PLASMA AND RED CELL RADIOIRON FOLLOWING INTRAVENOUS INJECTION

Turpentine Abscesses in Normal and Anemic Dogs*, ‡,§

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It is generally conceded that the *anemia of infection* is related to a disturbance in the production of hemoglobin, but the exact mechanism of the inhibition is uncertain. Possible alterations in the availability or internal metabolism of the necessary building materials and catalysts as well as the effects of variable stimuli to hemoglobin synthesis constitute some of the many facets of this still puzzling problem.

Robscheit-Robbins and Whipple (14) showed that in the anemic dog the increased production of hemoglobin following liver feeding or fasting was markedly impaired in the presence of sterile inflammation caused by turpentine, but that the low basal hemoglobin production on a standard salmon bread diet remained unchanged. These findings, together with observations on two dogs with spontaneous infection (endometritis) (14), indicated that neither impaired absorption nor increased destruction were necessarily responsible for the decreased hemoglobin production.

Radioactive iron has been used by several groups of investigators in attempts to determine whether alterations in the utilization of this element, *per se*, could account for the changes observed in association with infection or sterile inflammation. Hahn *et al.* (10) suggested impaired absorption as the cause of a markedly reduced utilization of orally administered radioactive iron in a small series of dogs given a single turpentine abscess. On the other hand, Wintrobe and his coworkers (15, 7) after studying the uptake of parenterally administered radioactive iron in pyridoxine- and iron-deficient pigs and in human subjects with chronic infection, concluded that iron was not the

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limiting factor in the impaired hemoglobin formation which is associated with the anemia of infection. Dubach *et al.* (3) demonstrated a low utilization of intravenous radioactive iron in a small group of patients with anemia and chronic infection or neoplasm.

The experiments to be presented in this report deal with the effects of multiple turpentine abscesses, produced consecutively over a 2 week period, on the utilization of intravenous radioactive iron in normal dogs and in dogs rendered anemic and iron-deficient by continued bleeding.

Normal dogs with sterile inflammation develop a progressive anemia which is associated with marked impairment in the uptake of radioactive iron by the red cells. This contrasts sharply with the absence of any alteration in the ability of the already anemic dogs on a standard basal diet to utilize the injected iron in the presence of turpentine abscesses.

The results of these experiments corroborate the findings of others concerning impaired hemoglobin formation in the development of the anemia of infection, but indicate that in the presence of the powerful stimulus due to severe depletion anemia the inhibitory effects of an inflammatory process can be overcome.

Methods

All dogs were mongrels and vaccinated against distemper. Normal, non-anemic, dogs were maintained on a diet of hospital scraps. Details relating to the production of anemia by repeated bleeding and the basal salmon bread ration fed to the anemic group have been previously described (17).

By the subcutaneous injection of 1 ml. of turpentine in the subscapular region at 3 to 4 day intervals, a more or less continuous sterile inflammatory reaction was maintained for 10 to 20 days.

Hemoglobin determinations were made by the cyanmethemoglobin method of Evelyn and Malloy (4) modified so that all the packed cells from a 5 to 10 ml. sample of blood were used.

The α, α'-dipyrridyl method of Kitzes, Elvehjem, and Schuette (12) was used to determine plasma iron concentration.

Radioactive iron, a mixture of Fe^{59} and Fe^{55}, was obtained from the Atomic Energy Commission.

Radioactive iron procedures were essentially those described by Hahn (8). Samples of red blood cells and plasma were first dry ashed in a muffle furnace at 550–600°C. Radioactive iron recovery with this method was 95 to 100 per cent complete. Specimens of feces, urine, and pus were prepared for electroplating by wet ashing followed by ether extraction (3).

Plasma volume determinations were made by the Evan's blue (5) method and the utilization of radioactive iron for hemoglobin synthesis was estimated from the calculated red cell mass corrected for the error introduced by the venous hematocrit (6, 11). The factor 0.82 suggested by Gibson *et al.* was actually used.

Radioactive iron was administered intravenously in gelatin (9).

EXPERIMENTAL OBSERVATIONS

Data pertaining to control experiments and to those in which sterile inflammation was induced by the subcutaneous injection of turpentine are shown for both non-anemic and anemic dogs in the tables.

Within 24 hours after the injection of turpentine, the animals, in addition to a local abscess, developed fever, lethargy, and some loss of appetite. The abscess became fluctuant in about 48 hours and usually ruptured in 3 to 4 days unless aspirated. Evacuation of pus was followed by a very rapid healing and an obvious return to normal.

Initial blood hemoglobin concentrations are listed in Table A, fifth column, and the lowest concentrations reached in the sixth column. The latter figures resulted from sampling alone in control experiments, and from a combination of sampling and sterile inflammation in the experiments involving turpentine injection. The average drop in hemoglobin concentration in *non-anemic* animals without inflammation was 8.1 per cent, in non-anemic animals with inflammation 42.0 per cent, in *anemic* animals without inflammation 8.5 per cent, and in anemic animals with inflammation 6.6 per cent. This point is graphically illustrated in Figs. 1 and 2, where a significant drop in hemoglobin concentration was seen only in the non-anemic dog following turpentine injection.

Plasma Iron Related to Anemia and Turpentine Injection.—From numerous observations in this and other laboratories it has been found that the average figure for plasma iron concentration in the normal dog is approximately 150 gamma per 100 ml. With chronic anemia due to bleeding and a diet low in iron plasma iron concentrations fell to about 50 gamma per 100 ml. or less, depending to some extent upon the degree of anemia (Table A).

Following repeated sterile, subcutaneous abscesses in non-anemic dogs the plasma iron showed a steady downward trend as anemia developed with sharper transient drops corresponding to periods of maximum body temperature. Anemic dogs on the other hand, showed only some fluctuations above and below the initially low plasma iron levels without any tendency to further lowering.

Plasma Iron Following Intravenous Injection.—Samples taken 10 minutes after the injection of radioactive iron were analyzed for total iron and radioactive iron. The total increment in circulating plasma iron averaged 70 per cent (range 54 to 88 per cent) of the amount injected (Table A, tenth column). In all but one instance less radioactive iron was present after 10 minutes in the circulation of the animals with inflammation than in the controls.

Although the iron-binding capacity of the plasma was not determined in this study, the maximal plasma iron concentrations (Table A, ninth column) were never greatly in excess of anticipated capacity levels and there was close agreement between the maximal concentration and the sum of the *initial* iron concentration and the 10 minute radioactive iron concentration.

In all instances total plasma iron and radioactive iron concentration dropped rapidly during the first 6 hours, the fall being a little slower in the non-anemic dogs. In all animals the residual radioactive iron in the plasma was insignificant

in amount 24 hours after the injection. Iron disappearance curves in two characteristic experiments are illustrated in Fig. 3. In no case was the rate of iron removal significantly modified by the presence of sterile inflammation.

Red Blood Cell Utilization of Intravenous Radioactive Iron.—Total radioactive iron in the circulating red blood cells, expressed as per cent of the adminis-

TABLE A

Effect of Turpentine Abscesses on Hemoglobin and Plasma Iron

Dog	Weight	Diet	No. of abscesses given	Hemoglobin		Radio-iron dose	Plasma iron		Maximum total plasma radioiron
				Initial	Minimal		Initial	Maximal	
	kg.			*gm. per cent*	*gm. per cent*	*mg.*	*gamma/ 100 ml.*	*gamma/ 100 ml.*	*per cent of dose*
Non-anemic dogs									
43–326	14.3	Kennel	5	14.7	8.4	2.5	141	500	67
43–326	14.3	"	0	14.4	13.2	2.5	130	449	88
45–36	15.5	"	0	15.2	14.7	1.5	174	463	75
45–36	15.2	"	4	15.7	9.1	2.6	80	529	66
47–79	17.6	"	0	14.1	12.3	2.6	140	350	81
47–79	16.6	"	3	15.7	9.5	3.2	150	372	71
Anemic dogs									
43–381	19.5	Salmon	0	5.0	4.7	3.2	42	285	64
43–381	"	Bread	3	5.9	5.5	3.2	41	263	55
43–381	"	"	0	4.4	4.4	4.5	34	315	54
44–206	12.7	"	0	6.8	4.8	2.1	60	322	72
44–206	"	"	4	5.2	5.1	2.1	48	257	76
44–206	"	"	0	3.6	3.3	4.5	50	353	54
43–31	15.0	"	0	6.1	6.1	4.2	51	466	82
43–31	"	"	3	4.4	3.9	4.5	36	497	74

tered dose, is shown at different time intervals after injection in Table B. In control experiments with both anemic and non-anemic animals a variable but significant incorporation of the labelled iron into the red cells was noted within 24 hours. In this control group maximal values were usually attained in from 1 to 2 weeks in anemic dogs, and in from 2 to 3 weeks in non-anemic dogs. It will be noted that the maximal incorporation of radioactive iron in the red cells of the anemic dogs ranged from 58 to 79 per cent of the dose injected. This is at variance with the 80 to 100 per cent utilization reported by Dubach,

Moore, and Minnich (3) who derived their figures from an estimated blood volume of 80 ml. per kilo body weight. In the present study, however, the observed blood volume in dogs made anemic by repeated bleeding was found to be about 65 ml. per kilo body weight.

In order to test the effect of sterile inflammation on the incorporation of the radioactive iron into red blood cells, injections of this material were given

TABLE B

Effect of Turpentine Abscesses on Red Cell Utilization of Intravenous Radioiron (1.5 to 4.5 mg. Dose)

Dog	No. of abscesses given	Utilization in relation to time, *per cent of dose*								
		1 day	2 days	4 days	1 wk.	2 wks.	3 wks.	4 wks.	5 wks.	6–8 wks.
Non-anemic dogs										
43–326	5	4	9	—	15	15	28	29	34	40
43–326	0	22	37	—	72	74	—	78	—	—
45–36	0	—	11	39	47	58	63	—	68	74
45–36	4	0	0	0	8	4	29	40	59	68
47–79	0	13	—	—	33	39	45	—	—	—
47–79	3	0	—	5	23	38	39	40	—	—
Anemic dogs										
43–381	0	4	15	35	47	59	61	—	—	—
43–381	3	6	—	36	70	69	70	—	76	—
43–381	0	—	—	43	44	55	64	67	—	—
44–206	0	2	9	30	41	58	57	—	—	—
44–206	4	0	—	29	46	75	73	73	—	—
44–206	0	7	—	41	52	55	62	72	—	—
43–31	0	—	58	—	75	76	76	—	—	—
43–31	3	20	—	—	54	75	79	78	—	—

to both non-anemic and anemic dogs 24 hours after the induction of the second of a series of turpentine abscesses.

The effects of this regimen on the red cell utilization of the intravenous radioactive iron in the non-anemic dog may be summarized as follows. During the period of active inflammation the rate of incorporation of the injected iron into circulating red cells was markedly diminished. In two experiments (dogs 43-326 and 45-36) when the inflammation was continued for 2 weeks the red cell utilization of the iron by the end of this period was 80 per cent and 93 per

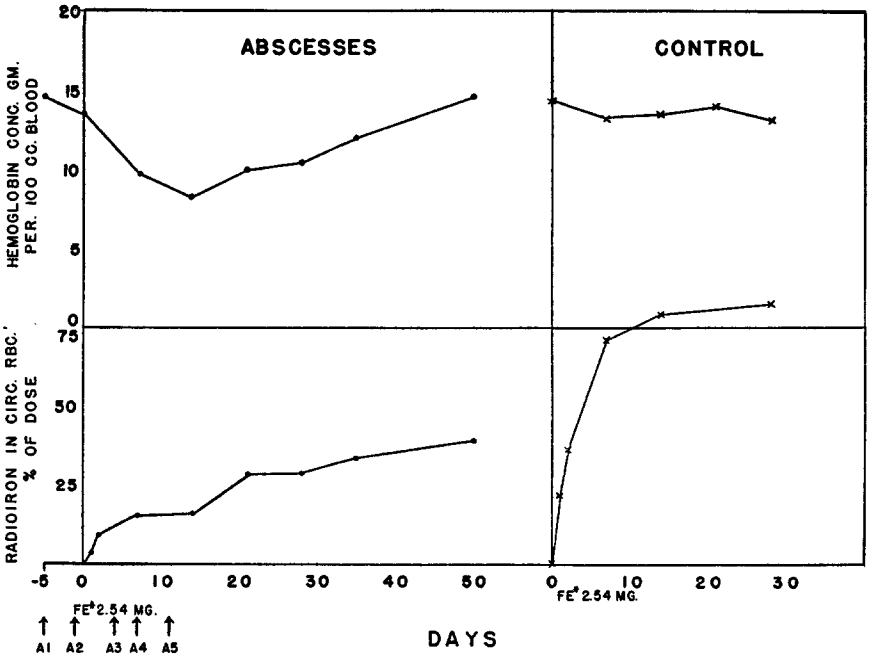

FIG. 1. Dog 43-326. Effect of turpentine abscesses on blood hemoglobin concentration and red cell utilization of injected radioiron in a non-anemic dog. A1, A2, etc. indicate the subcutaneous injection of 1 ml. of turpentine. Radioiron injected at time zero.

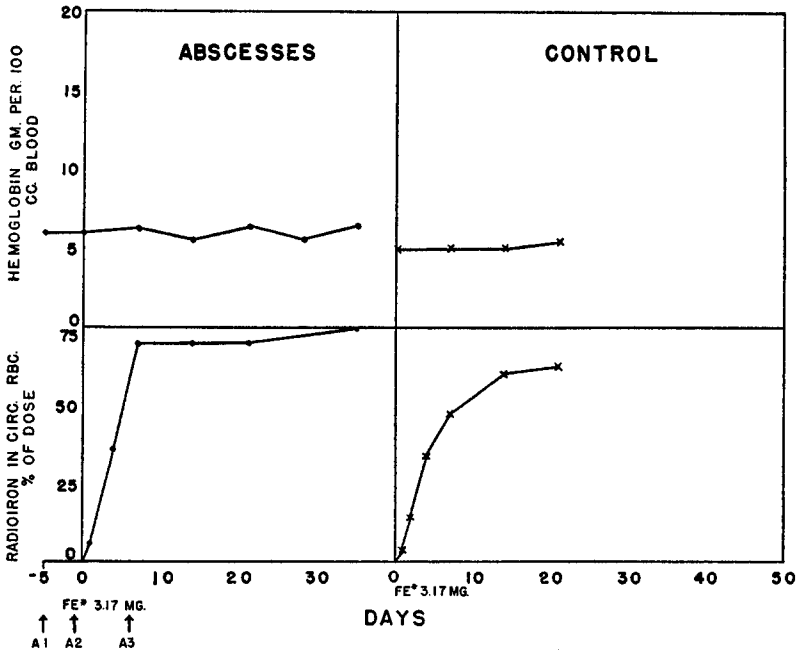

FIG. 2. Dog 43-381. Effect of turpentine abscesses on blood hemoglobin concentration and red cell utilization of injected radioiron in an anemic, iron-deficient dog. A1, A2, etc. indicate the subcutaneous injection of 1 ml. of turpentine. Radioiron injected at time zero.

cent lower than the control levels. In dog 47-79, on the other hand, with inflammation present for only 1 week, there was marked retardation of utilization during this 7 day period with a rise to the control level at the end of 2 weeks.

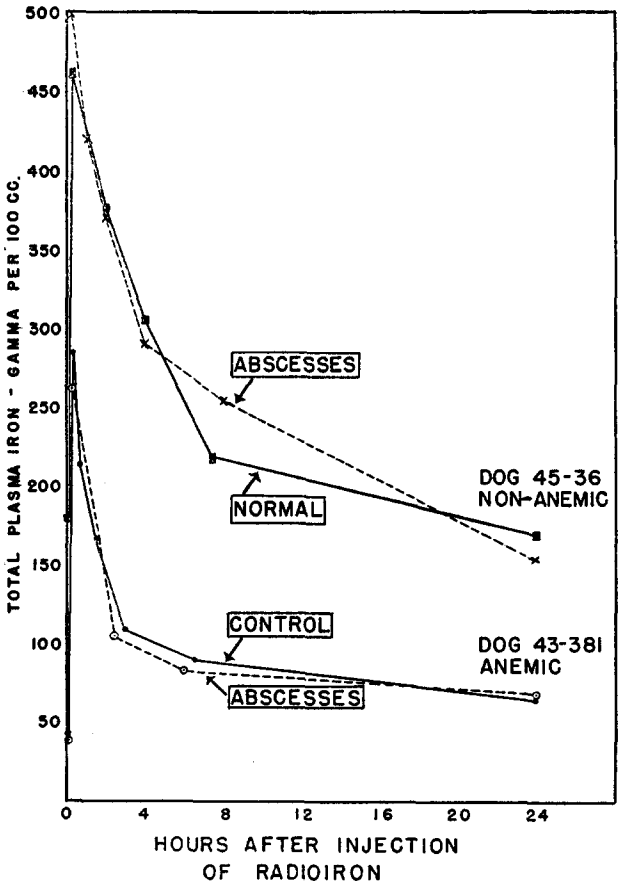

FIG. 3. Total plasma iron disappearance curves in non-anemic and anemic dogs with and without turpentine abscesses.

In these non-anemic dogs, the decreased utilization was associated with the development of a moderate anemia with the hematocrit and hemoglobin values falling about 40 per cent. In the postabscess period, recovery from the anemia occurred in 3 to 5 weeks and there was a tendency for the red cell radioactive iron ultimately to approach the control levels (Fig. 1 and Table B).

Dogs rendered anemic by bleeding and maintained on a basal diet low in

iron, when subjected to a similar series of turpentine abscesses reacted quite differently. In the presence of inflammation the red cells incorporated, if anything, more radioactive iron than in the control experiments, and there was no increase in the degree of anemia (Fig. 2 and Table B).

Additional observations made during the course of these experiments showed that insignificant amounts of radioactive iron, probably related to red cell contamination, appeared in the inflammatory exudates, and that if the presence of turpentine abscesses modified the urinary or fecal excretion of the intravenous iron the changes were too small to detect by the available methods. Total plasma protein concentrations showed a moderate decrease of up to 1.0 gm. per 100 ml., but there was no consistent specific alteration in the albumin/globulin ratio.

DISCUSSION

Sterile inflammation extending over a period of 2 weeks does not decrease the uptake of intravenous radioiron by the red cells or lower the blood hemoglobin concentration in anemic iron-deficient dogs on a basal diet low in iron. This agrees with the reported findings of Robscheit-Robbins and Whipple (14) that the hemoglobin output as measured by bleeding in the standard anemic dog on the same diet is unchanged by sterile inflammation. These observations suggest that under the conditions of iron deficiency due to bleeding and a diet low in iron the *maximal stimulus* to use all available materials for hemoglobin synthesis dominates the situation, and is capable of overcoming the inhibition caused by sterile inflammation. This contrasts with the decreased response to liver feeding in anemic dogs (14) in the presence of sterile inflammation. Under these conditions sufficient building materials are supplied but the dog is unable to synthesize hemoglobin at a maximal rate.

The inhibition of hemoglobin production observed in the non-anemic, non-iron-deficient dogs given turpentine abscesses, as indicated by the development of anemia and the delayed uptake of radioiron, can in no way be attributed to a lack of iron. This supports the view, expressed by Greenberg *et al.* (7) that iron is not the limiting factor in the anemia of infection, a view based upon the inability of intravenous infusions of iron to increase hemoglobin formation in patients with anemia and chronic infection.

It is interesting to speculate about the possible relationship between the ability of the anemic, iron-deficient dog to overcome the effects of sterile inflammation; and the influence of cobalt in preventing the development of anemia in growing rats given weekly subcutaneous injections of turpentine (16), and in improving the anemia in patients with chronic infections (1, 13). At least there is apparently an increased stimulus to erythropoiesis capable of negating the usual effects of inflammation in both instances.

From the data in the literature and the results of this study it is suggested

that the deleterious effect of inflammation on hemoglobin synthesis is most likely related to interference with some metabolic reaction rather than to a deficiency of some critical building material. The impairment whatever its nature, is only a relative matter since the anemias associated with infection or sterile inflammation are seldom severe or progressive beyond a certain point. Thus a sufficient stimulus to the bone marrow may overcome the usual inhibitory effects of such inflammatory processes.

A question raised by Hahn, Bale, and Whipple (10), concerns the possible rôle of decreased intestinal absorption of iron in the production of the anemia of infection. These authors found a marked reduction in red cell uptake of radioiron fed 24 to 48 hours after the induction of a single, subcutaneous turpentine abscess in a small series of dogs. Since, in the present study dogs under comparable conditions showed no impairment of their ability to utilize small doses of intravenous iron for hemoglobin synthesis, the findings of Hahn, Bale, and Whipple can best be explained by lowered absorption from the intestine. However, the behavior of the non-anemic dogs reported herewith, the studies of Dubach, Callender, and Moore (2), and those of Wintrobe and his coworkers would tend to minimize the importance of the intestinal factor as far as clinical anemias associated with infection are concerned.

CONCLUSIONS

Sterile inflammation induced by repeated subcutaneous injections of turpentine in non-anemic, non-iron—deficient dogs, leads to a fall in plasma iron concentration, the development of a moderate anemia, and a marked delay in the uptake by the red blood cells of intravenous radioiron.

Similar periods of inflammation in anemic, iron-deficient dogs on a diet low in iron cause no increase in the degree of anemia and no inhibition of red blood cell uptake of intravenous radioiron.

Radioiron appears only in traces in abscess exudates.

Intravenous iron disappearance curves following a single injection are uninfluenced by sterile inflammation in either anemic or non-anemic dogs.

The impairment of hemoglobin synthesis caused by inflammation is at most a relative matter, since the anemia that develops is seldom severe or progressive, and since the *inhibition can be overcome* if the marrow is sufficiently stimulated by the demands of a severe continuing anemia.

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