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The Kinetics of Serum Ferritin[1] (40081)

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Ferritin is widely distributed in body tissues where it constitutes the major form of storage iron. It was originally believed that ferritin is normally confined to intracellular compartments. However, it has been shown recently that minute amounts of ferritin can invariably be detected in human sera by sensitive immunologic measurements and that the level of the serum ferritin provides an accurate measure of iron stores (1–5). Compared with the rapid accumulation of data regarding the clinical significance of the serum ferritin, the physiology of this parameter is poorly understood. The reticuloendothelial system located in the liver, spleen, and bone marrow (RES) appears to be the major precursor compartment based on radioactive labelling measurements in the rat (6) and clinical correlations in man (5). Relatively large doses of recrystallized ferritin in rats are rapidly cleared by the hepatic parenchymal cell (7) and a rapid clearance has also been demonstrated in man although the major site of uptake was not identified (8). In the present study, a dog model was employed to define the kinetics of serum ferritin. The effect on ferritin clearance of the amount of material injected, its iron content, and its method of purification was studied.

Materials and methods. Studies were performed in adult mongrel dogs of either sex weighing between 13 and 19 kg. All animals were dewormed and kept under observation for at least 14 days prior to study. The animals were housed in individual cages and fed regular dog chow. To examine the effect of iron status on ferritin clearance, two animals were made iron deficient as determined by bone marrow examination after removing 450 ml of blood 7 and 14 days prior to study.

For kinetic measurements, animals were anesthetized with iv phenobarbital. One ml blood was drawn prior to and at frequent intervals for at least 30 min following the injection of ferritin. The rate of clearance was determined by least squares regression, assuming exponential clearance, and the recovery of injected ferritin was calculated from the extrapolated time zero value and an estimated plasma volume of 35 ml/kg.

Recrystallized ferritin was prepared from dog liver by the method of Mazur and Shorr (9). Five times recrystallized ferritin was stored at 4° as an $(NH_4)_2SO_4$ precipitate. Prior to use, aliquots were redissolved in 0.2 *M* borate buffer and extensively dialyzed against the same buffer. The final preparation was chromatographed on Sephadex G200 (Pharmacia, Upsula, Sweden). The concentration of ferritin was measured by Lowry's method (10) using a bovine serum albumin standard.

To prepare apoferritin, recrystallized ferritin was dialyzed overnight against 0.1 *M* acetate buffer, pH 5.2, and for a further 90 min against the same buffer containing 0.1 *M* thioglycollic acid. The colorless solution was then dialyzed against repeated changes of 0.05 *M* acetate buffer, pH 5.2, followed by deionized water and finally 0.2 *M* borate, pH 8.0 containing 0.15 *M* NaCl (borate buffer).

Recrystallized ferritin was tagged with ^{59}Fe by incubating 2 mg ferritin in 1 ml borate buffer with 150 μCi ^{59}Fe in 0.1 ml 1 *N* HCl containing 1 *M* freshly prepared ascorbic acid. The mixture was incubated at 4° for 2 hr with continuous oxygen agitation. Unbound ^{59}Fe was removed by 18 hr dialysis against 0.1 *M* EDTA followed by 24 hr dialysis against borate buffer. At that time 29% of the original ^{59}Fe was bound to ferritin and of this, 98% was precipitated by antiferritin antiserum. Recrystallized ferritin was iodinated with ^{125}I by the method of Hunter and Greenwood (11). Unbound radioiodine was removed chromatographically with Sephadex G25. Studies were also performed with crude (uncrystallized) ferritin prepared from dog

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liver and spleen. The latter tissues were homogenized in 4 vol cold 1 *N* NaCl, ultrasonicated for 1 min, heated to 70° for 10 min, and centrifuged at 25,000*g* for 1 hr at 4°.

Serum ferritin levels were measured by immunoradiometric assay (IRMA) as described by Miles *et al.* (12). The characteristics of this assay were similar to those previously reported for human serum ferritin measurements. In 19 normal animals the geometric mean serum ferritin was 24 ng/ml with a range from 7 to 75 ng/ml (± 1 SD 12–47 ng/ml).

Results. The clearance of recrystallized dog ferritin in doses greater than 500 μg was determined by changes in the serum ferritin level as measured by IRMA. A typical clearance curve with a dose of 5.2 mg recrystallized ferritin is shown in Fig. 1. The injected ferritin was cleared with a half time ($T_{1/2}$) of 6.6 min with a recovery of 101%. Following the exponential decline of 80–90% of the injected ferritin, the rate of clearance slowed considerably, presumably due to a return to the plasma compartment of a portion of the ferritin cleared initially. In all remaining studies clearance rates were determined from the initial linear segment of the curve.

Effect of dose. Since the level of serum ferritin in various clinical disorders ranges from 1 to several 1000 ng/ml, the effect on clearance of the amount of injected ferritin was examined. With doses greater than 400 μg, clearance could be determined from changes in the serum ferritin level as measured by IRMA. The results of studies performed with doses between 400 and 5,200 μg recrystallized ferritin are summarized in Table I. Clearance $T_{1/2}$ was uniformly less than 10 min, ranging from 5.8 to 8.7 with a mean of 7.1 min. There was no apparent relationship between the rate of clearance and either the quantity of injected ferritin or the basal serum ferritin level of the dog.

Recrystallized ferritin labelled with ^{125}I was used to study the clearance of a smaller amount of injected ferritin. With 3 μg of labeled ferritin, the clearance $T_{1/2}$ was 7.3 min, identical to the mean $T_{1/2}$ obtained with doses 100–1000 fold higher. In a final study this same dose of ^{125}I labelled recrystallized ferritin was mixed with 3000 μg unlabelled carrier ferritin prior to injection. A rapid $T_{1/2}$ of 6.9 min indicated no effect of this 1000-fold increase in injected dose.

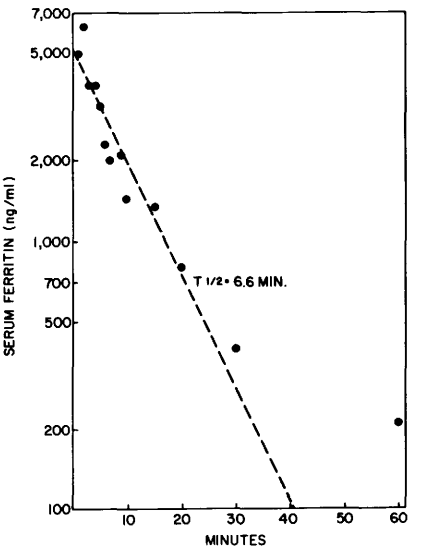

FIG. 1. The clearance of 5200 μg recrystallized ferritin. The interrupted line was determined by least square regression.

Ferritin iron clearance. A study was performed to determine whether iron contained in ferritin is cleared at the same rate as the protein moiety. Ferritin tagged *in vitro* with ^{59}Fe was injected in an amount sufficient to permit measurement of protein clearance by IRMA simultaneously. In the first study performed with 1200 μg recrystallized ferritin, the clearance $T_{1/2}$ for ^{59}Fe was 8.3 min as compared with 7.1 min for the ferritin protein (Fig. 2). The difference was not statistically significant. In a second study performed with 700 μg ferritin, similar clearance $T_{1/2}$ values of 7.9 and 7.6 min were observed for ^{59}Fe and ferritin protein respectively. In both these studies the residual plasma ^{59}Fe could be preciptated with an excess of antiferritin antiserum.

Effect of iron content. To determine the iron content of ferritin influences the clearance rate of the protein, the clearance of recrystallized ferritin was measured following complete removal of iron to obtain apoferritin. In two separate studies performed with 1000 and 1200 μg ferritin, the clearance $T_{1/2}$ increased significantly to 25 and 16 min respectively (Fig. 3A).

Effect of purification procedures. To deter-

TABLE I. THE RATE OF CLEARANCE OF RECRYSTALLIZED HEPATIC FERRITIN AS MEASURED BY IRMA.

Study No.	Dog wt.	Dose of ferritin	Preinjection serum ferritin	Serum ferritin increment	$T_{1/2}$	Recovery[a]
	(*kg*)	(*μg*)	(*ng/ml*)	(*ng/ml*)	(*min*)	(%)
1	26	5200	114	5808	6.6	101
2	16	3200	118	8818	5.8	154
3	25	700	20	625	7.0	78
4	26	650	120	501	7.4	70
5	16	400	180	975	8.7	136

[a] Based on a plasma volume of 35 ml/kg.

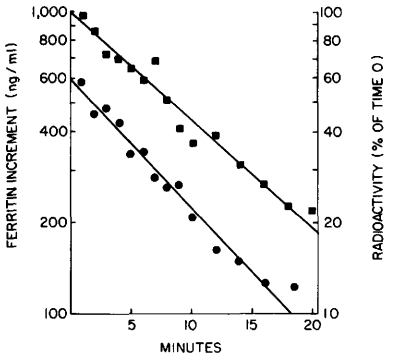

FIG. 2. The clearance of 1200 μg recrystallized ferritin tagged with ^{59}Fe. Clearance $T_{1/2}$ of the protein as measured by IRMA (●) was 8.3 min and the clearance of ^{59}Fe (■) was 7.1 min.

mine the possible effect of recrystallization on ferritin clearance, studies were performed with relatively crude ferritin recovered in the heated supernatant of dog liver or spleen homogenates. In two separate studies with dog liver ferritin, the clearance $T_{1/2}$ of 3010 and 2930 μg ferritin as determined by IRMA was 35 and 18 min respectively (Fig. 3B). Similar results were obtained with 1850 μg and 1300 μg ferritin from dog spleen; the clearance $T_{1/2}$ was 32 and 33 min respectively (Fig. 3C).

Effect of iron status. The serum ferritin level is roughly proportional to iron stores in man although it has not yet been determined whether these changes are due to differences in the entry or in the clearance of circulating ferritin. The latter possibility was examined by measuring the clearance of recrystallized ferritin in phlebotomized dogs. In two studies the clearance $T_{1/2}$ of 1200 μg recrystallized ferritin was 5.8 and 9 min which are similar to values obtained in iron replete animals.

Discussion. It has not been possible to directly measure the influx of serum ferritin in human subjects. However, there is circumstantial evidence that the RES is the immediate precursor of the circulating protein. For example, the sudden reduction in RES stores of iron which occurs following phlebotomy is reflected by an equally precipitous drop in serum ferritin. In a wide spectrum of clinical disorders there is an excellent correlation between serum ferritin and RES stores as estimated directly by histologic grading of a bone marrow aspirate (5). The sudden block in transferrin loading of iron by RES which occurs with induced fever or elective surgery produces an appropriate reciprocal rise in the serum ferritin (13). In patients with on-going infection or inflammation, the serum ferritin may be disproportionately elevated in relation to marrow iron stores although the correlation still holds (5). More direct evidence that the RES accounts for the major influx of ferritin to serum was obtained in rats by Siimes and coworkers (6). Following the injection of ^{59}Fe tagged red cells damaged by heating to ensure prompt removal by RES, radioactivity promptly appeared in circulating ferritin within 20–40 min. In contrast, no labelling of ferritin was observed following the administration of oral radioiron or ^{59}Fe hemoglobin–haptoglobin which is removed by the hepatic parenchymal cell (14). Thus, while other tissues might well contribute to circulating ferritin, the RES seems to be the major source.

Data on the clearance rate and sites of tissue uptake of circulating ferritin is also limited. It has been shown in rats that recrystallized ferritin tagged with ^{59}Fe is removed almost exclusively by the hepatic parenchymal cell (7). Relatively large doses above 200 μg are removed with $T_{1/2}$ of 30–40 min (15), whereas smaller doses below 1 μg are removed more rapidly with a half time of less

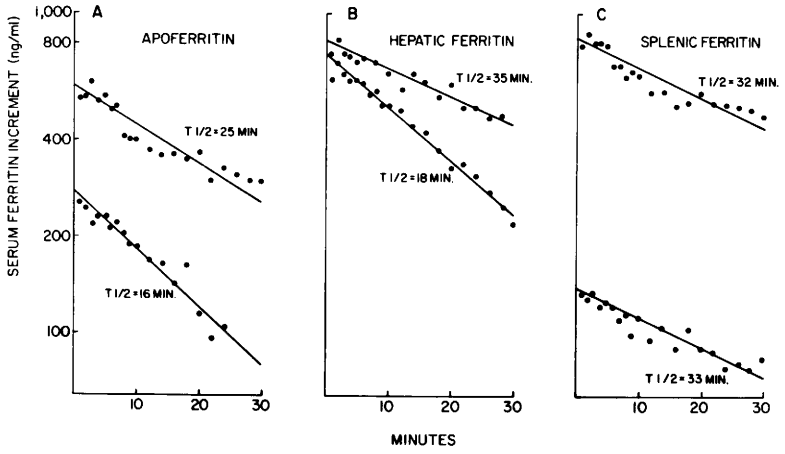

FIG. 3. The clearance of apoferritin (A), (crude noncrystallized) hepatic (B) and splenic (C) ferritin. The result of two separate studies are shown for each preparation. In all cases clearance was significantly prolonged as compared with recrystallized ferritin.

than 5 min (6). The only observation on the clearance rates of serum ferritin in man was obtained in newborn infants requiring exchange transfusions for hyperbilirubinemia (8). Based on the difference between the plasma ferritin of the transfused blood and in the infant both before and following blood transfusion, a very rapid $T_{1/2}$ between 2.5 and 5.5 min was observed in two infants with birth weights above 2000 g. In the infants with lower birth weights, below 1200 g, slower clearances of 9.1 and 34 min were seen. Because of this very rapid turnover of serum ferritin, it has been calculated that if the circulating protein has a normal iron content of 20–25%, the amount of iron transported through the serum as ferritin each day could equal the daily plasma iron turnover from the breakdown of senescent red cells.

None of the observations reported here are inconsistent with published data on serum ferritin kinetics. In the present study the clearance $T_{1/2}$ was invariably less than 10 min with doses ranging from 3 μg protein to 5.2 mg. This would suggest that in this animal model clearance of ferritin is not rate limiting and that any changes in the circulating level reflect differences in the rate of entry rather than the rate of removal from the circulation.

It was also observed in this study that ferritin iron is not selectively removed from the circulating protein but is cleared simultaneously. Moreover, because the serum radioactivity following ^{59}Fe ferritin injection was precipitable with excess antiferritin antiserum, there is not direct transfer of iron from ferritin to transferrin. However, it was observed that removal of iron from recrystallized ferritin resulted in a substantially slower clearance of the protein. This finding is of interest in light of recent studies in man which suggest that the circulating protein contains no iron (16). Serum ferritin turnover may be substantially slower than that observed with recrystallized ferritin in these studies and there may be no appreciable transport of iron through this compartment. Evidence was also obtained in this study that the method of purifying ferritin may significantly influence its rate of clearance. It is now known that both serum and tissue ferritin actually consist of a family of isoferritins which can be identified by isoelectric focusing (17, 18). Furthermore, studies in man indicate that recrystallization selects out predominantly basic isoferritins which are less heterogeneous than the mixture of isoferritins obtained without recrystallization (19). This may explain why recrystallized ferritin is cleared more rapidly than the ferritin recovered from tissue without crystallization. Obviously, any final interpretation of the kinetic data observed in this model must await more detailed characterization of the circulating protein.

Summary. The mean serum ferritin in normal dogs averaged 24 ng/ml which is similar to the level in normal man. The plasma clearance of recrystallized ferritin was determined

by changes in the serum level or by the clearance of ferritin tagged with ^{125}I or ^{59}Fe. Clearance half times was invariably less than 10 min with doses ranging from tracer quantities to several mg protein. The iron and protein moieties of ferritin were removed simultaneously, but clearance of the protein was slower when iron was removed before injection. The clearances of relatively crude hepatic or splenic ferritin preparations were also slower, suggesting that isoferritins may differ substantially in their rate of clearance.

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