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FERRITIN

VI. CONVERSION OF INORGANIC AND HEMOGLOBIN IRON INTO FERRITIN IRON IN THE ANIMAL BODY. STORAGE FUNCTION OF FERRITIN IRON AS SHOWN BY RADIOACTIVE AND MAGNETIC MEASUREMENTS*

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The properties of ferritin, an iron-protein compound found in the liver, spleen, and marrow of mammals, including man, have been reported in previous papers (1-3). Ferritin consists of a protein fraction, apoferritin, linked together with micelles of a special type of colloidal ferric hydroxide, the crystals containing as much as 23 per cent of iron. There are three possible states of ferric iron; namely, those with 1, 3, and 5 unpaired electrons in the outer electron shell. Magnetic measurements (3) have shown that iron in the micelles of ferritin is present in the rarely occurring state of 3 unpaired electrons per iron atom. This special property makes it possible to distinguish magnetically the iron of ferritin from any other form of ferric iron. The very high iron content of ferritin suggests that it functions as a storage compound. We have attempted to demonstrate this function by following the fate of iron labeled with the Fe^{59} radioactive¹ isotope injected intravenously as ferric ammonium citrate, as well as the fate of labeled iron contained as a constituent of the heme of the red blood cells. At the same time it was of interest to determine whether by injection of an excessive amount of iron containing 5 unpaired electrons (ferric ammonium citrate) the iron could be converted into a ferritin possessing higher magnetic susceptibility values per iron atom than that of regular ferritin.

Methods

The detailed procedures for the isolation of ferritin have been previously described (4), the tissue being ground with an equal volume of water, heated to 80°, filtered, and the filtrate treated with 35 gm. of ammonium sulfate per 100 ml. of filtrate. The brown precipitate obtained was dialyzed against distilled water, producing a brown solution which contained the

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ferritin and non-crystallizable ferritin. This solution we shall designate as F + NCF. The iron micelles of both ferritin and non-crystallizable ferritin fractions have been shown to be identical by magnetic measurement. The solution was tested for the presence of heme iron by adding pyridine and sodium dithionite and examining for the hemochromogen spectrum with a hand spectroscope. The characteristic bands were found to be very faint, indicating that only traces of heme iron were present.

A portion of the solution of F + NCF was treated to isolate crystalline ferritin and the material was further recrystallized. The twice crystallized material was centrifuged at a standard speed and the volume occupied by the material noted (4).

Measurements of the radioactivity were made on liver, spleen, marrow tissue, and on the material injected as well as on solutions of the F + NCF and the twice crystallized ferritin. The detailed methods of these determinations have been described (5-7). The material was ashed by the wet method, the iron separated by alkaline precipitation, electroplated onto tin cylinders, and counted. Activity is expressed in terms of scale-of-four counts per minute obtained by means of a "turret" type tube (5). Preparation of a donor whose hemoglobin is tagged has been described (7).

Magnetic measurements were made by a modification of the Gouy method described by Michaelis (8) as the "deflection method."

EXPERIMENTAL

The first experiment was carried out to determine whether there might be a direct conversion of the iron of ferric ammonium citrate administered intravenously into ferritin iron. Dog 42-998 was an adult mongrel female weighing 9 kilos having a hematocrit value of 45.2 per cent. A dose of 39 mg. of radioactive iron in the form of ferric ammonium citrate was given by vein and this dose repeated 4 days later. 13 days following the first injection the animal was sacrificed by viviperfusion (9). The tissues generally were well cleared of red blood cells as seen grossly, the hematocrit having been reduced to less than 0.8 per cent by the procedure. Aliquots were reserved for total and radioactive iron measurements and the remainder of the liver and spleen was reserved for isolation of the ferritin fractions. The F + NCF fraction was subjected to measurement of its magnetic susceptibility and then examined for total and radioactive iron content. The results are summarized in Table I.

In the second experiment it was desired to demonstrate whether ferritin iron of the liver and spleen could be derived from red cell hemoglobin iron. Dog 42-816 was a young, adult, female mongrel terrier taken from stock. It was given by vein 110 ml. of heparinized whole blood containing radioactive iron in the hemoglobin of the red blood cells. The following day the red cell hematocrit was 50 per cent and there were 2400 counts per minute

per 100 ml. of mixed red cells. The estimated cell mass was 280 ml., giving an estimated total circulating radioactivity of 6720 counts per minute. On this day and the day following, 300 mg. of acetylphenylhydrazine were administered subcutaneously to produce blood destruction. 5 days after the second dose the hematocrit had dropped to 19.6 per cent. At this latter time the plasma was quite icteric and the animal was sacrificed by injection of 2 ml. of chloroform intravenously. Just before death the red cell radioactivity was found to be 955 counts per minute per 100 ml. of cells. The estimated cell mass was 110 ml., which gave as a total circulating radio-

TABLE I
Ferritin Iron Formation from Ferric Ammonium Citrate by Vein (Dog 42-998)

	Total* Fe	Total activity	Specific activity	Distribution of labeled Fe as per cent injected Fe	Per cent Fe found which was tagged
	mg.	counts per min.	counts per min. per mg. Fe		
Injected ferric ammonium citrate.....	78.0	238,000	3050	100	
Liver (323 gm. fresh weight)	78.0	195,000	2500	82	82
“ F + NCF.....	27.3	62,000	2270	26	75
“ ferritin.....	7.72	17,800	2310	7.5	76
Spleen (21 gm. fresh weight)	4.95	821	166	0.35	5.4
“ F + NCF.....	1.91	466	244	0.20	8.0

Liver F + NCF measured magnetically at 27° shows a susceptibility of the iron contained in 1 ml. of the solution (0.736 mg.) of $+0.0779 \times 10^{-6}$ c.g.s., corresponding to a susceptibility per gm. atom of iron = 5910×10^{-6} , and hence to a magnetic dipole moment = 3.78 Bohr magnetons per gm. atom of iron, which is precisely the average value of previous determinations of regular ferritin.

* F + NCF (ferritin and non-crystallizable ferritin) and ferritin fractions found in the liver and spleen have been corrected to correspond to the amount which would have been isolated from whole organs.

activity 1050 counts per minute. This would indicate that 5670 counts per minute of radioactivity had been lost to the circulation owing to blood breakdown. The liver and spleen were removed and aliquots reserved for total and radioactive iron determination, the remainder being used for isolation of the ferritin fractions. The F + NCF and the twice crystallized ferritin fractions were examined for total and radioactive iron content. The results are summarized in Table II.

DISCUSSION

The data revealed by these experiments, although limited, clearly indicate that ferritin performs the function of storage for iron in the animal body.

Following injection as ferric ammonium citrate or liberation from red cells which were destroyed by the action of acetylphenylhydrazine, it has been demonstrated that such iron is used for the construction of new ferric hydroxide micelles of ferritin.

In the first experiment (Table I) 82 per cent of the iron injected as ferric ammonium citrate was shown to be taken up by the liver. The ferritin and non-crystallizable fraction (F + NCF) which was isolated contained 75 per cent of the iron of this fraction in the form of radioactive iron, showing a conversion of the ferric iron of the injected ferric ammonium citrate into ferritin iron. Magnetic² susceptibility determinations showed that the

TABLE II
Ferritin Formation from Hemoglobin Iron of Red Blood Cells (Dog 42-816)

	Total Fe	Total activity	Specific activity	Distribution of labeled Fe; total circulating activity after transfusion taken as 100 per cent	Distribution of labeled Fe; activity lost to circulation = 100 per cent	Per cent Fe which is radioactive
	<i>mg.</i>	<i>counts per min.</i>	<i>counts per min. per mg. Fe</i>	<i>per cent</i>	<i>per cent</i>	
Injected tagged blood (110 ml.).....	64.2*	6720*	105*	100		
Circulating blood after transfusion....	324	6720	20.7	100		19.8
Circulating blood before death.....	127	1050	8.3	15.6		7.9
Liver (335 gm.).....	138	3100	22.5	46.1	55	21.4
F + NCF.....	72.4	1925	26.5			25.3
Ferritin.....	12.4	332	26.8			25.5
Spleen (89 gm.).....	87.5	1150	13.2	17.1	20	12.5
F + NCF.....	26.1	328	12.6			12.0

* Estimated.

injected ferric iron containing 5 unpaired electrons had been converted into ferric hydroxide containing 3 unpaired electrons. Thus none of the in-

² Ferric ammonium citrate is an ionic compound containing 5 unpaired electrons per iron atom. When injected into the organism, the neutral reactions of the body fluids may bring about some hydrolysis of this compound with the resulting production of some ferric hydroxide which need not have the same magnetic susceptibility as the injected iron (3). The essential fact, however, is that the labeled iron of this F + NCF fraction shows precisely the same values for the magnetic susceptibility per iron atom as is found in all horse, human, and dog ferritins that have thus far been measured. The value for the magnetic susceptibility of ferritin iron is unique and readily distinguishes this iron from all other iron compounds normally occurring in the organism.

jected material in its original state could be detected in the isolated ferritin fraction by the magnetic method. The twice crystallized ferritin (F) of the liver had the same specific radioactivity as the F + NCF fraction, indicating the iron to be essentially the same in both fractions. The identity of the iron in the F + NCF and F fractions as determined by the radioactivity method corroborates previous findings of their identity as determined by the magnetic susceptibility method (3).

In the second experiment (Table II) red cells containing tagged iron as a constituent of their hemoglobin were injected and the resulting mixed cells of the circulation were destroyed with acetylphenylhydrazine. The dog was sacrificed after 6 days. During this time interval not only destruction of red cells but also undoubtedly some regeneration had occurred (10). The liver was found to have taken up 46 per cent of this labeled iron originally present in the circulation. 25 per cent of the total iron present in the F + NCF and in the twice crystallized ferritin fractions of the liver was present as labeled iron. The specific radioactivities of the liver and its ferritin fractions were somewhat higher than the circulating specific activity of the circulating red cells following transfusion of the tagged cells. The figures indicate that in this instance there was some preferential conversion of injected hemoglobin iron into ferritin iron owing possibly to a more rapid destruction of the injected erythrocytes. Of the labeled iron formerly in the circulation following transfusion, 17 per cent was found in the spleen. That the specific activity of the splenic iron is lower than that of the liver is an indication that a relatively large amount of non-radioactive iron was present in this organ of this normal dog prior to the transfusion and subsequent destruction of red cells.

The form in which iron is introduced appears to determine the distribution of this element in the viscera (11). In the first experiment the soluble ferric ammonium citrate was removed from the circulation almost wholly by the liver, only 0.35 per cent of the labeled iron having been taken up by the splenic tissue. In the second experiment, however, following breakdown of the red blood cells and their subsequent phagocytosis by the cells of the reticuloendothelial system there is an appreciable uptake of the iron by the spleen as well as the liver.

SUMMARY

Iron in the form of ferric ammonium citrate when administered by vein to the dog is readily converted into ferritin iron in the liver.

Iron derived from hemoglobin of the circulating red blood cells following the destruction of the cells by acetylphenylhydrazine is in part, at least, converted to ferritin iron in the liver and spleen.

The body is able to convert injected ferric iron of the form containing

5 unpaired electrons to ferric iron of the form containing 3 unpaired electrons, characteristic of ferritin.

It is concluded that ferritin iron acts in the capacity of storage iron in the animal body.

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