Brief Report

Commentary on Hemosiderin

By G. W. Richter and M. C. Bessis

The term hemosiderin ("Hämosiderin") was proposed by Neumann1 though others had earlier recognized the material to which the word refers, notably Perls.² Neumann's definition was clearly based on histologic and histochemical criteria: brown granules giving certain reactions for iron, particularly the Prussian (Berlin) blue test, as seen in situ in cells or tissues through the light microscope. Later microscopists³ have confirmed and extended the observations of Neumann.

The advent of electron microscopy has led investigators to inquire into the fine structure of the granules described by the earlier histologists and histochemists. For this purpose material has been examined in situ in cells as well as after isolation from cell homogenates.⁴⁻¹¹ Some of the findings and conclusions are summarized in this brief report.

As seen in situ in cells, typical, Prussian-blue-positive hemosiderin granules occur in at least five forms:

1. Closely packed cytoplasmic collections of particles of high electron-scattering power, ranging from about 8 Å to about 70 Å in diameter. The collections are often, but not always, enclosed in single (unit) cytoplasmic membranes. Sometimes there is more than one enveloping membrane. Many of the particles can be identified as the (FeOOH) micelles of individual ferritin molecules; these have characteristic size, shape and subunits, as first shown by Farrant.¹² Other particles in the aggregates are clearly not parts of ferritin molecules (figs. 1, 2). Electron diffraction studies have indicated that they are forms of Fe₂O₃, mainly α-Fe₂O₃.H₂O.¹¹ The cytoplasmic bodies as a whole vary greatly in size (up to several microns in diameter).

2. Closely packed cytoplasmic collections of dense particles without admixture of ferritin molecules, but otherwise similar to those described in (1). These also are commonly enclosed in single unit membranes.

3. More complex bodies in which material such as that found in the first and second types are mixed with osmiophilic substances (presumably lipids or "lipofuscin") or with other material of moderate electron-scattering power. These bodies sometimes contain myelin figures or other membranous complexes and have single or multiple unit membranes at their peripheries. Such compound bodies are included in the categories others have termed lysosomes, cytolysosomes, cytosomes etc. This type is frequently encountered in hepatic parenchymal cells in cases of hemochromatosis.

4. Crystals of ferritin with characteristic structure and lattice spacings.

From the Department of Pathology, Cornell University Medical College, New York, N. Y., the École de Médecine, Université de Paris, and Centre National de Transfusion Sanguine, Paris, France.


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Fig. 1.—Part of cytoplasm of rat macrophage cultured in vitro in a medium containing 0.1 per cent FeSO₄. Note that there are several organelles, measuring 1 to 2 μ in cross section, which contain innumerable tiny particles that measure about 60 Å in diameter. Note polymorphism of profiles of these organelles. One contains a complex internal system of membranes. Such organelles appear as Prussian-blue positive granules in the light microscope. Magnification: 59,000 x.

These occur particularly in the cytoplasm of reticular cells in erythroblastic islands of bone marrow (e.g., in man) but they may also be found elsewhere (e.g., in histiocytes of the spleen). These crystals may or may not be enclosed in distinct membranes.

(5) Collections of ferritin molecules without distinct crystalline (or “paracrystalline”) order, but disposed as described in (4).

We can add at this point that intracellular hemosiderin granules resulting from parenteral injections of certain preparations that contain Fe₂O₃ (e.g., iron-dextran, saccharated iron oxide) at first resemble those of type (2). Several days after injection, and subsequently, types (1), (4) and (5) can be found (figs. 1, 2).¹³

It has been established that ferritin gives a positive Prussian blue test, though the literature abounds with statements to the contrary. The interested reader is referred to papers by Thiéry¹⁴ and by Shoden and Richter.¹⁵

Ferritin molecules are often scattered about the cytoplasmic matrix in various sorts of cells, particularly in histiocytes and in parenchymal liver cells.
Fig. 2.—Section through the deposit of hemosiderin in a splenectomy patient. Magnification: 50,000 x.
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and very strikingly so in cases of iron overload. Such cells may give a diffuse cytoplasmic Prussian blue test if the reaction is carried out on properly fixed material and is critically controlled.

In isolating hemosiderin granules from cell homogenates the use of aqueous solvents can result in extraction of ferritin and apoferritin because these are soluble at low salt concentrations. This has been demonstrated with the aid of electron microscopy, especially by using negative staining procedures. It has also been shown chemically and serologically by using anti-ferritin sera. It should be emphasized that the findings just summarized relate only to certain aspects of the constitution of what has loosely been lumped together under the label of hemosiderin. As is well-known, chemical studies have pointed to the presence of various other constituents. This summary deals only with substances that give the Prussian blue test, which is the identifying feature of hemosiderin in situ in cells.

It seems clear that future studies of hemosiderin that are based on material obtained from cell homogenates must be controlled by electron microscopy as has been done in work with mitochondrial preparations, ribosomes, cell nuclei, etc. The residues remaining after extraction of cell homogenates should not be equated to the unextracted material.

In conclusion we should like to emphasize that the classical hemosiderin of the histologist is not a single or unique substance. The constitution of this material is quite variable. Removal of hemosiderin granules from cells for chemical analysis demands careful control based on morphologic criteria and is, in any case, not a substitute, but a concomitant of examination in situ in cells. One should bear in mind that in its natural state hemosiderin occurs in living tissues, not as a test tube isolate, or as a thin section.

SOMMARIO IN INTERLINGUA

Le "hemosiderina" classic del histologo non es un substantia homogenee. Le constitution de iste material es variabilissime. Le extraction de granulos de hemosiderina ab le cellulas con le objectivo de subjicer lo a analyses chimic require un precise controlo a base de criterios morphologic. In omne caso, illo non es le equivalente del examine del celularis in sito. On debe considerar que hemosiderina occurre naturalmente in tissu vive e non es un agente in un tubo de reaction.

REFERENCES


G. W. Richter, M.D., Department of Pathology, Cornell University Medical College, New York, N. Y.

M. C. Bessis, M.D., Ecole de Médecine, Université de Paris and Centre National de Transfusion Sanguine, Paris, France
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