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Non-Hematin Iron in Erythrocytes.

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The structure of ferritin, a crystalline iron-protein compound containing 23% iron, has been discussed in previous papers.[1,2,3] The iron appears to be present in the form of micelles of colloidal iron hydroxide characterized by Fe atoms containing 3 unpaired electrons, and markedly different from various synthetic colloidal iron hydroxides. Crystals containing this iron have been isolated in relatively high yield from spleen, liver and red bone marrow of various species[4] strongly suggesting that this iron arises from hemoglobin decomposition. Since the amount of non-hematin iron in horse spleen is so great (1.8 to 2.0% Fe in dry weight of spleen), it appeared that a histological study of this tissue might reveal the distribution and perhaps the origin of the iron.

1 Granick, S., *J. Biol. Chem.*, 1942, **146**, 451.

2 Granick, S., and Michaelis, L., *J. Biol. Chem.*, 1943, **147**, 91.

3 Michaelis, L., Coryell, Chas., and Granick, L., *J. Biol. Chem.*, 1943, **148**, 463.

4 Granick, S., *J. Biol. Chem.*, in press.

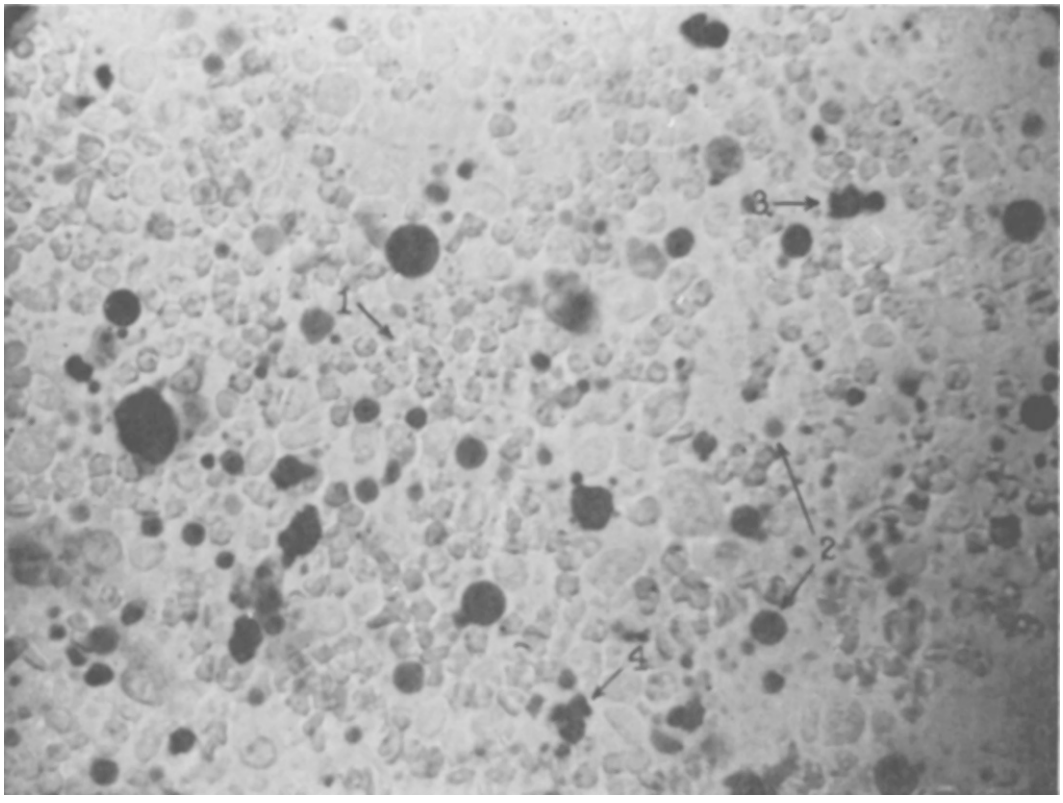

FIG. 1.
H_2S Reaction on Horse Spleen.
1→ Cluster of RBC with black dots.
2→ "Grey" cells.
3→ Hemosiderin granules.
4→ Macrophage containing hemosiderin granules.

A very sensitive test for inorganic iron including the colloidal ferric hydroxide of ferritin is the production of a black insoluble iron sulfide with H_2S. When a piece of horse spleen was teased out into a drop of 1% saline, most of the red blood cells appeared crenated. Cells occasionally appeared which were swollen, had a smooth outline and contained hemoglobin. On the addition of a drop of 1% saline containing H_2S to the tissue, some of the hemosiderin granules became black immediately (3, Fig. 1), thus indicating the presence of iron in them. Other hemosiderin granules remained yellow, indicating that they lacked the non-hematin iron. Some macrophages could be seen to contain large hemosiderin granules which turned black (4, Fig. 1). A number of the crenated erythrocytes developed several very tiny black spots scattered on their membranes (1, Fig. 1). In the presence of H_2S the swollen erythrocytes took on a smooth grey appearance which within a minute became finely granular and darker grey. These latter cells we designate as the "grey cells" (2, Fig. 1).

The "grey" cells have also been observed in horse bone marrow, spleen of guinea pig, spleen of a rabbit and in a human spleen. They have only been observed when the ferritin content appeared to be very high. It was only in the horse spleen that the grey cells could be seen to contain hemoglobin and could therefore be identified as erythrocytes.

In the H_2S reaction the vertebral bone marrow of a 3-year-old horse showed relatively fewer grey cells than did the same animal's spleen. In the marrow the grey cells varied in size from twice the diameter of normal erythrocytes down to 3-5 μ diameter. In a few of the polymorphs the granules also became dark with H_2S.

In the spleens of several guinea pigs with high ferritin content a few grey cells were observed with H_2S; also some black-staining hemosiderin granules in macrophages and some black extracellular granules were observed. In one rabbit spleen a few grey cells were also observed.

In the human material,[4] cases tested with H_2S included No. 10,699 through No. 10,714. Of these cases only those of No. 10,699, monocytic leukemia, and No. 10,709, lymphocytic leukemia, contained much iron in liver and spleen, and these are the 2 cases with the highest ferritin content yet observed. In No. 10,699, the spleen contained a few grey cells per field and a number of tiny pale grey spheres. The liver cells contained numerous heavy black granules. In No. 10,709, the spleen was engorged with erythrocytes among which were present many colorless globules about 1½ times the diameter of the erythrocytes. Only a few of these became pale grey with H_2S. The liver cells were filled with granules but only about 5% of the parenchyma cells contained granules staining grey with H_2S.

Discussion. A plausible explanation for the appearance of the grey cells is that they are degenerating erythrocytes in which the iron of hemoglobin has been set free. It has generally been assumed that red blood cells or their fragments are ingested by macrophages and then broken down. The evidence presented suggests another mechanism; the changes in the erythrocytes may be a function of secretions of the cells lining the sinuses of the spleen. These changes involve increased permeability and fragility and lead to the eventual decomposition of the heme and release of iron without the necessity of phagocytosis by macrophages.

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