text{SiO}_2 \cdot n\text{H}_2\text{O}$           | Algae/exoskeleton           |
| Magnetite       | $\text{Fe}_3\text{O}_4$                            | Bacteria/magnetotaxis       |
| Goethite        | $\alpha\text{-FeOOH}$                              | Chitons/teeth               |
| Lepidocrocite   | $\gamma\text{-FeOOH}$                              | Limpets, chitons/teeth      |
| Ferrihydrite    | $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ | Many organisms/Fe store     |
| Greigite        | $\text{Fe}_3\text{S}_4$                            | Bacteria/magnetotaxis       |

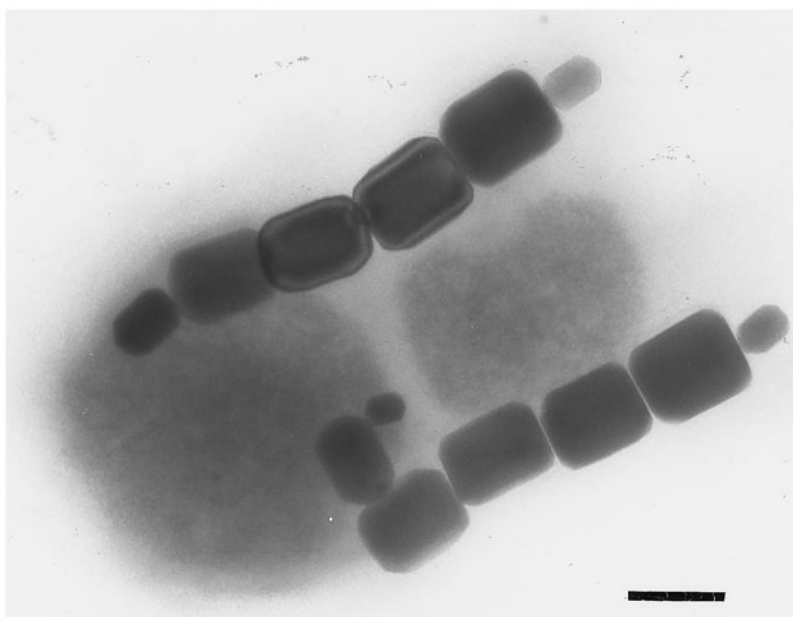


Fig. 2. Electron micrograph of magnetite crystals following cell rupture of an isolate of magnetotactic bacteria. Scale bar = 50 nm.

*Acanthopleura hirtosa* (Fig. 1a) and for the limpet *Patella laticostata* (Fig. 1b), together with the teeth of another mollusc, *Nerita atramentosa*, (Fig. 1c) where arrays of inorganic granules are embedded in the tooth matrix [19,20].

As noted in Table 1, magnetite also occurs in microorganisms: the magnetite crystals in a species of magnetotactic bacteria are shown in Fig. 2. Further details are available in the monograph devoted to iron biominerals [7]. Magnetite has also been reported in several species. Its presence in the human brain has stimulated a series of studies of iron biomineralization in the brain, particularly as it may be involved in certain kinds of epilepsy [21,22].

Recently, the biosynthesis of these inorganic materials and their associated organic structural matrices has attracted much attention from materials scientists, inspired by the insights of visionaries such as the late Derek Birchall, who urged: “It is as well, then, to look for fresh insights to biology at the wisdom encapsulated in the materials it uses” [23]. Several mechanisms by which such biominerals are formed have been proposed:

- biologically induced mineralisation;
- biologically controlled mineralisation;
- facilitated assembly via foams, emulsions and vesicles.

These have been considered in more detail elsewhere [6,24–30].

## 2. Cellular iron biominerals in tissues

### 2.1. Ferritin

Cellular iron biominerals can be differentiated as soluble or insoluble. The soluble form, ferritin, is an approximately spherical molecule with a central cavity or core within which the iron(III) oxyhydroxide particle is held [31–34]. The diameter of the cavity is about 8 nm. The protein shell, which is approximately 2 nm thick, renders the particle water soluble. In the normal condition, ferritin is the major iron biomineral found in tissues.

The structures of the mineral particles within mammalian ferritins have been found to be based on that of the mineral ferrihydrite ( $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$ ) [31] with variations in the degree of crystallinity and particle size being observed for ferritins from different human organs and disease states [32,33]. The electron dense cores can be visualised on the electron microscope as shown in Fig. 3. The protein shell surrounding the core is not seen in this imaging procedure (though it is readily apparent when the sample is negatively stained with phosphotungstic acid). The nanoscale iron particles are prevented from aggregation by the protein shells which also maintain them in solution.

### 2.2. Hemosiderin

The insoluble form of tissue iron deposits is known as hemosiderin. At higher levels of iron loading such as is found in iron overload disease, iron(III) oxyhydroxide particles are mostly found in insoluble aggregates associated with protein residues and, at times, some cellular debris. The heterogeneity of hemosiderin is apparent in the electron micrograph shown in Fig. 4.

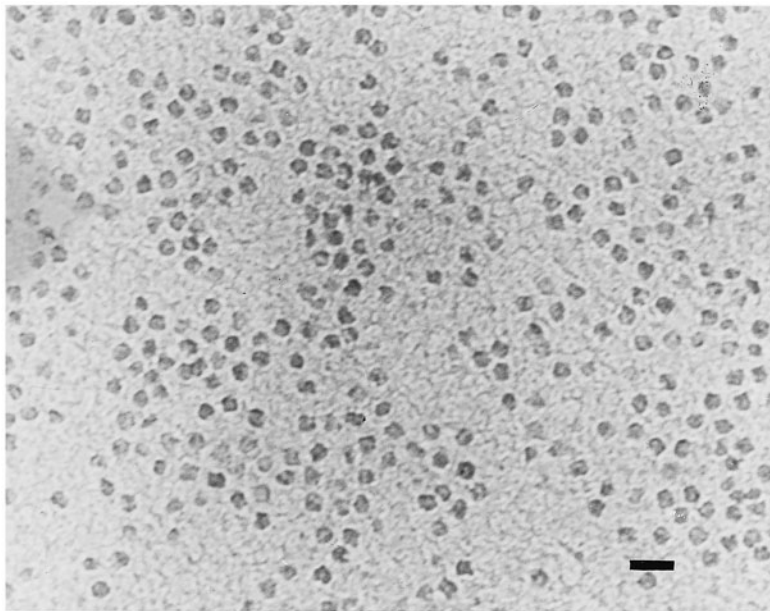


Fig. 3. Transmission electron micrograph of a preparation of ferritin. Scale bar = 20 nm.

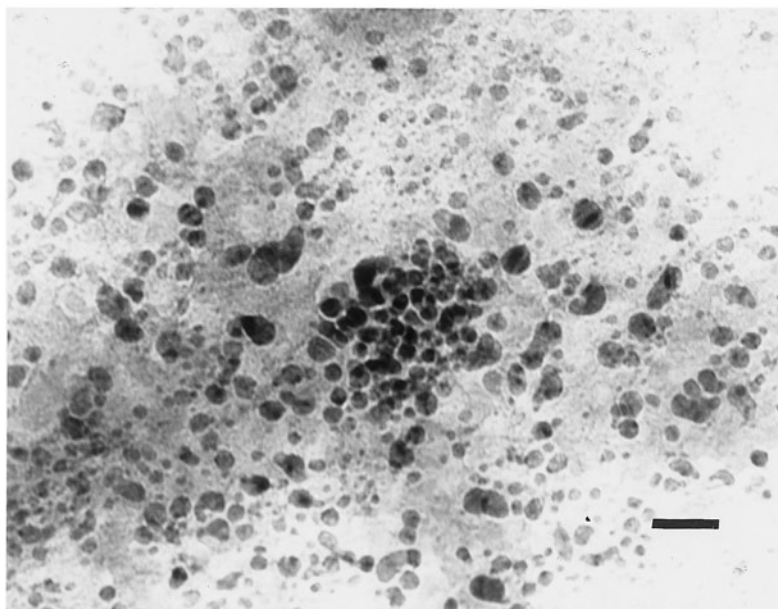


Fig. 4. Transmission electron micrograph of a preparation of hemosiderin. Scale bar = 20 nm.

Three quite different iron(III) oxyhydroxide mineral structures have been identified in hemosiderins from different human tissues in several case studies [32,35–42]. The three different structures are similar to (i) the mineral ferrihydrite, (ii) a highly defect and poorly crystalline goethite ( $\alpha$ -FeOOH), and (iii) non-crystalline hydrated iron(III) oxyhydroxides. Preliminary studies suggested that there may be relationships between the mineral form of the iron(III) oxyhydroxide particles of hemosiderin and the type of disease and/or the clinical treatment administered to the patient. However, insufficient numbers of patients in these case studies prevented confident conclusions being drawn about such relationships. In fact, a more recent study has questioned the validity of the proposed relationships [38] based on two further case studies. Thus, the question of whether the mineral structure of hemosiderin iron deposits is determined by the type of disease, the clinical treatment administered, or some other factor had, until recently, still not been satisfactorily answered.

The ultrafine particles of such iron biominerals can best be characterised by employing a range of instrumental techniques. These include electron microscopy, electron and X-ray diffraction, vibrational spectroscopy, X-ray absorption spectroscopy (both XANES and EXAFS), magnetic measurements and Mössbauer spectroscopy. We have found low temperature Mössbauer spectroscopy to be of particular value in distinguishing the various forms of nanoscale iron biominerals [43,44].

### 3. Case studies

In the present paper, these processes provide the conceptual framework for consideration of the nature and function of iron biominerals, particularly ferrihydrite and goethite, in three case studies:

- the tissue iron deposits formed in the iron overload associated with the genetic disease of thalassemia, a medical condition of global significance.
- the tissue iron deposits formed in the liver of the endangered tropical marine mammal, the dugong *Dugong dugon*.
- the granules formed in the tissue of the freshwater mussel *Velutunio angasi*.

#### 3.1. Case study 1: human genetic disease of thalassemia

Diseases such as the thalassemias and genetic hemochromatosis are examples of iron overload diseases. In both thalassemia and hemochromatosis, excess iron is deposited in the tissues in the form of ultrafine particles of iron(III) oxyhydroxide [32].

In the case of hemochromatosis, the iron overload is a primary symptom of the disease in that there is a genetic defect [45,46] resulting in a loss of control of iron absorption from the diet. Once diagnosed, the disease can be effectively treated by regular phlebotomy to remove iron. In the case of thalassemia, there is a genetic effect resulting in ineffective synthesis of hemoglobin leading to anemia [47]. The anemia, if left untreated, leads to increased erythropoiesis and increased absorption

of iron from the diet [48]. The increased iron absorption results in iron overload. Most cases of thalassemia major in wealthy countries are treated with regular red cell transfusions (to alleviate the anemia). While the blood transfusions suppress increased erythropoiesis and thus curtail excessive iron absorption from the diet, transfusions in themselves constitute a large influx of iron to the body. Thus, iron chelation therapy is normally administered in conjunction with red cell transfusion therapy. In less wealthy countries, however, thalassemic patients may receive few, if any, blood transfusions and no chelation therapy.

We have recently completed a detailed statistical analysis of Mössbauer spectral data directed to the question of whether the mineral structure of hemosiderin iron deposits is determined by the type of disease, the clinical treatment administered, or some other factor [49]. The study aimed to elucidate these questions by studying the form of iron(III) oxyhydroxide deposits in a relatively large number of patients from two quite distinct and identifiable groups, namely (i) seven Australian  $\beta$ -thalassemic patients, who have received multiple transfusions of packed red cells and regular chelation therapy and (ii) 12 Thai  $\beta$ -thalassemia/hemoglobin E patients, who have received few, if any, red cell transfusions and no chelation therapy. The thalassemia syndromes are widespread in south-east Asia, where they constitute a major public health problem [50,51].

Mössbauer spectra of spleen samples from these patients were recorded with samples at 78 K. Typical spectra are shown in Fig. 5. All spectra showed a relatively intense central doublet with spectral parameters (Table 2) indicative of paramagnetic or superparamagnetic high-spin Fe(III). Many of the spectra also clearly showed a sextet signal (Fig. 5b). Some spectra showed an additional low intensity doublet which could be attributed to heme iron. Spectral parameters for the sextet component observed for the group of spleen samples with the highest sextet-signal to noise ratio are shown in Table 3. Data for other tissue samples, liver and pancreas, are given elsewhere [49].

On the basis of previous reports from our laboratory and others [52–58], these spectral components can be identified as being due to polynuclear iron(III) oxyhydroxide deposits in the tissue. This is consistent with the fact that the predominant form of iron found in iron loaded tissues is usually hemosiderin [35]. The doublet component in the spectrum has parameters consistent with (a) ferritin, (b) non-crystalline hemosiderin, (c) hemosiderin based on the structure of the mineral ferrihydrite, or (d) the doublet component associated with hemosiderin based on the structure of the mineral goethite (the doublet component being due to those hemosiderin particles with a smaller magnetic anisotropy energy) [32,36,37]. As such it is not possible to unambiguously identify the source of this signal and the signal may be due to a combination of the above forms of iron. However, the sextet component in the spectrum can be identified as being due to the presence of hemosiderin based on the structure of the mineral goethite since this is the *only* form of tissue iron deposit known to give a Mössbauer spectral sextet component with these parameters at 78 K [38–40].

Sextet signals in the thalassemic spleen samples have significantly higher values of the fraction of the non-heme Mössbauer signal in the form of sextet at 78 K ( $F_s$ )



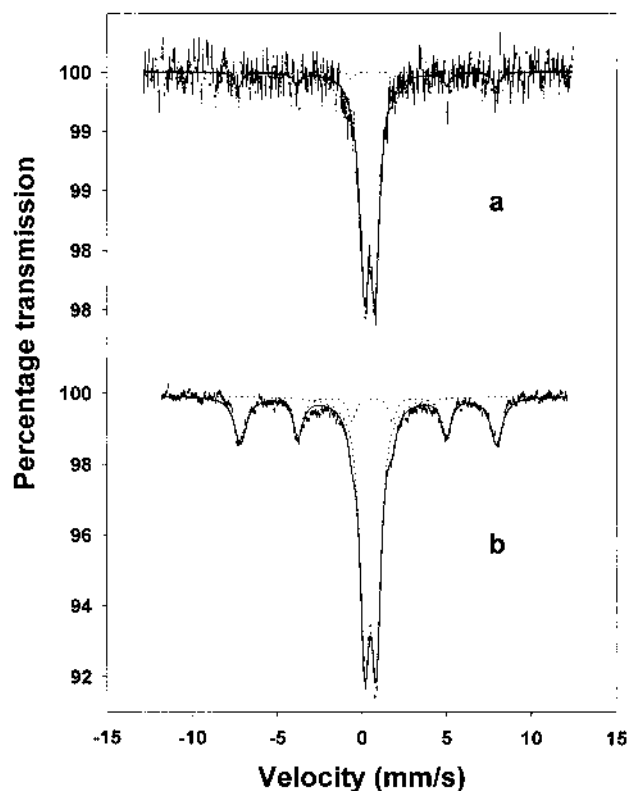


Fig. 5. Mössbauer spectra of (a) a sample of Thai  $\beta$ -thalassemia/Hemoglobin E spleen and (b) a sample of Australian  $\beta$ -thalassemia spleen at 78 K.

in the Australian  $\beta$ -thalassemia spleens than the Thai  $\beta$ -thalassemia/Hb E spleens (Table 3 and Fig. 6). This indicates that the fraction of non-heme iron in the goethite-like form is significantly greater in the Australian  $\beta$ -thalassemia spleens than in the Thai  $\beta$ -thalassemia/Hb E spleens. This study thus provided the first statistically based comparison of the hemosiderins in different thalassemia patient groups.

Table 3

Mean values and ranges for Mössbauer spectral parameters of sextet components in thalassemic spleen tissues at 78 K (from Ref. [49])<sup>a</sup>

| Tissue | $\delta$  | $\Delta E_Q$   | $B_{\text{hf}}$ | $\Gamma$  |
|--------|-----------|----------------|-----------------|-----------|
| Spleen | 0.47      | −0.21          | 47.0            | 0.86      |
| Range  | 0.42–0.51 | −0.28 to −0.13 | 46.5–47.4       | 0.73–0.99 |

<sup>a</sup>  $\delta$  is the centre shift in  $\text{mm s}^{-1}$ ,  $\Delta E_Q$  is the quadrupole perturbation,  $B_{\text{hf}}$  is the magnetic-hyperfine-field splitting in T, and  $\Gamma$  is the full linewidth at half height of the outer lines of the sextet in  $\text{mm s}^{-1}$ .

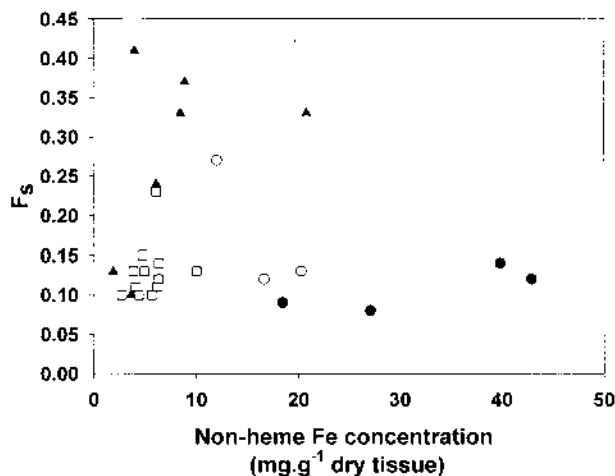


Fig. 6. The fraction  $F_s$  of the non-heme Mössbauer signal in the form of sextet versus non-heme iron concentration in human tissues.  $\square$ , Thai  $\beta$ -thalassemia/Hb E spleen;  $\blacktriangle$ , Australian thalassemia spleen;  $\bullet$ , Thai  $\beta$ -thalassemia/Hb E liver;  $\circ$ , Thai  $\beta$ -thalassemia/Hb E pancreas.

There could be several reasons for the difference in chemical speciation of spleen iron between the two groups of thalassemia patients studied including: (a) the different genotypes of the two groups; (b) the effect of blood transfusions; (c) the effect of chelation therapy; (d) differences in diet. Experiments on animal systems loaded with iron both by hypertransfusion and by dietary sources [59,60] may help identify which factors may have the most influence on the speciation.

The fact that there is different chemical speciation of iron in tissues between different identifiable groups of patients has implications for the treatment and management of iron overload diseases. The reactivities of the different iron oxide forms are expected to be different. Ferrihydrite is less stable than goethite. Its solubility product is about  $10^{-38}$ – $10^{-39}$  compared with  $10^{-42}$ – $10^{-44}$  for goethite [61]. Although the goethite-like form of hemosiderin is likely to be more soluble than goethite itself, hemosiderin from transfusional iron loaded patients has been shown to release iron less readily than the ferrihydrite cores of ferritin when exposed to various chelators [52,62,63]. Thus, the ferrihydrite form of hemosiderin may be more toxic to cells than the goethite-like form, on an atom for atom basis, because of its higher solubility. On the other hand, it should be easier to chelate and remove from the body than the goethite-like form. This may partly explain why the Australian patients had a higher fraction of their non-heme spleen iron in the goethite-like form. More of the ferrihydrite form may have been removed by the chelation therapy, the remaining deposits thus being enriched in the goethite-like form. As new chelation regimes continue to be developed, using chelators other than desferrioxamine, the only chelator in current routine clinical use, it may be possible to target different forms of hemosiderin present in different tissues. These data clearly delineate the presence of different forms of iron in thalassemia tissue

and raise provocative questions and opportunities for further research directed to both increasing our understanding of the underlying molecular pathology of iron overload diseases and to improving patient care.

### 3.2. Case study 2: marine mammal *Dugong dugon*

The sea mammal dugong, *Dugong dugon* (Müller) is an herbivorous marine mammal [64,65]. The dugong is found in tropical regions of the ocean, extending through the subtropical and tropical coasts of the Indian and western Pacific Oceans. Dugongs spend much of their time feeding on the seagrass in the coastal waters. The dugong is a large mammal that can weigh up to 400 kg and be 3 m in length [64]. Since the dugong spends all of its life in the coastal marine environment, it is vulnerable to anthropogenic activity, through pollution, injury through collisions with marine vessels, traditional hunting and, particularly, loss of habitat and the availability of seagrass, the main source of nutrition for the animal. Access to tissue samples for analysis is quite limited.

The initial observation of high liver tissue levels of iron [65] has been confirmed together with the rather surprising observation that this high level of iron appears not to damage the dugong liver tissue [66]. The levels of iron in four samples of dugong from tropical waters of Australia are shown in Table 4 and can be compared with that reported for thalassemia liver iron of  $32200 \mu\text{g g}^{-1}$  dry wt. [49]. A light micrograph of the dugong liver is shown in Fig. 7, where the electron dense deposits of hemosiderin are readily seen.

Hemosiderin isolated from dugong tissue has been studied by variable temperature Mössbauer spectroscopy over the range 17–200 K. The data are shown in Fig. 8. As discussed in detail above in Case Study 1, the significant temperature to note is 78 K, where a sextet component is clearly present. Curve fitting allows the spectral parameters to be determined (see Table 5) including the relative spectral areas of the doublet and sextet components. In particular, the sextet component has grown to 66% of spectral area at 17 K. At 78 K, it is already 35% of the spectral area, comparable to that of the highest Fs values shown in the thalassemia data of Fig. 5.

The presence of such a high fraction of the goethite form of hemosiderin in the dugong liver tissue is a matter of some surprise. In the thalassemia situation, this

Table 4  
Tissue iron concentrations ( $\mu\text{g g}^{-1}$  dry wt.) in four specimens of the dugong *Dugong dugon*<sup>a</sup>

| Specimen | 1     | 2     | 3     | 4     |
|----------|-------|-------|-------|-------|
| Liver    | 47100 | 19300 | 12700 | 71100 |
| Spleen   | 7600  | n/a   | n/a   | n/a   |
| Heart    | 340   | n/a   | n/a   | n/a   |
| Kidney   | 1220  | n/a   | n/a   | n/a   |

<sup>a</sup> Specimens 1, 2 and 3 were collected from animals drowned in Shark Bay, Western Australia; specimen 4 came from traditional hunting in Gulf of Carpentaria, Northern Territory, Australia.

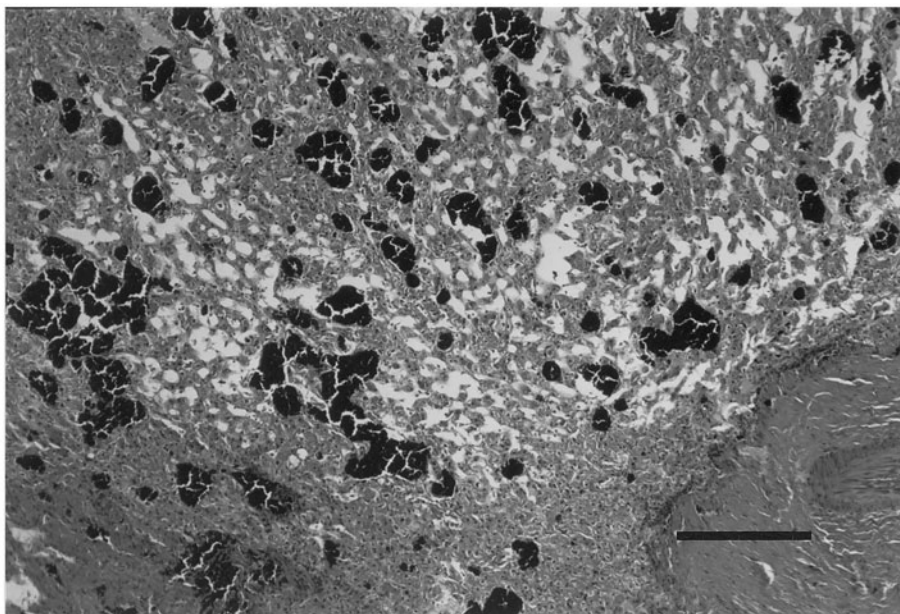


Fig. 7. Light micrograph of liver tissue of dugong *Dugong dugon*, unstained section. Scale bar = 100  $\mu\text{m}$ .

form of hemosiderin is found predominantly in the transfusional overload tissue. Dugongs receive their iron by the dietary route and it suggests that the dugong physiology provides for the synthesis of the thermodynamically more stable and presumably less toxic, form of iron(III) oxyhydroxide. The mechanisms by which this is achieved are not yet known.

More extensive environmental reports on the heavy metal composition of dugong liver, seagrass and sediments, as well as the characterisation of the liver iron biominerals, ferritin and hemosiderin, are in preparation.

Table 5

Mössbauer spectral parameters of a sample of freeze-dried dugong liver tissue at temperatures of 17 and 200 K<sup>a</sup>

| Doublet component |          |              |          |       | Sextet component |              |          |                 |       |
|-------------------|----------|--------------|----------|-------|------------------|--------------|----------|-----------------|-------|
| T (K)             | $\delta$ | $\Delta E_Q$ | $\Gamma$ | A (%) | $\delta$         | $\Delta E_Q$ | $\Gamma$ | $B_{\text{hf}}$ | A (%) |
| 200               | 0.41     | 0.67         | 0.72     | 100   | —                | —            | —        | —               | 0     |
| 17                | 0.49     | 0.54         | 1.50     | 34    | 0.46             | −0.25        | 0.78     | 48.2            | 66    |

<sup>a</sup>  $\delta$  is the centre shift in  $\text{mm s}^{-1}$ ,  $\Delta E_Q$  is the quadrupole perturbation,  $B_{\text{hf}}$  is the magnetic-hyperfine-field splitting in T, and  $\Gamma$  is the full linewidth at half height of the outer lines of the sextet in  $\text{mm/s}$ . A% is the percentage spectral area of each subcomponent.

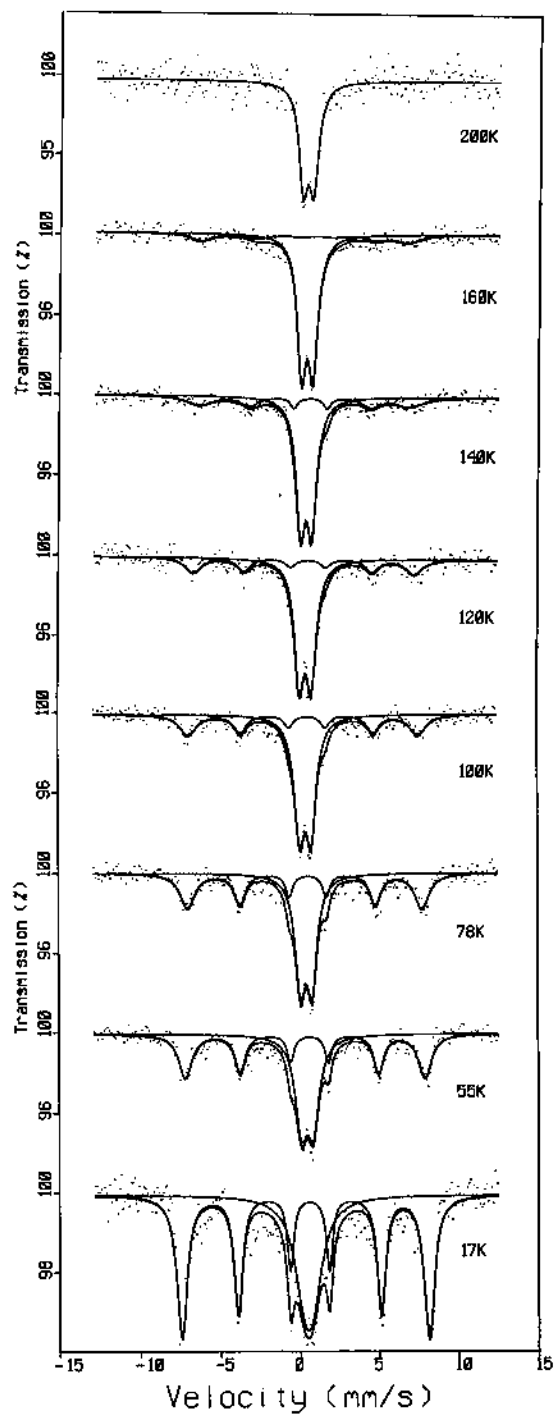


Fig. 8. Mössbauer spectra of liver tissue of dugong *Dugong dugon* at temperatures ranging from 17 to 200 K.

### 3.3. Case Study 3: freshwater mussel, *Velusunio angasi*

The freshwater mussel, *Velusunio angasi*, occurs in the stream beds of creeks and billabongs (lake-like water bodies) in tropical regions of the Northern Territory, Australia. In appearance, it is black shelled (analogous to the common marine mussel *Mytilus (Galloprovincialis) edulis*) about 5 cm long and 3 cm wide. It is part of the traditional diet of Aboriginal Australians living in the region. In creeks, near the Ranger uranium mine, it is known to concentrate radionuclides, particularly  $^{226}\text{Ra}$ , within insoluble granules in its tissue [67,68]. These granules also contain appreciable amounts of Ca, Mg, Ba, P, Fe and Al. In this case study of iron biominerals, the nature and role of iron in the mussels is of interest.

The granules are readily noted on dissection as brown inclusions in and surrounding almost all organs. Typical iron levels in the whole animal and major organs are given in Table 6 together with iron levels in the water column near the collection sites. Clearly, most of the iron in the water is particulate [69]. The iron in the mussel is widespread in tissues, but, on isolation, greater than 80% is found in the insoluble fraction of the isolate. Under the electron microscope, granules appear as aggregates of fine particles of micron and submicron size, which, by X-ray analysis, are found to contain Fe. The particles are flocculate with a comparatively homogeneous size distribution.

Speciation of the iron was determined by Mössbauer spectroscopy. The spectrum at liquid helium (4.2 K) consists of a doublet with spectral parameters indicating that the iron is present as iron(III). As shown in Fig. 9, the spectrum shows a partial transformation of the doublet to a sextet at 1.5 K, indicating the onset of magnetic ordering between these two temperatures. The very low temperature for this ordering indicates that the magnetic exchange interactions are quite weak. This is consistent with the iron atoms in the material being not as closely packed as in a well-ordered lattice of a mineral. In addition, there are significant amounts of P present in the granules and in the water column (data not shown) and phosphate groups could serve as bridging groups between the iron atoms, separating them sufficiently to weaken the exchange interactions appreciably. Phosphate and pyrophosphate are known to occur in granular form in other organisms [20]. The spectral characteristics are very similar to those of nanoscale (< 10 nm) hydrated iron(III) phosphate particles found in other biological systems such as bacterial ferritin proteins.

Table 6

Mean iron levels in the freshwater mussel *Velusunio angasi* ( $\text{mg g}^{-1}$  wet weight) and water ( $\mu\text{g l}^{-1}$ ) of Magela Creek, Northern Territory, Australia

| Mussel        |     | Water                            |     |
|---------------|-----|----------------------------------|-----|
| Whole animal  | 2.7 | Filtered (< 0.45 $\mu\text{m}$ ) | 95  |
| Gills         | 3.1 | Particulate                      | 990 |
| Mantle        | 3.9 |                                  |     |
| Visceral mass | 5.6 | % Particulate                    | 91  |

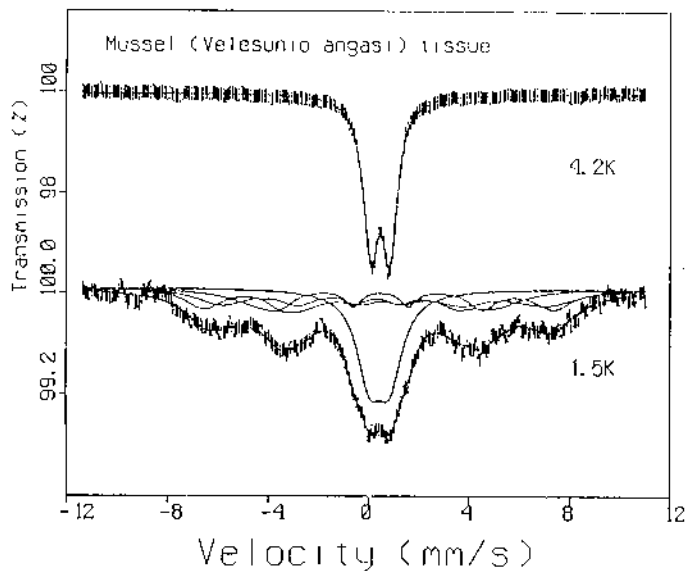


Fig. 9. Mössbauer spectra of tissue granules of freshwater mussel *Velutunio angasi* at 4.2 and 1.5 K.

The particulate iron present in the water column, with its large surface area, could provide a pathway for adsorption of ions such as the radionuclides prior to uptake by the mussels. Other pathways are also possible. The particulate iron is filtered by the mussel and subsequently incorporated in the granules, undergoing some biotic transformation in the process. The extent of this bioprocessing is much less than in the iron biominerals considered in the previous case study, where synthesis from soluble precursors provided the biominerals of ferritin and hemosiderin. The case of the freshwater mussel, *Velutunio angasi* provides a cautionary note to studies of biominerals, since the particulate iron from the water column is the result of abiotic, not biotic, processes.

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### References

- [1] U. Schwertmann, R.M. Cornell, *Iron Oxides in the Laboratory. Preparation and Characterization*, VCH Verlagsgesellschaft, Weinheim, 1991.

- [2] R.W. Fitzpatrick, H.C.W. Skinner (Eds.), *Iron and Manganese Biomineralization Processes in Modern and Ancient Environments*. Catena Supplement 21 (1992).
- [3] S. Mann, J. Webb, R.J.P. Williams, *Biomineralization. Chemical and Biochemical Perspectives*, VCH Verlagsgesellschaft, Weinheim, 1989.
- [4] S. Weiner, H.A. Lowenstam, *On Biomineralization*, Oxford University Press, Oxford, 1989.
- [5] K. Simkiss, K.W. Wilbur, *Biomineralization: Cell Biology and Mineral Deposition*, Academic Press, London, 1989.
- [6] R.B. Frankel, R.P. Blakemore, *Iron Biominerals*, Plenum Press, New York, 1991.
- [7] J. Webb, L.R. Brooker, M.V. Nesterova, A. Lee, W. Chua-anusorn, D.J. Macey, T.G. St. Pierre, *S. Afr. J. Chem.* 50 (1997) 195.
- [8] R.S. Steneck, L. Watling, *Mar. Biol.* 68 (1982) 299.
- [9] J. Webb, D.J. Macey, S. Mann, Ref. [3], (1989) 345.
- [10] H.A. Lowenstam, *Bull. Geol. Soc. America* 73 (1962) 435.
- [11] L.A. Evans, D.J. Macey, J. Webb, *Philos. Trans. Royal Soc.* B329 (1990) 87.
- [12] L.A. Evans, D.J. Macey, J. Webb, *Mar. Biol.* 109 (1991) 28.
- [13] L.A. Evans, D.J. Macey, J. Webb, *Acta Zoologica* 75 (1993) 75.
- [14] D.J. Macey, L.R. Brooker, *J. Morphology* 230 (1996) 33.
- [15] J.L. Kirschvink, H.A. Lowenstam, *Earth Planetary Sci. Lett.* 44 (1979) 193.
- [16] N.W. Runham, *Quart. J. Microsc. Sci.* 102 (1961) 371.
- [17] S. Mann, C.C. Perry, J. Webb, B. Luke, R.J.P. Williams, *Proc. Roy. Soc. Lond B* 227 (1986) 179.
- [18] T.G. St. Pierre, S. Mann, J. Webb, D.P.E. Dickson, N.W. Runham, R.J.P. Williams, *Proc. Roy. Soc. Lond. B* 228 (1986) 31.
- [19] D.J. Macey, L.R. Brooker, V. Cameron, *Molluscan Research* 18 (1997) 33.
- [20] M.G. Taylor and K. Simkiss, Ref. [3] (1989) 427.
- [21] J.L. Kirschvink, A. Kobayashi-Kirschvink, B.J. Woodford, *Proceedings of National Academic of Sciences* 89 (1992) 7683.
- [22] J. Dobson, M. Fuller, S. Moser, H.G. Wieser, J.R. Dunn, J. Zoeger, in: L. Deecke, C. Baumgartner, G. Stroink, S.J. Williamson (Eds.), *Advances in Biomagnetism*, Elsevier, Amsterdam, 1994.
- [23] J.D. Birchall, Ref. [3], (1989) 491.
- [24] H.A. Lowenstam, *Science* 211 (1981) 1126–1129.
- [25] S. Mann, G.A. Ozin, *Nature* 382 (1996) 313.
- [26] C.T. Kresge, *Adv. Materials* 8 (1996) 181.
- [27] S. Mann, D. Walsh, *Chem. Brit.* (1996) 31.
- [28] G.A. Ozin, *Adv. Materials* 8 (1996) 184.
- [29] S. Mann, *Adv. Materials* 8 (1996) 183.
- [30] J.D. Birchall, Ref. [3] (1989) 491.
- [31] P.M. Harrison, F.A. Fischbach, T.G. Hoy, G.H. Haggis, *Nature* 216 (1967) 1188.
- [32] T.G. St. Pierre, J. Webb, S. Mann, in: S. Mann, J. Webb, R.J.P. Williams (Eds.), *Biomineralization: Chemical and Biochemical Perspectives*, VCH Verlagsgesellschaft, Weinheim, 1989, p. 295.
- [33] T.G. St. Pierre, K.C. Tran, J. Webb, D.J. Macey, B.R. Heywood, N.H. Sparks, V.J. Wade, S. Mann, P. Pootrakul, *Biol. Metals* 4 (1991) 162.
- [34] P.M. Harrison, P. Arosio, *Biochim. Biophys. Acta* 1275 (1996) 161.
- [35] M.P. Weir, J.F. Gibson, T.J. Peters, *Cell Biochem. Function* 2 (1984) 186.
- [36] S. Mann, V.J. Wade, D.P.E. Dickson, N.M.K. Reid, R.J. Ward, M. O'Connell, T.J. Peters, *FEBS Lett.* 234 (1988) 69.
- [37] D.P.E. Dickson, N.M.K. Reid, S. Mann, V.J. Wade, R.J. Ward, T.J. Peters, *Biochim. Biophys. Acta* 957 (1988) 81.
- [38] R.J. Ward, M. Ramsey, D.P.E. Dickson, C. Hunt, T. Douglas, S. Mann, F. Aouad, T.J. Peters, R.R. Crichton, *Eur. J. Biochem.* 225 (1994) 187.
- [39] T.G. St. Pierre, K.C. Tran, J. Webb, D.J. Macey, P. Pootrakul, D.P.E. Dickson, *Hyperfine Interac.* 71 (1992) 1279.
- [40] T.G. St. Pierre, K.C. Tran, J. Webb, D.J. Macey, P. Pootrakul, in: S. Suga, H. Nakahara (Eds.), *Mechanisms and phylogeny of mineralization in biological systems*, Springer-Verlag, Tokyo, 1991, p. 291.



- [41] J. Webb, T.G. St. Pierre, K.C. Tran, W. Chua-anusorn, D.J. Macey, P. Pootrakul, *Inorg. Chim. Acta* 242–243 (1996) 121.
- [42] W. Chua-anusorn, T.G. St. Pierre, J. Webb, D.J. Macey, P. Yansukon, P. Pootrakul, *Hyperfine Interac.* 91 (1994) 905.
- [43] J. Webb, T.G. St. Pierre, in: G.J. Long, F. Grandjean (Eds.), *Mössbauer Spectroscopy Applied to Inorganic Chemistry*, vol. 3, Plenum, New York, 1989.
- [44] E. Murad, J.H. Johnston, in: G.J. Long (Ed.), *Mössbauer Spectroscopy Applied To Inorganic Chemistry*, vol. 2, Plenum Publishing Corporation, New York, 1987, p. 507.
- [45] J.N. Feder, A. Gnirke, W. Thomas, Z. Tsuchihashi, D.A. Ruddy, A. Basava, F. Dormishian, R. Domingo, M.C. Ellis, A. Fullan, L.M. Hinton, N.L. Jones, B.E. Kimmel, G.S. Kronmal, P. Lauer, V.K. Lee, D.B. Loeb, F.A. Mapa, E. McClellan, N.C. Meyer, G.A. Mintier, N. Moeller, T. Moore, E. Morikang, C.E. Prass, R.K. Wolff, et al., *Nature Genetics* 13 (1996) 399.
- [46] L.W. Powell, E.C. Jazwinska, *New. Eng. J. Med.* 335 (1996) 1837.
- [47] D.R. Higgs, *Quart. J. Med.* 86 (1993) 559.
- [48] P. Pootrakul, S. Hungspenges, S. Fucharoen, D. Baylink, E. Thompson, E. English, M. Lee, J. Burnell, C. Finch, *New. Eng. J. Med.* 304 (1981) 1470.
- [49] T.G. St. Pierre, W. Chua-anusorn, J. Webb, D. Macey, P. Pootrakul, *Biochim. Biophys. Acta* 1407 (1998) 51.
- [50] P. Wasi, *Clinical Haematology* 10 (1981) 707.
- [51] S. Fucharoen, P. Winichagoon, *Hemoglobin* 21 (1997) 299.
- [52] S.H. Bell, M.P. Weir, D.P.E. Dickson, J.F. Gibson, G.A. Sharp, T.J. Peters, *Biochim. Biophys. Acta* 787 (1984) 227.
- [53] J.N. Rimbart, F. Dumas, C. Kellershohn, R. Girot, P. Brissot, *Biochimie* 67 (1985) 663.
- [54] J.N. Rimbart, F. Dumas, G. Richardot, C. Kellershohn, *Hyperfine Interac.* 29 (1986) 1439.
- [55] T.G. St. Pierre, D.P.E. Dickson, J.K. Kirkwood, R.J. Ward, T.J. Peters, *Biochim. Biophys. Acta* 924 (1987) 447.
- [56] T.G. St. Pierre, R.K. Pollard, D.P.E. Dickson, R.J. Ward, T.J. Peters, *Biochim. Biophys. Acta* 952 (1988) 158.
- [57] M. Chorxy, R. Meczynski, T.J. Panek, J. Radioanal. Nucl. Chem. Letters 128 (1988) 201.
- [58] D.P.E. Dickson, R.K. Pollard, B. Borch-Iohnsen, R.J. Ward, T.J. Peters, *Hyperfine Interac.* 42 (1988) 889.
- [59] S.C. Andrews, M.C. Brady, A. Treffry, J.M. Williams, S. Mann, M.I. Clenton, W. de Bruijn, P.M. Harrison, *Biol. Metals* 1 (1988) 33.
- [60] W. Chua-anusorn, D.J. Macey, J. Webb, P. de la Motte Hall, T.G. St. Pierre, *BioMetals* 12 (1999) 103.
- [61] U. Schwertmann, R.W. Fitzpatrick, *Catena Supplement* 21 (1992) 7.
- [62] R.J. Ward, M.J. O'Connell, D.P.E. Dickson, N.M.K. Reid, V.J. Wade, S. Mann, A. Bomford, T.J. Peters, *Biochim. Biophys. Acta* 993 (1989) 131.
- [63] M.J. O'Connell, R.J. Ward, H. Baum, T.J. Peters, *Biochem. J.* 260 (1989) 903.
- [64] G.E. Heinsohn, J. Wake, H. Marsh, A.V. Spain, *Aquaculture* 12 (1977) 235.
- [65] G.R.W. Denton, H. Marsh, G.E. Heinsohn, C. Burdon-Jones, *Mar. Biol.* 57 (1987) 201.
- [66] W. Chua-anusorn, T.G. St. Pierre, G. Black, J. Webb, D.J. Macey, D. Parry, *Hyperfine Interactions* 91 (1994) 899.
- [67] R.A. Jeffree, R.D. Simpson, *Comp. Biochem. Physiol.* 79A (1984) 61.
- [68] S.G. George, T.L. Coombs, B.J.S. Pirie, *Biochim. Biophys. Acta* 716 (1982) 61.
- [69] B.N. Noller, *Chem. Aust.* 59 (1992) 403.