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The Presence of Ferritin in the Duodenal Mucosa and Liver in Hemochromatosis.

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The etiology of hemochromatosis is unknown.[1] Recently, work on ferritin has indicated that this substance is a normal iron storage protein of the body. Ferritin, by virtue of its storage property also appears to function in the regulation of iron absorption from the gut. The opportunity was presented of examining whether a relationship might exist between factors governing ferritin formation and those which lead to huge deposits of iron in hemochromatosis.

Case history: Presbyterian Hospital unit number 861951. Admitted March 15, died May 6, 1947.

The patient was a housewife of 56, who had had diabetes mellitus and an enlarged liver for seven years. *B. coli* peritonitis, and bilateral oophorectomy in 1945. She developed congestive heart failure a year before admission, without valve lesions or hypertension. Bronzing of the skin was not noticed until it was called to her attention. During her stay in the hospital auricular fibrillation and ascites appeared. Her diet was difficult to control, as she was disoriented at times. Small doses of standard insulin were used. The laboratory findings were: RBC 4,400,000, Hgb. 13.0 g, WBC 11,900, differential normal. Serum alkaline phosphatase 10.1 Bodansky units, urea N 14 mg%, bilirubin, trace, cholesterol 213 mg%, albumin 3.2%, globulin 2.4%, cephalin flocculation +, prothrombin time 24 sec., blood sugar varied from 70 to 200 mg%, serum CO_2 content 55 vol.%. A biopsy from the left upper quadrant of the abdomen showed normal muscle, but hemosiderosis of the skin compatible with hemochromatosis. She died after 6 weeks, in congestive failure.

Autopsy number 15,292: Death 12:15 a.m., autopsy 2:00 a.m. Organs placed in ice-box, delivered to the Rockefeller Institute 9 a.m. The findings were typical of hemochromatosis, both in the gross and microscopically, with marked cirrhosis of the liver, and fibrosis of the myocardium. There was an old mural thrombus at the apex of the left ventricle, infarcts of liver, spleen and right kidney, and an embolus in the left common iliac artery. A terminal bronchopneumonia and other incidental findings were noted. The liver weighed 2,210 g, spleen 220 g, and heart 340 g. The pancreas showed moderate siderosis, the islets were not remarkable. The pituitary showed a large scarred area in the pars anterior.

Experimental. Ferritin was isolated from the liver and spleen by a procedure described previously.[2] The human ferritin precipitates from solution as brown spheroids, instead of well-defined crystals when $CdSO_4$ is added to a solution of ferritin. Further purification was attempted by dissolving the $CdSO_4$-precipitate in ammonium sulfate (4 g/100 cc). On increasing the ammonium sulfate concentration up to 10 g/100 cc, a brownish slimy material could be centrifuged away. The remaining deep brown solution precipitated out completely on dialysis against distilled water. This precipitate was dissolved by bringing it to pH 5 with acetate buffer and the properties of the resulting solution were examined.

Addition of $CdSO_4$ to an aliquot of the solution resulted in a precipitate of brown spheroids with no suggestion of crystalline shape, indicating that this material was still impure.

* The writers are indebted to Doctors Randolph West and H. P. Smith of the Departments of Medicine and Pathology at Columbia University for the clinical record and the pathological material studied.

1 Sheldon, J. H., *Hemochromatosis,* Oxford University Press, London, 1935.

2 Granick, S., *J. Biol. Chem.,* 1943, **149,** 157.

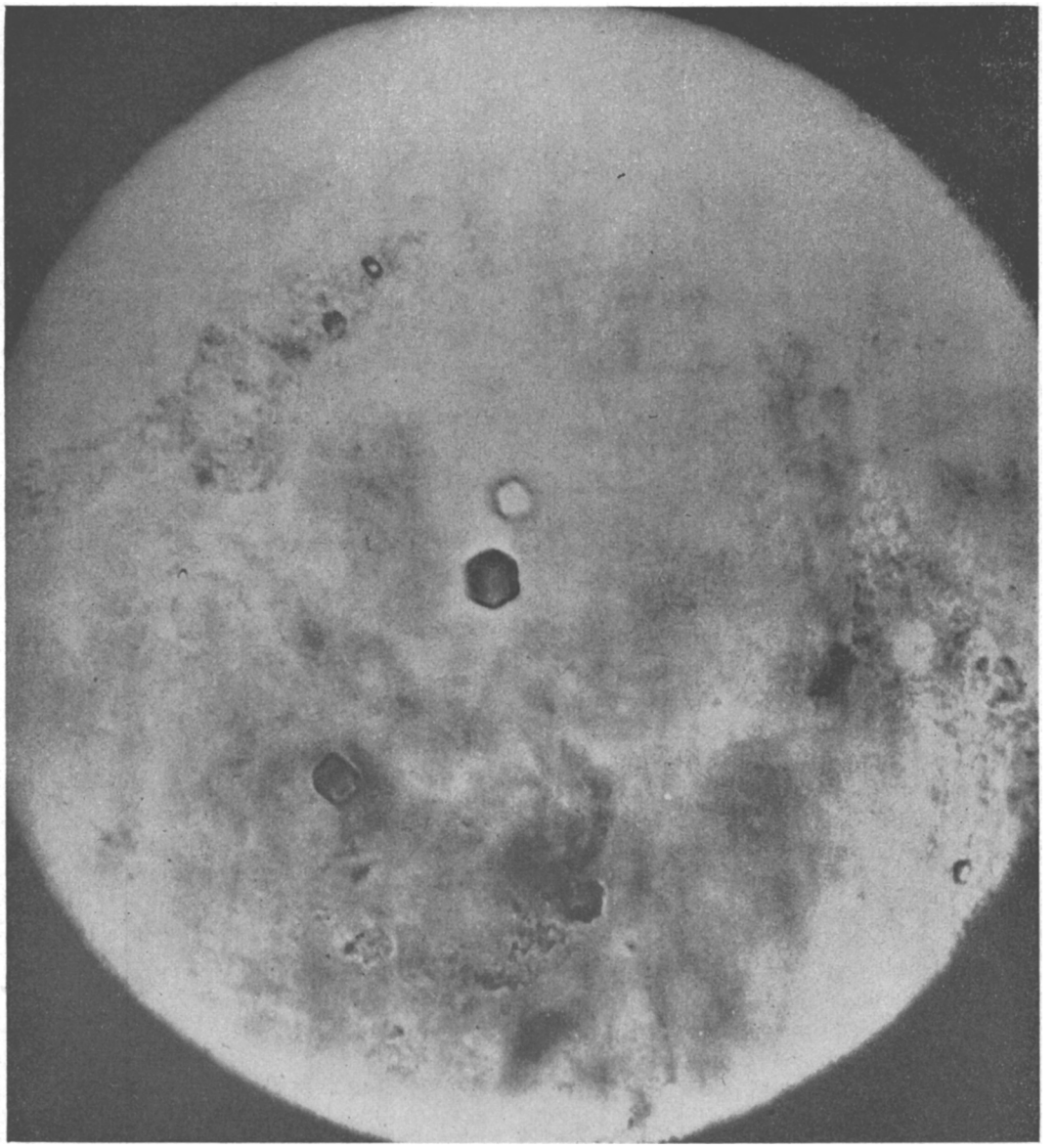

FIG. 1.

The solution contained 5.73 mg Fe/cc and 0.858 mg P/cc, or a ratio of 3.7 Fe : 1 P atom. The phosphorus appeared to belong to the iron hydroxide-phosphate micelles of ferritin since all of the phosphorus and iron could be dialyzed away on treating this ferritin with hydrosulfite at pH 4.6. This material was higher in P than was horse ferritin where the ratio is 8 or 9 Fe to 1 P atom. The ferritin contained 16.5% by dry weight of iron.

Magnetic measurements on this sample gave a value of 3.90 Bohr magnetons (B.M.) per g atom of Fe, the susceptibility being measured at 25°C. This value agrees within the limits of error with the value obtained for ferritin iron from horse and dog.[3] A sample of normal human ferritin kept in the ice-box for 3 years gave a value of 3.94 B.M.

[3] Michaelis, L., Coryell, C. D., and Granick, S., *J. Biol. Chem.*, 1943, **148**, 463.

Reprecipitation of the brown solution with $CdSO_4$ yielded about 1.2 cc of packed globules per 100 g of fresh liver. This is the highest content of ferritin yet obtained from human livers.[2] Ferritin was also isolated from the spleen and had properties of solubility and precipitability with $CdSO_4$ similar to those of the liver material. However, only about 0.11 cc of packed reprecipitated ferritin globules per 100 g fresh spleen were isolated.

A homogenate, prepared from a piece of liver by grinding in a Waring blender and further comminuting in a TenBroeck grinder was subjected to magnetic measurements. It had a value of 4.0 B.M. per g Fe atom. The iron in this homogenate consisted for the most part of ferritin and of hemosiderin granules, with a relatively negligible contribution from the iron porphyrin pigments. It is interesting that this value is not essentially different from the value of ferritin itself, probably indicating that the hemosiderin granules in the liver contain iron in the same structural configuration as in ferritin. A somewhat lower value for hemosiderin granules was obtained from horse spleen (B.M.=3.4).[3]

The duodenal mucosa was tested for ferritin by adding $CdSO_4$ to scrapings of the mucosa on a glass slide and examining it after 24 hours. Brown crystals of ferritin were observed in this material (Fig. 1). The crystals were better defined, with sharper outlines, than any human ferritin crystals seen previously. The presence of the crystals is evidence for the presence of ferritin in the duodenal mucosa. Normal human mucosa has not yet been examined for the presence of ferritin.

Discussion. The fact that ferritin can be found in the duodenal mucosa, spleen, and especially abundantly in the liver, indicates that hemochromatosis is not brought about by a failure to produce the specific protein apoferritin. The magnetic measurements on the ferritin of the liver also indicate that the structural configuration of the iron hydroxide-phosphate of the ferritin is normal, although its phosphorus content appears to be somewhat high.

The idea has often been suggested that the mechanism governing the intake of iron through the intestinal mucosa might be damaged or non-functional in hemochromatosis. If ferritin is assumed to play a role in the regulation of iron absorption[4] then the presence of ferritin in the duodenal mucosa does not support the idea that the regulatory mechanism of iron-absorption is directly affected.

In general, the normal blood picture in hemochromatosis and also the high level of iron in the serum suggest that the transport of iron is adequate.

The extremely high ferritin content of the liver and the normal magnetic values for ferritin iron indicate that the mechanism for iron storage is functioning at capacity.

Conclusion. The cause of hemochromatosis does not appear to be a defect in the formation of ferritin in the duodenal mucosa or the liver.

4 Granick, S., *J. Biol. Chem.*, 1946, **164**, 737.