THE ANEMIA OF INFECTION. III. THE UPTAKE OF RADIOACTIVE IRON IN IRON-DEFICIENT AND IN PYRIDOXINE-DEFICIENT PIGS BEFORE AND AFTER ACUTE INFLAMMATION [1]

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(Received for publication September 21, 1946)

In the first (1) of this series of studies on the pathogenesis of the anemia of infection, it was pointed out that there must be in association with infection a derangement in the intermediate metabolism of iron. This conclusion was based on the observation that in cases of infection associated with anemia, the plasma iron content is markedly lowered below the normal; this appears early, and is found even before anemia has developed. The injection of staphylococci or of turpentine in dogs was shown (2) to be associated with hypoferremia, and evidence was presented (1) which indicated that absorption of iron is probably adequate when infection is present, the hypoferremia being accompanied by a rapid removal of iron from the plasma. The present report, and those which follow, describe experiments which were designed to study the nature of the disturbance in the internal metabolism of iron.

When iron deficiency is present, anemia develops which is accompanied by marked hypoferremia. If iron is then given intravenously (3) it is taken up rapidly and quantitatively for erythropoiesis. The anemia associated with pyridoxine deficiency, on the other hand, is characterized by inability to use iron, with the result that it is deposited in the tissues and marked hyperferremia develops (4). Thus these conditions represent wide extremes in the ability to use iron for hemoglobin formation. It was thought that some insight might be gained as to the nature and severity of the alteration in iron metabolism associated with infection if hemoglobin synthesis and the uptake of radioactive iron by the red corpuscles were studied before and after acute inflammation was induced in these widely different types of anemia.

To measure the capacity to utilize iron for hemoglobin synthesis, 2 widely different quantities of radioactive iron (Fe^{59}) have been injected intravenously in the experimental animals. The smaller amount, 10 to 17 μg. per kgm. body weight, corresponds to the quantity which, in the normal human, may possibly be absorbed each day (5), namely 0.7 to 1.2 mgm. This is considerably lower than the amount of iron liberated from hemoglobin breakdown per day, which may amount to 25 mgm. The larger dose of Fe^{59} we have employed, 225 to 330 μg. per kgm. body weight, corresponds approximately to the amount derived each day from hemoglobin catabolism. Provided iron metabolism in the pig is similar to that in man, the smaller injection of Fe^{59} may therefore be regarded as much below the amount which the hemopoietic system can use, while the larger amount slightly taxes the metabolic system, since it presumably doubles, approximately, the quantity of iron which is available for the formation of hemoglobin.

In order to make certain that the radioactive iron appearing in the blood was present in hemoglobin, in a number of instances hemin was crystallized from the blood of animals which had received Fe^{59}. The radioactivity of the crystalline material was then compared with the activity in the whole blood per mgm. of iron.

METHODS

The experimental animals were growing pigs, similar to those used in studies already reported from this laboratory (6).

Inflammation or infection was produced in the following manner. Sterile abscesses were produced by intramuscular or subcutaneous injections of 4 ml. of turpen-

[1] The work described in this paper was commenced under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Utah. The expenses of this investigation were also defrayed in part by grants from the United States Public Health Service, Parke, Davis and Company and the Upjohn Company.

tine. Bacterial infection was induced by the daily intramuscular injection of 5 ml. of a mixed culture of *S. aureus* and *E. coli*. To produce burns, the bottom of a 125 ml. Erlenmeyer flask containing boiling water was placed on the flank of the animal for 20 seconds.

For intravenous injection the radioactive iron was prepared in the following manner. An aliquot of the Fe^{59}, obtained as $FeCl_3$ in hydrochloric acid, was reduced with ascorbic acid. To this 2 N sodium hydroxide was added drop by drop, until the solution just became violet brown. The solution was then diluted to volume with water. If a greater quantity of iron per unit volume was desired, non-radioactive $FeCl_3$ was added before reduction with ascorbic acid. The purity of the radioactive iron employed was ascertained by the rate of decay.

Hemin was isolated from blood samples by the method of Schalfejeff (8) as follows. A sample of blood cells was allowed to drip for a period of 15 minutes from a small separatory funnel into a 50 ml. centrifuge tube containing 3 volumes of glacial acetic acid saturated with sodium chloride. The tube was kept at 90 to 95° C. by using a hot plate, and the solution was agitated constantly with an electric stirrer. After it had cooled to room temperature, the material was allowed to stand over night in the refrigerator. The hemin was centrifuged down and, in sequence, it was washed with 50 per cent acetic acid, distilled water, 95 per cent ethyl alcohol (twice) and ether (twice). The yield of crystalline hemin was 50 to 70 per cent. In order to determine the quantity of hemin, the dry material was dissolved in a known volume of 0.1 NaOH, and a dilution of this solution was read in the photoelectric colorimeter at 520 mμ using a standard curve constructed with hemin of known purity as determined by iron measurement and spectrophotometric analysis. The hemin solutions were then digested and prepared for counting just as in the case of blood. Along with the hemin isolation and analysis, Fe^{59} analyses were made of the same original blood, the plasma being removed prior to preparation.

Hemoglobin determinations were carried out on the samples taken for Fe^{59} estimation. The volume of blood used was noted in order to calculate the number of grams of hemoglobin analyzed. The photoelectric oxyhemoglobin method of Bell, Chambers and Waddell (9) was employed, the instrument having been standardized by several procedures, including the oxygen capacity method (10) and the hemin method (11).

Calculation of Fe^{59} uptake. In studies with human subjects the uptake of Fe^{59} is usually calculated as follows (12):

$$\text{Total circulating hb.} = \frac{\text{blood volume}}{100} \times \text{grams hb. per 100 ml. blood;}$$

$$\text{Circulating counts} = \text{total circulating hb} \times \text{counts per gram hb.}$$

Many workers have assumed that the total blood volume is 80 ml. per kgm. body weight, believing that this assumption is at least as accurate as most total blood volume estimations made by calculation from the measured plasma volume and volume of packed cells (12).

In the case of growing pigs we have encountered considerable difficulty because of obvious shifts in plasma volume. In such pigs, especially in anemic animals, the hemoglobin per 100 ml. of blood shows wide and rapid variations. As a result, with sharp changes of hemoglobin one may get such impossible values as negative uptakes well beyond the range of error. In order to overcome this difficulty, we have devised a method of calculation which is based on the following assumptions.

(*1*) The total circulating hemoglobin is relatively constant, or increases or decreases consistently, and under our experimental conditions does not vary as much as is indicated by the changes in hemoglobin per 100 ml. of blood.

(*2*) The variations of the total circulating hemoglobin as obtained by the formula,

$$\frac{\text{weight of animal (kgm.)} \times 80}{100} \times \text{hb. per 100 ml.,}$$

range about the true value in a homeostatic manner. Accordingly, in these studies the body weight and the hemoglobin, in grams per 100 ml. of blood, have been plotted for each pig against time. From these values the total circulating hemoglobin has been calculated, and this also has been plotted on the graph. A line has then been drawn through the estimated average of the plotted values for the total circulating hemoglobin. It is assumed that this line presents a picture of the true alterations in total circulating hemoglobin. When for the calculation of Fe^{59} uptake it has been necessary to know the total circulating hemoglobin, the value has been taken from the appropriate point on this mean curve.

In the case of iron-deficient pigs, the actual mass of circulating hemoglobin was also determined by assuming that in such animals Fe^{59} is completely used for hemoglobin formation in 7 to 14 days or less. The following formula may be applied when Fe^{59} utilization is complete:

$$\frac{\text{Total counts injected intravenously}}{\text{counts per gram hemoglobin}} = \text{total circulating hb.}$$

The correlation of the estimated values for total circulating hemoglobin with those obtained by the radioactive method is shown in Figure 1. Since both methods entail sources of error the degree of correlation found is of interest.

RESULTS

Figure 2 illustrates the type of uptake curves obtained in different pigs. The complete data are recorded in Tables I to IV.

It will be observed that the iron-deficient animals usually showed rapid uptake, with little difference in the degree of uptake of the small and large doses of iron. This was to have been expected, since it is well known that such animals

can utilize iron very rapidly, even when it is given in large quantities. On the other hand the pyridoxine-deficient pigs were able to use a good percentage of the small dose only, a negligible percentage of the larger dose being used. In terms of absolute quantities of iron, the uptake was of the same order of magnitude when the large or the small dose was given. In the pyridoxine-deficient pig, hemoglobin formation is limited because of deficiency of this vitamin (4). That the iron not used in hemoglobin formation is stored, is indicated by the observations illustrated in Figure 3,

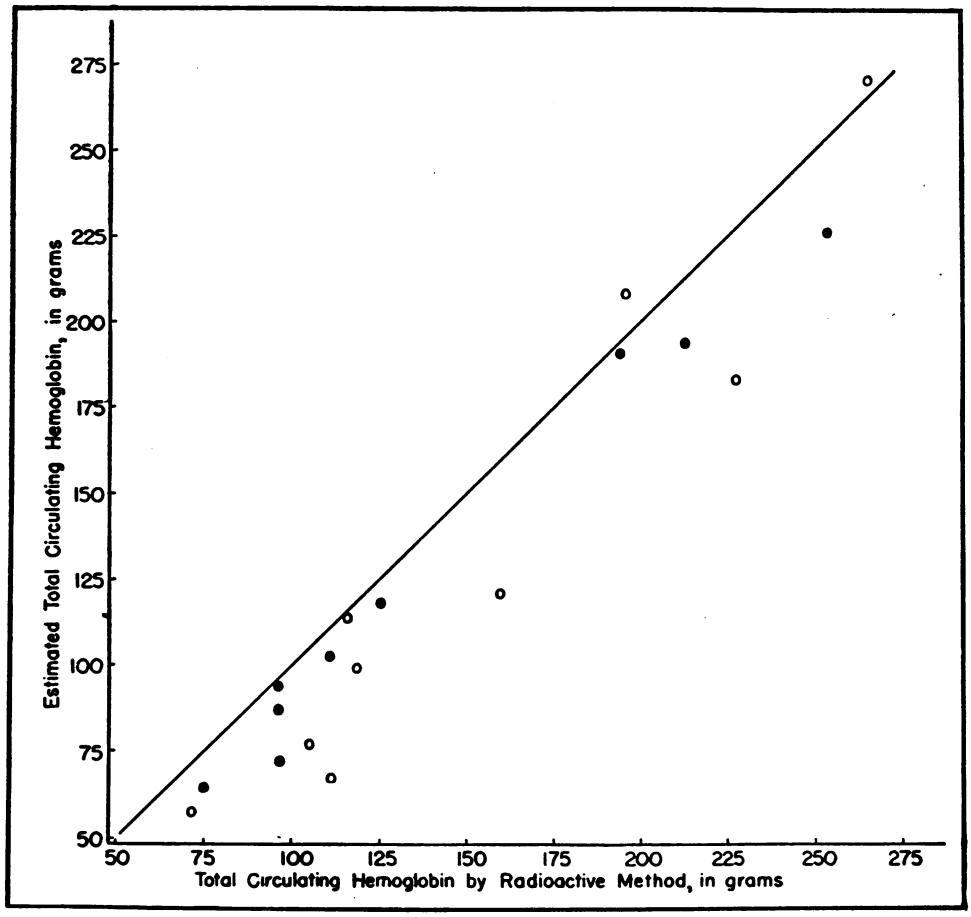

FIG. 1. THE CORRELATION OF TOTAL CIRCULATING HEMOGLOBIN AS ESTIMATED FROM A LINE BEST FITTING THE PLOTTED VALUES FOR TOTAL CIRCULATING HEMOGLOBIN (SEE TEXT) ("ESTIMATED TOTAL CIRCULATING HEMOGLOBIN") WITH THE TOTAL CIRCULATING HEMOGLOBIN AS DETERMINED FROM THE DILUTION OF RADIOACTIVE IRON INJECTED INTRAVENOUSLY IN IRON-DEFICIENT PIGS

The closed circles represent the correlation with the values based on the first injection of radioactive iron, and the open circles refer to values after a second injection. In these calculations the total amount of radioactive iron injected in the animal has been divided by the number of counts per gram. The necessity for assuming that all of the injected Fe^{59} has been incorporated in the hemoglobin, even when more than one injection has been made, limits the accuracy of this method. The trend for higher values for total circulating hemoglobin, as measured by the radioactive method as compared with those based on the estimated values, can be explained, at least in part, by failure to use Fe^{59} completely for hemoglobin formation. Consistent with this view is the fact that the best correlation was found with the values obtained after the first injection of radioactive iron, when the likelihood of complete utilization of iron for hemoglobin formation was greater.

in which Fe^{59} uptake by the red cells is seen to increase sharply when pyridoxine is supplied.

The uptake of iron by the red cells of the normal animals was intermediate between that observed in the pyridoxine-deficient and the iron-deficient pigs. In the normal mature pig the small dose was used more rapidly than the large one.

Figure 4 shows the effect of acute inflammation on the rate of hemoglobin formation in iron-deficient pigs. It will be seen that 5 of the 6 pigs showed retarded hemopoiesis. Since the otherwise normal iron-deficient animal is capable of completely utilizing for hemoglobin synthesis much larger doses of Fe^{59} than those used in this experiment, retardation of the uptake of 10 μg. Fe^{59} per kgm. must be regarded as indicating severe depression of hemopoietic function. Pig 9–24, in Figure 2, is an example of the effect of a more chronic infection on the rate of uptake of Fe^{59}. This animal had been attacked by a dog and received severe lacerations on the legs which subsequently became chronically infected. It may be noted that in this pig a larger dose of Fe^{59} was utilized even more poorly than the smaller one.

Figure 5 illustrates the extent to which infection impairs the response to therapy with iron in iron-deficient pigs. Two iron-deficient pigs, one of which was receiving daily injections of a bacterial

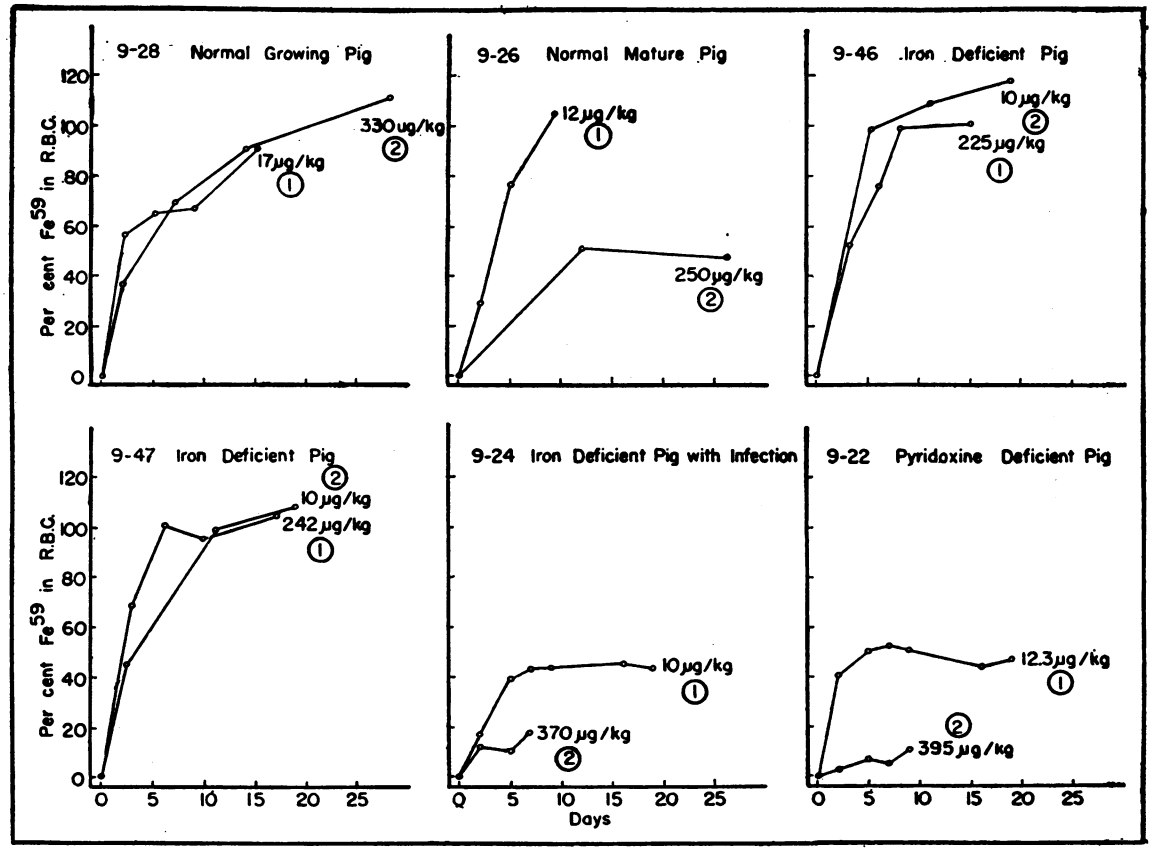

FIG. 2. UPTAKE OF INTRAVENOUSLY INJECTED RADIOACTIVE IRON (Fe^{59}) BY RED CELLS OF NORMAL GROWING AND MATURE PIGS AND IN PIGS WITH IRON DEFICIENCY OR PYRIDOXINE DEFICIENCY

A small and a large amount of iron was given in each instance. The order in which these were given is indicated as (*1*) and (*2*).

Note the more rapid uptakes of iron by the iron-deficient animals (9–46, 9–47) as compared with the normal ones, the less complete uptake of the large dose by the normal mature pig (9–26) as compared with the growing normal pig (9–28), and the poor uptake of iron by the pyridoxine-deficient pig (9–22).

The marked impairment of uptake of iron produced by infection in an iron-deficient pig (9–24) as compared with the uptake in a similar animal without infection (9–47), should be noted.

TABLE I

Uptake of small doses of intravenously injected Fe^{59} by the red corpuscles of normal and of pyridoxine-deficient pigs

Pig number	Normal					Pyridoxine deficient			
	9–25	9–26	9–28	9–29	9–30	9–22	9–39	9–40	9–40
Age, days	300	263	146	146	146	146	66	66	80
Weight (kgm.)	97.0	75.0	43.1	38.0	37.1	22.0	14.3	11.6	14.7
Previous injections of Fe^{59}, number	3	2	0	0	0	0	0	0	1
Previous injections of Fe^{59}, amount (mgm.)	40.83	12.57	0	0	0	0	0	0	0.12
Days since previous injection	37	96	0	0	0	0	0	0	14
Fe^{59} injected, amount (mgm.)	0.97	0.90	0.74	0.60	0.62	0.27	0.14	0.12	0.15
Days after injection	Percentage uptake								
2	34.1	28.3	56.7	26.3		41.5	5.0	43.0	
3									21.3
5	75.4	76.7	64.7	42.6	60.6	50.1	15.3	52.5	
7				45.9	62.8	52.6			
9		105.0	66.9	40.5	67.0	50.6	13.3	63.0	
11								45.8	
12	51.3								
15			92.0	55.1					
16					69.4	44.5			
19				65.3	67.1	47.1			
25									31.2
26	65.5								
28									42.0

TABLE II

Uptake of large doses of intravenously injected Fe^{59} by the red corpuscles of normal and of pyridoxine-deficient pigs

Pig number	Normal					Pyridoxine deficient		
	9–25	9–26	9–28	9–29	9–30	9–22	9–42	9–42
Age, days	263	300	167	167	169	166	66	80
Weight (kgm.)	83.6	99.4	56.2	49.7	48.3	26.6	9.0	10.9
Previous injections of Fe^{59}, number	2	3	1	1	1	1	0	1
Previous injections of amount (mgm.)	19.83	13.47	0.74	0.60	0.62	0.27	0	2.25
Days since previous injection	96	96	21	21	21	21	0	14
Fe^{59} injected, amount (mgm.)	21.0	24.8	18.55	8.65	15.95	7.80	2.25	2.73
Days after injection	Percentage uptake							
2	0		36.4	25.5	34.4	2.2	1.6	0
4								10.3
5	72.8				29.5	6.9	8.0	
7			69.5	61.0	59.0	5.0		
9	58.2					10.2	5.5	
11								10.8
12		50.8						
14			91.0	98.5	81.7		5.3	
25								6.8
26		46.7						
28			111.0	127.6				19.4
36					92.8			

TABLE III

Uptake of small doses of intravenously injected Fe^{59} by the red corpuscles of iron-deficient pigs

Pig number	9–25	9–26	9–27	9–46	9–47	9–48	9–49	9–50	9–51
Age, days	146	146	146	84	84	84	67	67	67
Weight (kgm.)	36.8	29.3	37.9	25.9	27.0	25.2	20.0	23.7	34.5
Previous injections of Fe^{59}, number	0	0	0	1	1	1	0	0	0
Previous injections of Fe^{59}, amount (mgm.)	0	0	0	5.75	6.75	5.23	0	0	0
Days since previous injection	0	0	0	17	17	17	0	0	0
Fe^{59} injected, amount (mgm.)	0.48	0.27	0.40	0.26	0.27	0.25	0.20	0.24	0.35
Days after injection	Percentage uptake								
2	22.2	37.0	33.0	37.8	46.7	84.0			
3							82.6	59.0	66.0
5	52.2	54.1	42.2	98.4		127.6			
6							74.7	76.8	84.0
7	63.0	65.4	42.2						
9	57.0	92.5	58.7						
10							89.0		
11				110.0	98.5	134.0			
15	92.0		63.2						
17								97.0	98.5
19	88.7		90.5	120.0	107.0	138.0			

TABLE IV

Uptake of large doses of intravenously injected Fe^{59} by the red corpuscles of iron-deficient pigs

Pig number	9–25	9–26	9–27	9–46	9–47	9–48	9–49	9–50	9–51
Age, days	167	167	167	67	67	67	84	84	84
Weight (kgm.)	46.4	37.2	48.3	23.0	23.3	20.9	21.3	28.2	42.7
Previous injections of Fe^{59}, number	1	1	1	0	0	0	1	1	1
Previous injections of Fe^{59}, amount (mgm.)	0.48	0.27	0.40	0	0	0	0.20	0.24	0.35
Days since previous injections	21	21	21	0	0	0	17	17	17
Fe^{59} injected, amount (mgm.)	19.35	12.30	16.17	5.24	5.65	4.89	5.33	7.05	10.70
Days after injection	Percentage uptake								
2	59.0	28.8	33.9				48.3	43.8	60.0
3				53.3	69.5	82.3			
4	75.7								
5		55.8	65.3				65.2	58.5	102.0
6	91.2			77.0	100.5	102.0			
7			86.2						
8	99.0			100.0					
9		61.5	83.5						
10					95.5	109.0			
11							79.0	75.6	120.0
13	112.0								
14		62.5							
15				102.0					
17					104.0				
19								84.3	101.0

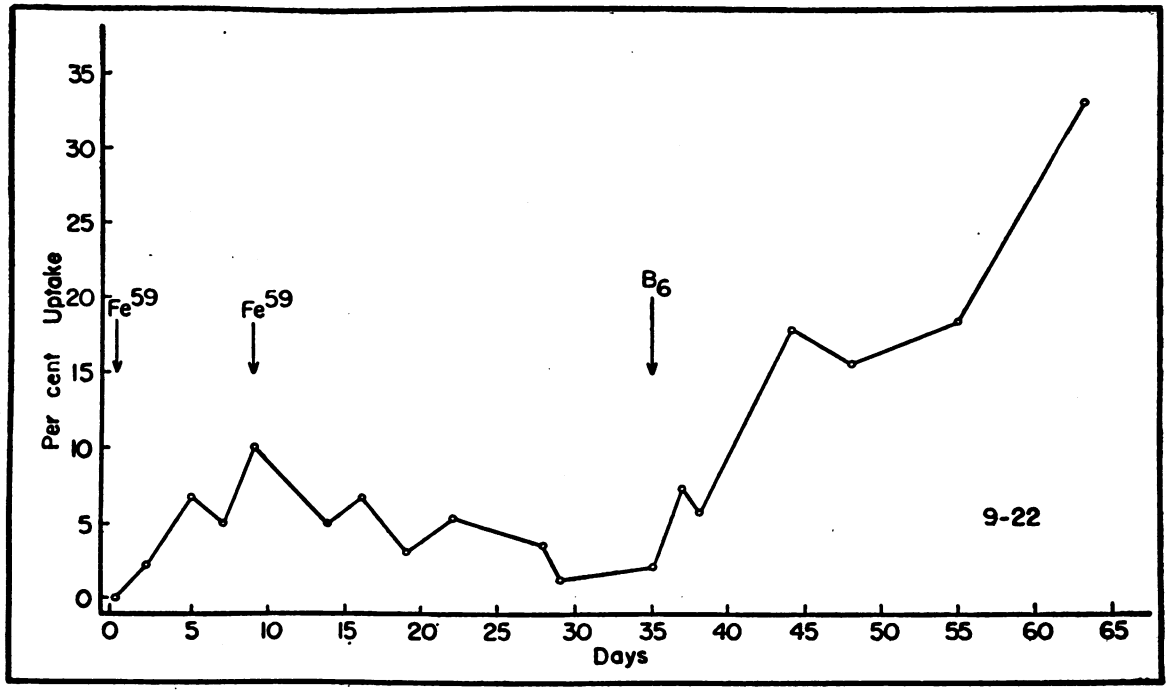

FIG. 3. UPTAKE OF INTRAVENOUSLY INJECTED RADIOACTIVE IRON (Fe^{59}) BY THE RED CELLS OF A PYRIDOXINE-DEFICIENT PIG BEFORE AND AFTER THE ADMINISTRATION OF PYRIDOXINE (B_6)

Two injections of Fe^{59}, each 250 μg. per kgm. body weight, were made. The percentage uptake has been calculated on the basis of the sum of the 2 doses.

That the iron which failed to enter the red cells before pyridoxine was given had been stored, is indicated by the appearance of the radioactive iron in the red cells after normal hemoglobin formation was made possible by the administration of pyridoxine.

mixture (*S. aureus* and *E. coli*), were both given intravenously 100 mgm. of iron per day for 4 days. The uninfected pig (9–49) showed prompt and rapid hemoglobin synthesis. From calculations of the total circulating hemoglobin before and after treatment, it is estimated that 83 per cent of the iron was utilized for hemoglobin formation. On the other hand, the animal receiving the bacterial mixture did not respond significantly to iron therapy until the bacterial injections were stopped and penicillin was used.

In Figure 6 is shown the effect of infection on the response of pyridoxine-deficient pigs to vitamin B_6. One pyridoxine-deficient pig (9–40) was given daily intramuscular injections of a bacterial mixture. Both were given pyridoxine intramuscularly. As will be seen, the animal receiving the bacterial mixture formed hemoglobin but slowly, even though large amounts of pyridoxine were given, whereas the non-infected pig synthesized hemoglobin promptly.

In Table V is presented evidence that Fe^{59} appearing in the blood cells is present as hemoglobin iron. The considerable variation in some instances, well beyond the usual error in the recovery and counting of Fe^{59} in a sample of blood (7), may be ascribed to the small quantity of hemin isolated and thus to the unusually small number of counts with which we were dealing. In spite of this technical difficulty, it will be noted that the average values for these animals showed approximately 100 per cent of the red cell Fe^{59} to be hemoglobin iron. The procedure employed for isolating and purifying the hemin could not possibly have carried through Fe^{59} which was not in the hemoglobin structure originally.

DISCUSSION

It is clear from the present studies that marked retardation of hemopoiesis occurs as a result of inflammation. This is in agreement with the findings of Robscheit-Robbins and Whipple (13)

who showed that a sterile abscess will diminish the production of new hemoglobin in the anemic dog when liver is being fed. That so powerful a stimulus as intravenous iron therapy for an iron- deficient animal, or pyridoxine for a B_6-deficient pig, cannot overcome the retardation or does so slowly and incompletely, attests to the severity of the effect of inflammation. Hemoglobin production normally has priority over other protein production (14). Any process which has precedence over hemoglobin formation can be expected to be important to the survival of the animal.

There is no reason to assume that the mechanism whereby hemoglobin formation is retarded by acute inflammation is different from that associated with chronic infection, even though anemia more commonly accompanies the latter than the former. That time is required for anemia to develop suggests that blood destruction is not a significant factor; instead, as red corpuscles wear out, they fail to be replaced. The "life cycle" of the red corpuscle is thought to be about 125 days (15).

It is of interest in connection with the pathogenesis of the anemia of infection that the pyridoxine-deficient pig which represents, in a sense, a state of supersaturation with iron, is incapable of synthesizing hemoglobin efficiently in the presence

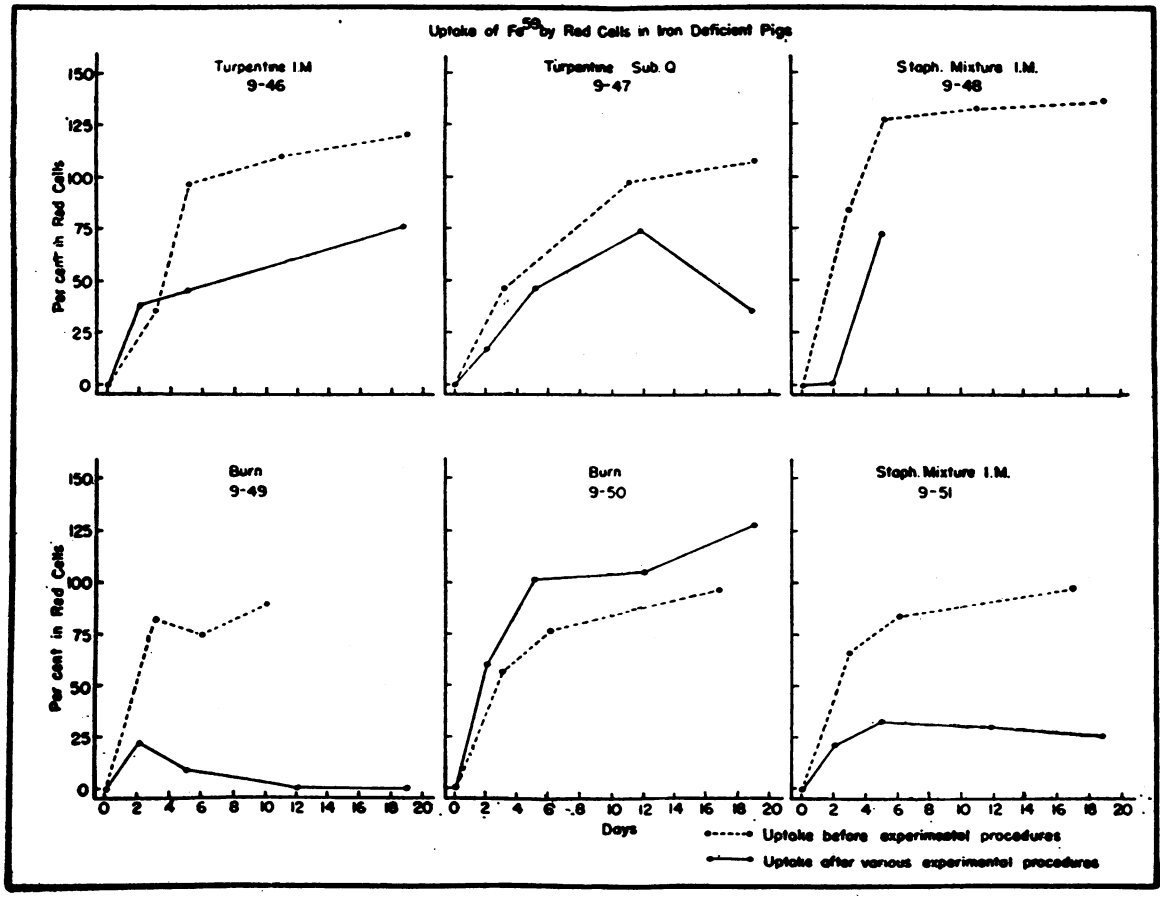

FIG. 4. THE INFLUENCE OF ACUTE INFLAMMATION ON THE UPTAKE OF INTRAVENOUSLY INJECTED RADIOACTIVE IRON BY THE RED CELLS OF IRON-DEFICIENT PIGS

In all instances small amounts of iron were given (10 μg. per kgm. body weight). The interrupted lines represent the uptake before the experimental procedure was carried out and the continuous line indicates the uptake after inflammation was produced.

Note that in every instance but one, a marked retardation of uptake occurred whether turpentine or a bacterial culture was injected, or a burn was produced.

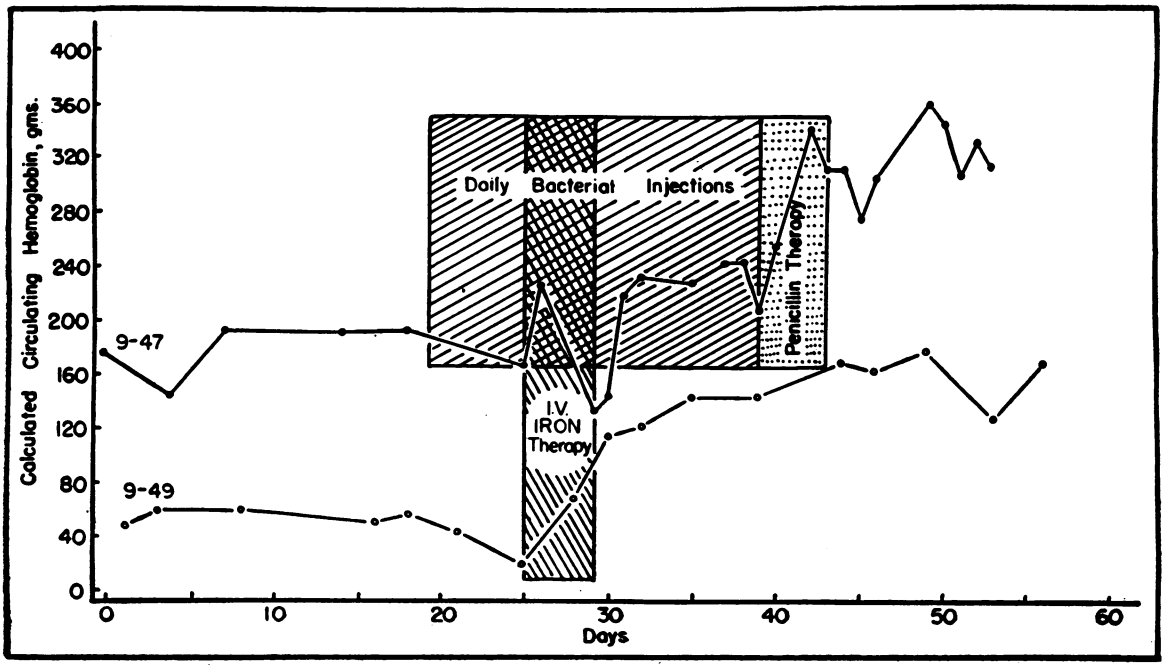

FIG. 5. THE EFFECT OF INFLAMMATION PRODUCED BY DAILY BACTERIAL INJECTIONS ON THE HEMOPOIETIC RESPONSE OF IRON-DEFICIENT PIGS TO THE ADMINISTRATION OF IRON

In the non-infected animal (9–49) the intravenous administration of 400 mgm. iron, given as ferric chloride reduced by ascorbic acid, and then brought to neutrality with alkali, was followed by a prompt and almost complete utilization of the iron for hemoglobin formation as estimated from the rise in calculated total circulating hemoglobin. In the infected animal, on the other hand, the rise above the average level preceding iron administration was comparatively small until the injections were discontinued, the abscesses were drained, and penicillin was given. Following such treatment the hemoglobin rose to the expected level.

of infection, even when pyridoxine is given. This suggests that a limitation of iron is not the primary cause of the anemia of infection. In the paper (16) which follows, additional evidence is presented which indicates that this anemia does not result from a lack of iron.

Comment may be made concerning the usefulness of the radioactive technic as a measure of retarded hematopoiesis. Observation of the rate and extent of the uptake of intravenously administered Fe^{59} is useful, particularly, in circumstances in which retarded hemoglobin formation is suspected but anemia is not present. Theoretically, at least, it should also be helpful in the study of cases where diminished red cell destruction exists to compensate for impaired hemoglobin formation. In using radioactive iron for such purposes, it is important to employ such amounts as will test the functional efficiency of the hemopoietic system, and yet will not be so great that the major portion of the iron given must necessarily be shunted into the iron stores.

SUMMARY

1. The uptake of radioactive iron, injected intravenously, has been studied in normal growing and mature pigs, in iron-deficient and in pyridoxine-deficient pigs, and in such animals when inflammation was produced by various means.

2. For this purpose, 2 different quantities of iron were used, one corresponding to the amount assumed to be absorbed daily from the diet, the other corresponding to the estimated amount derived from normal hemoglobin catabolism. These quantities have been selected in order to test the functional capacity of the hemopoietic system.

3. It has been shown by the isolation of hemin that the radioactive iron appearing in the blood is present in the hemoglobin moiety.

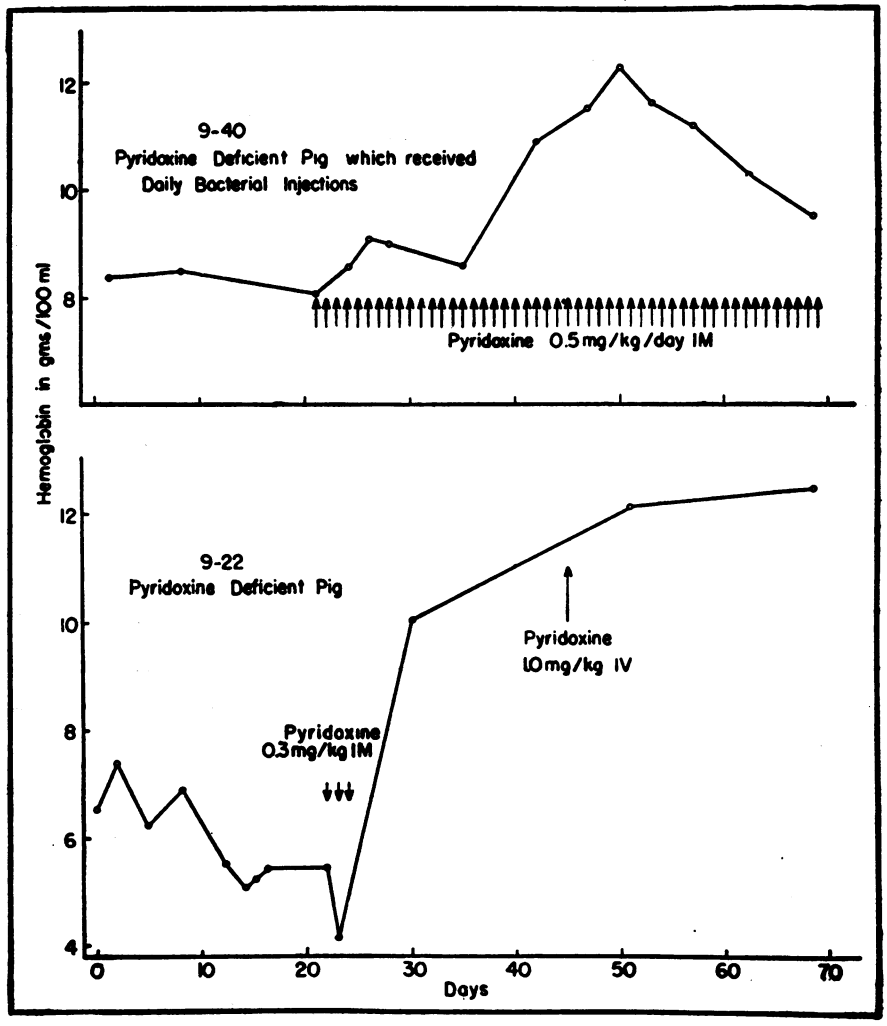

FIG. 6. THE EFFECT OF INFLAMMATION PRODUCED BY DAILY INTRAMUSCULAR INJECTIONS OF BACTERIAL CULTURES ON THE HEMOPOIETIC RESPONSE OF PYRIDOXINE-DEFICIENT PIGS TO THE ADMINISTRATION OF PYRIDOXINE

In the non-infected animal (9–22) the injection of pyridoxine was followed by a prompt and marked rise in hemoglobin, whereas in the pig in which infection was produced (9–40), the response was slow, small in amount, and was not maintained, even though much larger amounts of pyridoxine were given.

4. It has been demonstrated that the uptake of radioactive iron is rapid and complete in iron deficiency; slow, and even negligible, if large doses are used, in pyridoxine deficiency; and intermediate, between these extremes in normal animals.

5. In spite of the pronounced avidity of the iron-deficient animal for iron, in the presence of inflammation the uptake of iron is markedly impaired.

6. In pyridoxine deficiency the rapid and efficient uptake of iron which follows the administration of pyridoxine, is markedly reduced by the presence of infection.

7. It is concluded that the anemia of infection is caused by impaired hemoglobin production. Anemia does not appear at once, however, for it is only when outworn red corpuscles must be replaced that the defect is noticeable.

The radioactive iron used in these studies was obtained from the Massachusetts Institute of Technology through the courtesy of Dr. Robley D. Evans.

TABLE V

Conversion of intravenously injected Fe^{59} to hemoglobin iron in pigs

Animal no.	Status	Day following Fe^{59} injection	Percentage uptake of Fe^{59} by RBC	Percentage of RBC Fe^{59} which is hemoglobin iron
9–22	Pyridoxine deficient	5	50	101
		7	53	101
		9	51	116
9–24	Iron deficient + infection	7	43	106
		9	43	119
9–26	Iron deficient	2	37	104
		5	54	122
		7	65	97
9–30	Normal	5	61	126
9–42	Pyridoxine deficient	2	24	102
9–46	Iron deficient	2	62	89
9–47	Iron deficient	2	37	109
9–49	Iron deficient	2	59	103
9–50	Iron deficient	2	62	95
Average Fe^{59} as hemoglobin iron				107

Calculations;

a. Counts per mgm. hb. iron

$$= \frac{\text{Counts per gram hb. in original blood}}{3.39}.$$

b. Counts per mgm. hemin iron

$$= \frac{\text{Counts per 10 mgm. crystallized hemin}}{0.86}.$$

c. Percentage of RBC Fe^{59} which is hemoglobin iron

$$= \frac{b}{a} \times 100.$$

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