THE ANEMIA OF INFECTION. V. FATE OF INJECTED RADIOACTIVE IRON IN THE PRESENCE OF INFLAMMATION[1]

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Since hypoferremia is so consistently associated with the anemia of infection (1), and since injected iron appears to be diverted from the plasma in infection (1, 2), it seemed desirable to determine the fate of the diverted iron.

In this study, radioactive iron was injected intravenously into normal rats and in rats with acute inflammation. The blood and tissues were then analyzed after a period of time for radioactivity. Preliminary studies were made in which only the blood and liver were analyzed in order to determine the appropriate amount of iron to employ. Radioactive iron was also injected intravenously into dogs, and analyses were carried out on a number of tissues, including the exudates.

METHODS

The radioactive iron used in these studies was injected as ferric chloride in physiological saline. For the rats, the concentration was adjusted so that about 0.2 ml. was injected for each 100 grams body weight. In all the rats the same tuberculin syringe was employed for this purpose. In order to be certain that the solution did not enter subcutaneously, the vein on the lateral aspect of the tail was exposed under nembutal anesthesia and the solution was injected intravenously. In one experiment Fe^{59} was injected intraperitoneally.

Inflammation was produced by the intramuscular injection of 0.5 ml. of turpentine. Robscheit-Robbins and Whipple (3) have shown that the resulting sterile abscess can be compared with a bacterial abscess. In some animals, 0.5 ml. of a mixed culture of *S. aureus* and *E. coli* was injected intramuscularly. Injections of bacteria or turpentine were made about 2 hours before Fe^{59} was administered.

The methods for preparing and electroplating the Fe^{59} from the biological samples have been described elsewhere (4). Blood volume was estimated by assuming that the normal mammal possesses 80 ml. blood per kgm. body weight. Hemoglobin was determined in a representative sample of the tissue to be analyzed for radioactivity by a modification of the method of Greenberg and Erickson (5). The radioactivity attributable to the tissue hemoglobin was then subtracted from the total radioactivity of the tissue.

Blood samples were taken in the rats from the abdominal aorta. Blood hemoglobin determinations were made by the photoelectric oxyhemoglobin method of Bell, Chambers and Waddell (6).

The white rats used for these studies were virgin females obtained from the Sprague-Dawley Company and from the Carworth Farms. The dogs were mongrels secured locally.

RESULTS

Table I summarizes the results of analyses of the blood and liver of rats which received Fe^{59} intravenously. It will be seen that usually the rats which had been subjected to acute inflammation showed more Fe^{59} in the liver than was the case in the control animals. On the other hand, the control rats showed better uptake into the red cells than those with inflammation. In general, the sum of the radioactivity in the blood and liver of the

TABLE I

Distribution of injected (i.v.) Fe^{59} in rats 48 hours after injection

Number of rats	Experimental procedure	Amount Fe^{59} injected	Proportion found in		
			Blood	Liver	Blood plus liver
		γ per 100 grams	*per cent*	*per cent*	*per cent*
3	Turp. i.m. 1 ml.	10	15.2	59.5	74.8
3	None	10	35.2	36.3	71.5
4	Turp. i.m. 0.5 ml.	10	9.1	61.1	70.2
5	None	10	24.3	38.5	62.8
4	Turp. subcut. 0.5 ml. Bact. mix. 0.25 ml.	10	33.7	25.5	59.3
4	Bact. mix. i.m. 0.25 ml.	10	22.0	32.8	54.8
3	Turp. subcut. 0.5 ml.	10	39.7	22.0	61.7
4	Turp. subcut. 0.5 ml.	10	46.8	17.2	64.0
4	None	10	38.8	25.5	64.3
3	Turp. i.m. 0.5 ml.	3	26.1	37.8	63.9
3	None	3	39.1	24.6	63.7

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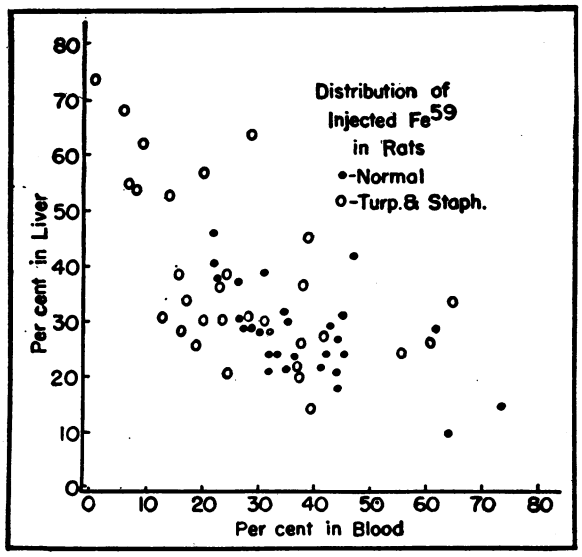

FIG. 1. TO ILLUSTRATE THE INVERSE CORRELATION OF THE QUANTITY OF RADIOACTIVE IRON IN THE BLOOD AND THAT IN THE LIVER FOLLOWING ITS INTRAVENOUS INJECTION BOTH IN NORMAL RATS AND IN RATS RECEIVING BACTERIAL OR TURPENTINE INJECTIONS

control animals was approximately equal to the same sum in the animals with inflammation. This is graphically illustrated in Figure 1.. In this figure the percentage of Fe^{59} in the liver of each animal is plotted against the corresponding Fe^{59} percentage in the blood. It may be observed that the points for the control animals and for those with inflammation fall along the same general line of correlation. When the iron was not found in the blood, it was in the liver. In no case, however, did the values for normal animals fall in the upper portion of the curve.

It should be pointed out that certain animals injected with turpentine apparently did not exhibit retardation of uptake of Fe^{59} by the red cells. The results in these animals together with their controls have been included in Figure 1. Sprague-Dawley rats were apparently considerably more sensitive to turpentine inflammation than were those of the Carworth Farm. Unfortunately, at the time these experiments were conducted it was very difficult to obtain the former animals. In these rats the liver and blood Fe^{59} percentages approached those of the normal animals. However, whether or not diversion to the liver occurred, the reciprocal relation of blood and liver Fe^{59} was maintained.

TABLE II

Distribution of Fe^{59} (25 μg. per 100 grams) injected intraperitoneally in rats

Rat no.	Experimental procedure	Time after injection	Proportion found in			
			Blood	Liver	Spleen	Total
		hours	*per cent*	*per cent*	*per cent*	*per cent*
1	Control	66	24.3	9.3	0.2	34
2	Control	66	18.7	6.4	0.8	28
3	Control	66	25.8	9.5	0.6	36
Average			22.9	8.4	0.5	32
4	Turp. i.m. 1 ml.	66	18.7	13.3	3.4	35
5	Turp. i.m. 1 ml.	66	15.7	9.7	1.1	26
6	Turp. i.m. 1 ml.	66	17.9	12.0	0.7	31
Average			17.1	11.7	1.7	31
7	Control	90	26.9	8.1	*	35**
8	Control	90	30.6	3.9	0	35
9	Control	90	28.5	*	0.7	35**
Average			28.6	6.0	0.4	35
10	Turp. i.m. 1 ml.	90	24.9	11.5	1.0	37
11	Turp. i.m. 1 ml.	90	19.8	9.3	*	29**
12	Turp. i.m. 1 ml.	90	17.5	10.8	0.7	29
13	Turp. i.m. 1 ml.	90	22.4	9.4	1.0	33
Average			21.1	10.3	0.9	32

* Sample lost.
** Calculating by assuming lost sample to be equivalent of average finding in other animals.

In Table II are shown the results of an experiment in which Fe^{59} was injected intraperitoneally, rather than intravenously, in rats. Analyses of the blood, liver and spleen were made on ½ the animals at approximately 3 days, and on the other ½ approximately 4 days following the injection of Fe^{59}. It will be seen that the distribution of the Fe^{59} tended to follow the same pattern as that observed after intravenous injection, except that in the latter case more was recovered in the period of time the animals were studied. It cannot be said with any assurance that an increase occured in the relative percentage of Fe^{59} in the spleen in the animals with inflammation as compared with their controls.

Table III presents an experiment in rats in which analyses were made of the Fe^{59} content of normal and inflamed muscles together with analyses of the radioactivity of the livers and spleens. As will be observed, very little Fe^{59} could be accounted for in either the inflamed or normal muscle 48 hours following the turpentine injection. On the other hand, the liver and blood again ac-

TABLE III

Distribution of injected (i.v.) Fe^{59} 10 μg. per 100 grams body weight

Rat no.	Experimental procedure	Proportion of injected Fe^{59} found in				
		Leg muscle (per gram wet weight)		Whole blood	Liver	Blood plus liver
		Normal leg	Inflamed leg			
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	Staph. / *E. coli* i.m.	0.6	1.1	28	31	59
2	Turp. i.m.	0.0	0.4	48	14	62
3	Turp. subcut.	0.0	4.4	38	21	59
4	Turp. Staph. / *E. coli* i.m.	1.0	2.0	31	30	61
5 to 8	None			39	25	64

counted for most of the Fe^{59}. Apparently a small increase in the proportion of Fe^{59} takes place in the inflamed muscle.

Table IV presents the results of an experiment in which Fe^{59} was injected into 2 dogs, one of which had received turpentine intramuscularly. The dose of iron employed was much larger than those used in the rat experiments. It will be seen that most of the Fe^{59} was found in the liver even in the normal dog. This is in agreement with the results of Hahn and coworkers (7) who noted that the major portion of a large dose of intravenously injected Fe^{59} is deposited in the liver, mainly as storage iron. It will be noticed that the percentage of Fe^{59} in the blood of the normal animal was more than twice that in the blood of the animal with inflammation. In addition it will be observed that more Fe^{59} was found in the spleen of the dog with inflammation than in the normal animal. The spleen is said not to enter into iron metabolism to the degree that the liver is concerned (8). It is of interest, finally, that no significant quantity of Fe^{59} was found in the inflamed muscle, in the exudate of the sterile abscess, or in the excreta. It should be mentioned that the analyses of the stools and bone marrows were made difficult by the presence of inorganic salts. The results are to be regarded only as approximate indices of the proportion of Fe^{59} carried to the stool and the bone marrow.

DISCUSSION

These results indicate that in infection a signficant proportion of injected radioactive iron is not diverted away from the usual organs engaged in iron metabolism. Since the liver is the major iron-storing organ it is to be expected that most of the iron would be found there. It is of interest that Sandberg, Gross and Holly (9, 10) analyzed the spleen and liver from human autopsy specimens in chronic disease accompanied by anemia, and in various types of cancer with and without associated anemia. They found large accumulations of iron and copper in the liver and spleen, even when anemia was absent; and, in the presence of anemia, in amounts out of proportion to the degree of ane-

TABLE IV

Distribution of injected (i.v.) Fe^{59} in dogs

Experimental procedure and amt. Fe^{59} given		Proportion of injected Fe^{59} found in							
		Blood	Liver	Spleen	Plasma	Muscle per gram dry wt.	Stool and urine	Marrow per gram dry wt.	Total
day		*per cent*	*per cent*	*per cent*	*perc ent*	*per cent*	*per cent*	*per cent*	*per cent*
1	No turp. Fe^{59} 0.44 mgm. per kgm.								
2	Fe^{59} 0.44 mgm. per kgm.								
3	Sacrificed	5.89	86.70	2.04	0.20	0.0002	0.10	0.004	95
1	Turp. 10 ml. i.m. Fe^{59} 0.31 mgm. per kgm.								
2	Fe^{59} 0.31 mgm. per kgm.								
3	Sacrificed	2.30	81.30	4.40	0.04	normal 0.0006 inflamed 0.0040	0.05	0.004	88

The inflammatory exudate in an animal given turpentine contained 0.1 per cent of the injected Fe^{59} per 100 ml.

mia. The present studies indicate that while more iron is found in the liver and spleen in infection, the increase in these tissues apparently corresponds to the quantity of iron not used for hemoglobin formation as a result of the retarded hemopoiesis. As has been shown in the preceding paper (2), the diversion which occurs, while very real, apparently is not the primary cause of the anemia of infection.

The greater quantity of iron found in the inflamed tissues as compared with that in the normal tissues was not of such an amount as to account for the persistent hypoferremia which accompanies infection. Menkin (11) found an accumulation of intravenously administered iron in the inflammatory tissues of rabbits. However, although his figures indicate that an average of 67 per cent more iron was present in the inflamed tissue after iron was injected than before, quantitatively the total amount of iron concerned was very small. He injected about 10 mgm. of iron and found 6.5 mgm. per 100 grams of dry inflamed tissue. From his description it seems unlikely, however, that more than 2 grams of dry tissue was inflamed; thus only 0.13 mgm. could be accounted for in this way. This represents but 1.3 per cent of the 10 mgm. of iron injected. This is of the same order of magnitude as was found in our studies.

It might be argued that this quantity of iron, if removed continuously, could produce hypoferremia in a patient with chronic infection. It does not seem plausible, however, that a comparatively small area of inflammation could take up sufficient iron to produce significant hypoferremia and impair hemoglobin synthesis. The data which have been presented suggest that it is the diversion of iron to the liver and spleen which is correlated with the hypoferremia.

With further reference to the fate of plasma iron diverted in the presence of inflammation, it may be pointed out that Hahn *et al* (12) showed that the urine and bile contain negligible quantities of iron injected intravenously in doses even larger than those used in the present study. In balance studies, Schaefer (13) found no evidence of increased excretion of iron in children with infection. It may be noted also that in a previous paper of this series (14), an iron-deficient pig with infection was described which was given large therapeutic doses of iron intravenously. This animal apparently did not excrete a significant quantity of iron, since on relief of the infection all of the injected iron could be accounted for as hemoglobin.

Our data offer no evidence as to the reason for the diversion of the iron from the plasma to the liver and, to a much smaller extent, to other tissues. It must be assumed that this diversion of iron is intimately related to some process related to infection. The present studies indicate that the lack of iron is not the fundamental factor in the pathogenesis of the anemia of infection. The presence of excess erythrocyte protoporphyrin and serum copper in cases of infection associated with anemia (1) suggests that no limitation exists in these substances. By elimination, the protein moiety of hemoglobin may be suspected as being involved. This view is tenable, furthermore, because it is well known that a disturbance in protein metabolism occurs in infections. It has been shown that in acute infections the urine nitrogen is very greatly increased (15), the same being true, though to a lesser extent, in chronic infections. It is generally recognized that the plasma albumin concentration is an index of protein reserves (16). Since globin is derived from these reserves (17), the fact that in chronic infection the plasma albumin concentration may be low (18) is probably significant in relation to the pathogenesis of the anemia of infection.

SUMMARY

1. Radioactive iron was injected intravenously in normal rats and dogs, and in animals with acute inflammation, in order to determine the fate of the iron.

2. A number of rats with inflammation showed retarded hemopoiesis and increased deposition of Fe^{59} in the liver and spleen as compared to the control animals.

3. There was good inverse correlation between the Fe^{59} in the liver and in the blood in both the normal rats and in the animals with inflammation.

4. Analyses of the inflamed tissues for Fe^{59} indicated that no significant quantity of iron is diverted to the inflamed area.

5. Insignificant percentages of large doses of intravenously injected iron were found in the exudate of a sterile abscess and in the excreta of a dog that had received turpentine intramuscularly.

6. It has been concluded that the major diversion of plasma iron in infection is to the ordinary storage tissues, mainly the liver.

7. This diversion is related to the hypoferremia.

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