CORRESPONDENCE

"Normal" Serum Ferritin—A Caution

To the Editor:

We have found that serum from patients with severe iron-storage disease may erroneously appear to have normal serum ferritin concentrations by a two-site immunoradiometric assay. If these sera are assayed at high dilutions, it is found that they indeed do contain vastly increased levels of ferritin. To obviate the potential error of assigning a normal ferritin value to a patient with a true level that is pathologically increased, we recommend that assays be carried out at two dilutions on *all* serum specimens.

We use the two-site immunoradiometric ferritin assay described by Miles et al.[1] or a rapid modification of this assay which we have recently developed.[2] Serial double dilutions are made from a stock solution of purified ferritin prepared from human liver. These are used as reference standards extending from 100 to 0.05 ng ferritin/tube. Using 10 μl of serum, this range represents serum ferritin concentrations of 10,000–5 ng/ml.

In the two-step assay the ferritin to be measured is immobilized by reacting it with human antiferritin coating the wall of a polystyrene test tube. Then ^{125}I-labeled human antiferritin is placed in the tube to react with the immobilized ferritin. After washing the tube the amount of radioactivity remaining should reflect the amount of immobilized ferritin, so that with increasing ferritin the tube counts should rise progressively. At very high levels, however, a paradoxical fall is observed. Standard curves are parabolic in the high-dose range (Fig. 1). This parabolic "hook effect" is believed to be caused by binding of ferritin to low-affinity solid-phase antibody and its subsequent removal by higher affinity ^{125}I-labeled antibody.[1] In our experience, the extent of the "hook" varies with different batches of ^{125}I-labeled antibody. It may be minimized or abolished by repeated washing of tubes to remove rapidly dissociating antigen[1] before the addition of labeled antibody, but the procedure is time consuming and it is not generally carried out.

Sera were assayed from three patients with untreated idiopathic hemochromatosis. All had liver biopsies which showed periportal fibrosis and a marked increase in stainable iron. Sera were also assayed from two patients with iron overload following multiple transfusions for refractory anemia. Four normal sera were assayed. They were selected to represent matched pairs with the hemochromatosis sera on the basis that the undiluted normal sera gave similar tube counts to the hemochromatosis sera. Serum specimens were assayed undiluted and at various dilutions.

Results obtained on sera from the five patients with hemochromatosis are shown in Table 1. In each case counts from tests on undiluted serum indicated a value in the normal range. The counts obtained on diluted specimens indicated a much higher, abnormal amount of ferritin. When the normal sera were diluted there was an appropriate reduction of counts.

In the diagnostic laboratory the practical

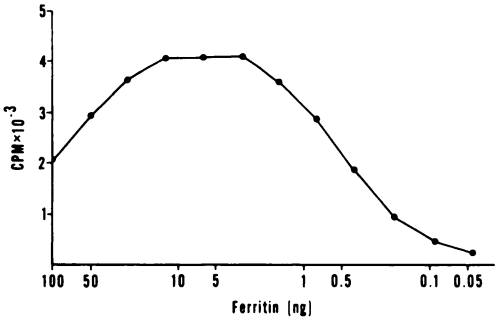

Fig. 1. Standard curve for ferritin assay showing a pronounced "hook" effect. Purified human liver ferritin was diluted to provide representative amounts in each tube. These values when multiplied by 100 correspond to serum concentrations. "Normal" region lies on the right. Note that 3000 cpm might be read out to represent 4500 or 90 ng/ml of serum.

Table 1. Alternative Ferritin Readings on Opposite Sides of Standard Curve (in Fig. 1)

Serum	Dilution	^{125}I (cpm)*	Ferritin Readout From Standard Curve (ng/ml)		
			Left Side of "Hook"	Right Side of "Hook"	Dilution-adjusted† Serum Ferritin Concentration (ng/ml)
J.L.	Undiluted	3490	2900	140	140
(idiopathic	1:8	3870	1700	250	2000
hemochromatosis)	1:64	2970	4700	87	5570
J.W.	Undiluted	3150	4000	105	105
(idiopathic	1:8	3740	2100	190	1520
hemochromatosis)	1:64	2970	4700	87	5570
J.C.	Undiluted	3580	2650	155	155
(idiopathic	1:8	3570	2700	150	1200
hemochromatosis)	1:64	1770	—	38	2430
H.C.	Undiluted	2590	6600	65	65
(transfusional	1:8	2920	5000	94	752
hemosiderosis)	1:64	3100	4200	100	6400
	1:512	780	—	15	7680
B.D.	Undiluted	3500	2800	145	145
(transfusional	1:8	3900	1600	240	1920
hemosiderosis)	1:64	2300	8100	54	3460
Normals					
1	Undiluted	3500	2900	140	140
	1:8	900	—	18	144
2	Undiluted	3050	4500	95	95
	1:8	620	—	12	96
3	Undiluted	3600	2600	160	160
	1:8	1100	—	22	176
4	Undiluted	2600	6200	66	66
	1:8	450	—	9	72

*Mean of triplicate determinations.

†Calculated from reading on the right side of the "hook."

problem posed by the hook phenomenon is a failure to identify abnormal sera. The pitfall may be avoided by routinely performing assays on all unknown sera at two dilutions. When a diluted specimen shows a paradoxical increase in the tube count (e.g., patient J.L. at 1:8 in Table 1) it can be reassayed at a further dilution. This dilution should be sufficient to give a dose response that falls within the working range of the assay, which lies on the right side of the hook shown in Fig. 1 (J.L. at 1:64).

It is not practical to run unknown sera only in the diluted state. This practice would destroy precision at the lower end of the assay range where discrimination between normal and deficient sera is important.

The high-dose hook phenomenon in the serum ferritin assay has been recognized,[1] but its implication in obtaining ambiguous single readings on unknown specimens has not been alluded to. A commercially obtainable test kit for serum ferritin (Fer-iron, Ramco Laboratories, Houston, Texas) does not take into account the potential pitfall arising from the hook effect. The importance of paying attention to this pitfall has been highlighted by the recognition that hemochromatosis may in some cases be associated with a normal serum ferritin concentration.[3] Failure to compensate for the hook phenomenon could result in missing the diagnosis in patients who actually have high concentrations of serum ferritin reflecting serious iron overload.

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Estimation of Bone Marrow Cellularity

To the Editor:

The report by Gruppo et al.[1] claiming inconsistencies in the estimation of bone marrow cellularity by the "aspirate" is misleading. Although the data presented are correct and valid, the procedure they used (coverslip preparation) should not have been synonymous with "aspirate," as it merely represents a smear preparation. For the reasons explained by the authors (admixture with peripheral blood) and indicated as early as 1957 by Agress,[2] a smear preparation is totally inadequate to estimate bone marrow cellularity. However, use of the aspirate is not limited to the preparation of smears: the particles can be gathered with a pipette (or by some other method),[3-5] allowed to clot, fixed in formalin, and processed for embedding in paraffin.[2] Sections through the aggregate provide excellent preparations in which at least 7–8 bone marrow units with preserved bone marrow cell-fat architecture are clearly delineated from the surrounding red blood cell clot.[6,7] This technique has been used by numerous investigators[8-11] and provides a rapid, accurate, and reproducible method for the estimation of bone marrow cellularity on bone marrow aspirates.

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