THE KINETICS OF IRON METABOLISM IN NORMAL GROWING SWINE*

BY W. N. JENSEN,‡ M.D., J. A. BUSH,§ M.D., HELEN ASHENBRUCKER, G. E., CARTWRIGHT, M.D., AND M. M. WINTROBE, M.D.

(From the Department of Medicine, College of Medicine, University of Utah, Salt Lake City)

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The availability of radioactive iron (Fe^{59}) of high specific activity and the development of the scintillation counter enabled Huff *et al.* (1) to devise a technique for determining the turnover rates of iron through plasma and red cells.

This method involves the intravenous injection of a tracer dose of Fe^{59} containing a quantity of carrier iron sufficiently small that the plasma iron concentration will not be elevated measurably. The Fe^{59} radioactivity disappears from the plasma according to the equation of a first order reaction and the time at which half of the injected dose has disappeared from the plasma ($T\frac{1}{2}$) is determined by plotting the counts of serial plasma samples on semilogarithmic paper. The plasma iron turnover rate (PITR) in milligrams per day may be calculated from the formula:

$$\frac{0.693 \times PV \times PI \times 24}{T\ 1/2} = PITR$$

in which PV is the plasma volume in milliliters, PI is the plasma iron concentration in milligrams per milliliter, 24 is the number of hours per day.

On subsequent days the red cells are assayed for Fe^{59} activity until a constant fraction of injected radioactivity is present in the erythrocytes. The red cell iron turnover rate (RBC ITR) in milligrams per day is calculated from the formula:

$$PITR \times Fe_c^{59} = RBC\ ITR$$

in which PITR is the plasma iron turnover rate in milligrams per day, Fe_c^{59} is the maximum fraction of injected Fe^{59} incorporated into the erythrocytes.

The total red cell iron (RBC I) in mg. may then be determined from the formula:

$$\frac{TBV}{100} \times Hb \times 3.4 = RBC\ I$$

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§ Postdoctoral Research Fellow, National Heart Institute, United States Public Health Service.

in which TBV is the total blood volume in milliliters, Hb is the hemoglobin concentration in gm. per 100 ml. of blood, 3.4 is the iron equivalent of hemoglobin (3.4 mg. iron per gm. of hemoglobin).

Since the daily turnover of iron through the red cells (RBC ITR) is known, the fraction of red cell iron renewed each day (RBC IRR) may be determined from the formula:

$$\frac{\text{RBC ITR}}{\text{RBC I}} = \text{RBC IRR}$$

The reciprocal of this is the average red cell life span.

The ferrokinetic technique has been used to study iron kinetics in normal human subjects and in patients with hematologic disorders (1–9). Since various types of experimental anemia can be produced readily in swine, this technique offered a promising avenue of approach to a better understanding of the nature of these disorders. The results of ferrokinetic studies in anemic swine will be the subject of a later paper (10). The purpose of this report is to present the results of ferrokinetic studies in normal growing swine.

Methods

Twenty-seven swine of the Chester-White breed were used in this study. The animals were housed in individual cages and handled in the manner described previously (11).

Twenty-three of the swine were fed the "normal" diet (12) consisting of crude casein, sucrose, lard, and swine salt mix along with supplementary vitamins and minerals. 4 of the animals were fed evaporated milk with added iron and copper as described previously (13).

Determinations of the red cell mass and estimates of the plasma and total blood volume were performed once or twice on each animal by a modification (13) of the method of Reid and Orr (14).

Hematologic studies (red cell and reticulocyte counts, hemoglobin and volume of packed red cell determinations, and calculations of corpuscular constants) were performed on the 1st and 8th days of the ferrokinetic study.

Plasma iron was determined by the method of Hamilton *et al.* (15). Hemin was isolated by the method of Fischer (16) and recrystallized and purified in the manner described by Shemin, London, and Rittenberg (17). Tissue hemoglobin was determined according to the method of Greenberg and Erickson (18).

The Fe^{59} was supplied as ferric chloride on allocation from the United States Atomic Energy Commission. 5 to 20 μc. of Fe^{59} containing 3 to 6 μg. of carrier iron were buffered to pH 6.0 with 4 per cent sodium citrate, and then allowed to incubate at room temperature for 30 minutes with the pig's own plasma. The swine were anesthetized with 5 per cent pentobarbital and 5 ml. of the plasma containing the Fe^{59} was injected into an ear vein. A standard was prepared by diluting 1 ml. of the remaining plasma-Fe^{59} complex to 250 ml. with water. Blood samples were obtained from the jugular vein every 15 minutes for the 1st hour and thereafter at 30 minute intervals. 1 ml. aliquots of the plasma samples were counted in a well-type scintillation counter. Each sample was counted for 4,096 counts. The remaining plasma was retained for plasma iron analysis. Uptake of Fe^{59} into the red cells was measured by counting 1 ml. of hemolyzed whole blood each day. The red cells were not washed, since no significant radioactivity was demonstrated in the plasma after day 0. In order to correct for radioactive decay, a 1 ml. aliquot of the standard was counted each day.

In vivo counting was performed on 4 of the animals. A mobile scintillation counter was employed for this study and counts were made over the liver, spleen, and sacral bone marrow.

Before the isotope was injected, the points of maximal liver and spleen dullness were determined by percussion, the hair was shaved, and the points were marked with India ink. In addition, a point over the sacrum about 6 cm. cephalad to the base of the tail was similarly shaved and marked. Care was taken to place the counter over the appropriate marked site in an identical manner at the time of each *in vivo* counting. After injection of the radioactive plasma, repeated counts were obtained as rapidly as possible over each site. These counts were then plotted on semilogarithmic paper. Since 100 per cent of the injected isotope was present in the plasma at time 0, extrapolation of the line through the sequential points obtained during the first 15 minutes after injection permitted an estimation of the activity in each organ at time 0, when 100 per cent of the dose was in the blood. Blood samples were obtained at the time of each subsequent *in vivo* counting. The gross count was corrected for activity due to blood circulating through each organ in the following manner (4): the count in the organ at time 0, the time when 100 per cent of the isotope was in the plasma, was multiplied by the fraction of the injected dose in the blood at the time of each *in vivo* counting. The product was then subtracted from the gross *in vivo* count in order to determine the activity actually in the organ tissue.

EXPERIMENTAL PROCEDURE AND RESULTS

Data from which the kinetic calculations have been made for the 18 swine are shown in Table I. The weights of the animals ranged from 8.6 to 97.0 kg. with a mean of 28.8 kg. During the period of study the rate of growth

TABLE I

Data from Which Ferrokinetic Calculations Were Made

Pig No.	Body weight	Growth rate	Plasma volume	Red cell volume	Volume of packed red cells	Hemoglobin	Plasma iron
	kg.	*kg./day*	*ml./kg.*	*ml./kg.*	*ml./100 ml.*	*gm./100 ml.*	*ug./100 ml.*
13–18	27.0	0.38	44.6	30.2	37.6	13.9	295
13–26	11.0	0.21	60.3	35.4	37.8	13.5	186
13–32	30.0	0.44	40.3	32.8	37.6	12.8	207
13–33	24.8	0.10	37.3	29.3	36.0	14.4	195
13–42	32.3	0.41	33.9	26.7	40.1	13.6	241
13–74	39.6	0.50	50.6	26.1	36.7	11.3	137
13–75	20.0	0.20	40.6	31.6	35.2	14.2	192
13–76	21.6	0.28	54.9	36.9	34.4	13.7	121
13–79	15.0	0.30	57.6	32.4	36.8	12.4	153
13–80	8.6	0.16	56.0	24.9	37.3	11.4	168
13–89	92.2	0.55	37.2	23.8	47.0	13.0	166
13–91	97.0	0.30	38.9	20.0	41.2	13.0	95
13–95	10.6	0.16	44.2	30.7	34.2	12.8	107
13–96	11.0	0.25	54.7	44.9	38.3	12.5	147
14–01	18.2	0.30	54.2	28.6	36.6	11.6	198
14–04	18.4	0.22	56.8	28.0	34.7	11.0	101
14–35	20.8	0.64	55.2	30.8	36.1	10.7	123
14–37	20.6	0.50	49.9	34.0	39.4	13.6	150
Mean....	28.8	0.33	47.7	30.4	37.6	12.7	166
Range...	8.6–97.0	0.10–0.64	33.9–60.3	20.0–44.9	34.2–47.0	10.7–14.4	95–295
S. D.....	25.2	0.15	8.5	5.4	3.0	1.0	52

was marked, averaging 0.33 kg./day. The values for volume of packed red cells and hemoglobin concentration represent the means of a number of determinations on each animal. The plasma iron concentrations ranged from 95 to 295 μg./100 ml. These values are similar to those which have been obtained previously (19) in this laboratory.

Disappearance of Radioiron from the Plasma.—A single rate of clearance of Fe^{59}, as illustrated in Fig. 1 A, was noted in three animals. Two rates, as

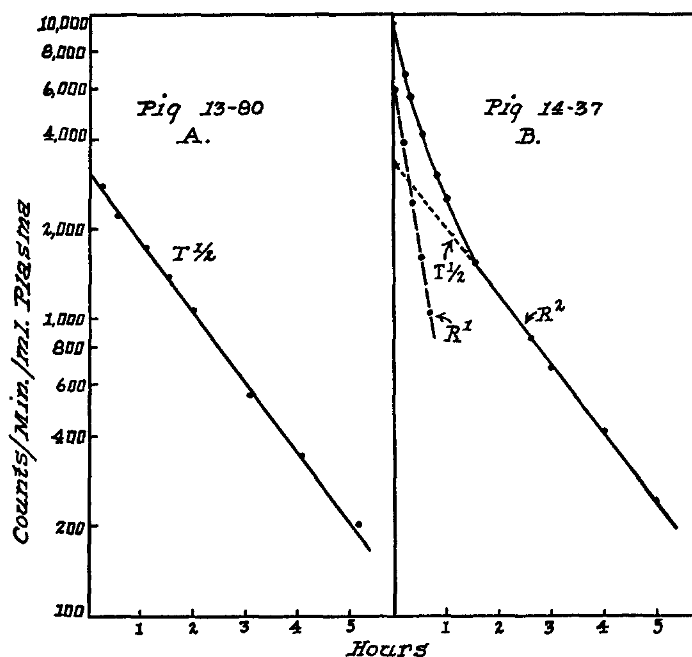

FIG. 1. Disappearance of Fe^{59} from the plasma of two pigs. Pig 13-80 exhibited a single rate of clearance of Fe^{59} (1 A). Pig 14-37 exhibited two rates of clearance of Fe^{59} (1 B). The first rate (R^1) was determined by substracting the extrapolated values of R^2 from the observed values prior to R^2. $T\frac{1}{2}$ represents the time at which the concentration of Fe^{59} in the plasma had decreased to half of its initial value (R^2 was used for this determination in 1 B).

illustrated in Fig. 1 B, were observed in the remaining 15 animals. In the swine exhibiting one rate, the T ½ was determined by noting the time at which the concentration of isotope had decreased to half of its initial level. When there were two rates of Fe^{59} disappearance, the line of the second rate was extrapolated to time 0, and the intercept of this line with the ordinate was considered to be the concentration of isotope at time zero for the determination of the $T\frac{1}{2}$.

Two possible explanations for the presence of more than one rate of Fe^{59} disappearance seemed likely enough to warrant investigation. The first possi-

bility, which was suggested by Bothwell *et al.* (20) as a cause of more than one rate of Fe^{59} disappearance in patients with hemochromatosis, is that the iron-binding capacity of the plasma with which the tracer was incubated may have been exceeded. If such were the case it is possible that the Fe^{59} which was not bound to transferrin might be cleared *in vivo* at an excessively rapid rate.

To test this possibility, the disappearance of Fe^{59} from the plasma of a single pig was determined in the usual manner and again on the following day with an aliquot of the plasma-Fe^{59} complex that had been dialyzed against distilled water for 24 hours (10 per cent of the radioactivity appeared in the dialysate). The two plasma Fe^{59} disappearance curves were similar (Fig. 2). In addition, a curve of disappearance of Fe^{59} was determined on a single ani-

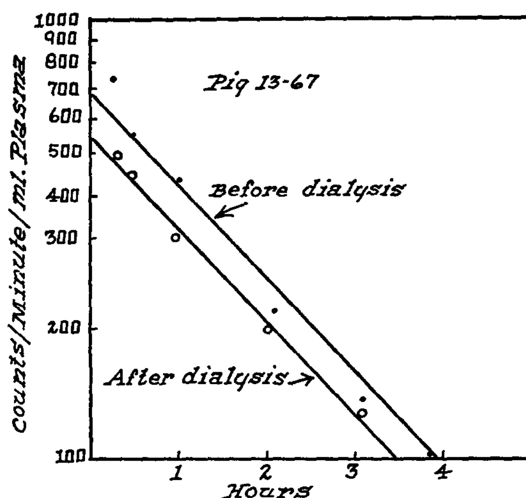

FIG. 2. Disappearance of plasma-bound Fe^{59} before and after dialysis

mal on two successive days with Fe^{59} bound to plasma on 1 day and with citrate buffered Fe^{59} chloride without prior incubation with plasma on the 2nd day. The two curves obtained were identical, indicating that even if none of the iron were bound to transferrin *in vitro*, this occurred promptly *in vivo*, and thus could not explain the occurrence of two different rates.

The second possibility that seemed likely to us was that the plasma iron pool might be in equilibrium with an extravascular iron pool. If such were the case, immediately after the injection of the Fe^{59} the rate of clearance of the isotope would represent the sum of the rates of uptake by the bone marrow and the other iron pool; however, when equilibrium would be attained with the other pool, the rate of clearance would be due only to uptake by the bone marrow. If there were such an iron pool, it seemed likely that this would be in the liver.

To test this possibility, the hepatic veins of 4 pigs were catheterized. This was accomplished by passing a cardiac catheter through the right atrium and down the inferior vena cava into

the hepatic vein. Simultaneous hepatic and jugular vein samples were analyzed for radioactivity after injection of plasma-bound Fe^{59}. The disappearance rates of Fe^{59} from the plasma obtained from the jugular vein and the hepatic vein were identical in all 4 swine. One of these is shown in Fig. 3. An additional pig was subjected to serial liver biopsy following injection of the isotope. A 6 cm. incision in the abdominal wall was made below the right costal margin, and 2 gm. samples of liver tissue were obtained surgically at appropriate intervals after the injection of plasma-bound Fe^{59}. Plasma samples were obtained in the usual manner. A portion of each liver sample was placed in a weighed cuvette which was then reweighed and counted. The remainder of the sample was analyzed for hemoglobin content. At the termination of the

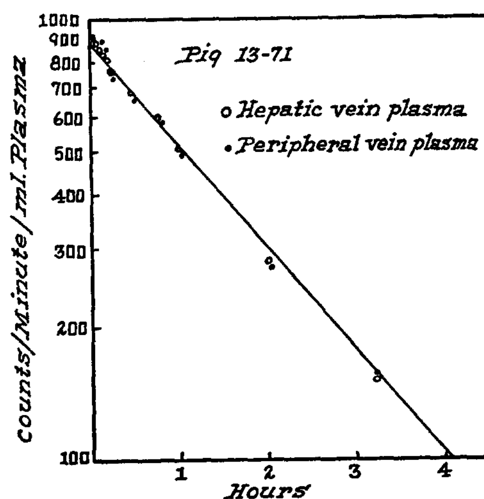

FIG. 3. Disappearance of Fe^{59} from plasma obtained simultaneously from hepatic and peripheral vein.

experiment the animal was sacrificed and the remainder of the liver was weighed. The amount of Fe^{59} present in the liver tissue was then determined on each specimen in the following manner:—

$$\text{CPM/gram of liver tissue} \times \text{liver weight (grams)} = \text{CPM/liver}$$

$$\frac{\text{Hb/gram of liver tissue} \times \text{liver weight (grams)}}{\text{Hb}_B} \times 100 = \text{milliliters blood in liver}$$

in which Hb_B is the blood hemoglobin in gm./100 ml.

$$\frac{\text{milliliters blood in liver}}{\text{TBV}} \times \frac{\text{per cent of injected Fe}^{59}\text{ in plasma}}{100} \times \text{CPM injected} = \text{CPM due to blood in liver}$$

in which TBV is the total blood volume in milliliters.

$$\text{CPM/liver} - \text{CPM due to blood in liver} = \text{CPM in liver tissue}$$

The disappearance of Fe^{59} from the plasma and its appearance in the liver are shown in Fig. 4. During the first 45 minutes the rate of clearance of the

isotope from the plasma and its rate of uptake by the liver were more rapid than in the remainder of the experiment. When the increases in the proportion of isotope in the liver during the intervals between plasma samplings

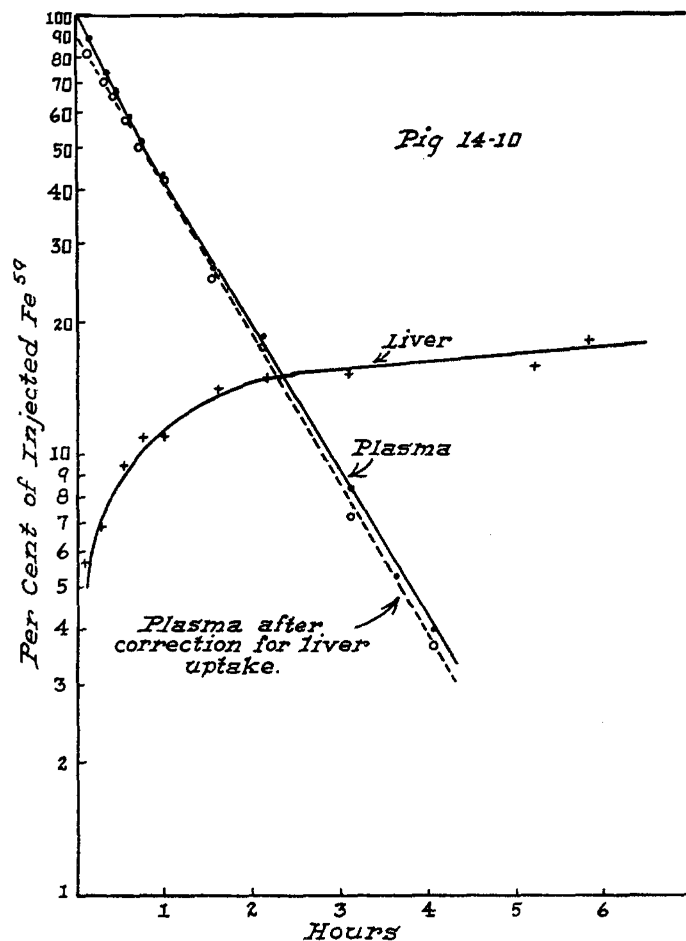

FIG. 4. Disappearance of Fe^{59} from plasma compared to uptake of Fe^{59} by liver. The activity in the plasma was corrected for uptake by the liver in the following manner: The proportion of the administered isotope which was incorporated into the liver prior to the time of the first plasma sample was subtracted from the proportion observed in that sample. Subsequent plasma samples were corrected by the subtraction of the increase in liver activity occurring since the previous plasma sample.

are subtracted from the observed percentage of injected activity in the plasma at each time, a straight line is obtained (Fig. 4). This study suggests that the initial rapid rate of disappearance of Fe^{59} from the plasma, was due to

rapid uptake of the isotope by the liver. Since correction of the data for the variation in the rate of uptake by the liver results in a straight line with a slope almost identical to that of the second rate, the latter was used for the determination of the $T\ ½$.

TABLE II

Ferrokinetic Data on Swine

Pig No.	T½* Hours	PITR‡	Per cent of injected Fe^{59} incorporated into RBC	RBC IIR§	Fe incorporated into RBC due to growth	RBC ITR‖	RBC life span
		mg./kg. day		*mg./kg. day*	*mg./kg. day*	*mg./kg. day*	*days*
13–18	1.33	1.64	93	1.52	0.49	1.03	34
13–26	1.12	1.66	100	1.66	0.85	0.81	56
13–32	1.13	1.10	94	1.03	0.45	0.58	56
13–33	1.17	1.03	72	0.74	0.13	0.61	67
13–42	1.25	1.08	72	0.78	0.36	0.42	67
13–74	1.05	1.10	83	0.91	0.18	0.73	40
13–75	1.02	1.27	77	0.98	0.35	0.63	53
13–76	0.78	1.42	95	1.35	0.55	0.80	56
13–79	1.12	1.31	98	1.28	0.76	0.52	71
13–80	1.33	1.17	100	1.17	0.43	0.74	45
13–89	1.67	0.61	95	0.58	0.16	0.42	62
13–91	1.53	0.40	100	0.40	0.03	0.37	71
13–95	0.88	0.90	95	0.85	0.50	0.35	91
13–96	0.72	1.52	94	1.43	0.70	0.73	53
14–01	1.57	1.13	88	0.99	0.54	0.45	77
14–04	1.06	0.90	98	0.88	0.39	0.49	67
14–35	1.47	0.76	95	0.72	0.40	0.32	100
14–37	1.28	0.97	100	0.97	0.38	0.59	67
Mean......	1.19	1.11	92	1.01	0.42	0.59	63
Range......	0.72–1.67	0.40–1.66	72–100	0.40–1.66	0.03–0.85	0.32–1.03	34–100
S.D........	0.26	0.34	9	0.34	0.40	0.19	16

* The time at which half of the isotope initially present had disappeared from the plasma.

‡ Plasma iron turnover rate.

§ Red cell iron incorporation rate.

‖ Red cell iron turnover rate.

The half times of plasma Fe^{59} disappearance ($T\ ½$) are shown in Table II. The mean value for the group of 18 normal pigs was 1.19 ± 0.26 hours.

Plasma Iron Turnover Rate (*PITR*).—The PITR was determined by the formula cited earlier. The plasma iron value used in this determination was the mean of three or more individual determinations performed on plasma samples obtained prior to the $T\ ½$. The plasma volume was that obtained on day 0.

The values for the PITR's of the 18 normal swine are tabulated in Table II. The mean value was 1.11 ± 0.34 mg. per kg. day.

Uptake of Radioiron into Red Cells.—The per cent of injected Fe^{59} incorporated into red cells was determined by the following formula:—

$$\frac{\text{CPM/milliliter blood} \times \text{TBV}}{\text{CPM injected}} \times 100$$

in which TBV is the total blood volume in milliliters, and CPM injected is the counts per minute per milliliter of standard × dilution of standard × milliliters injected.

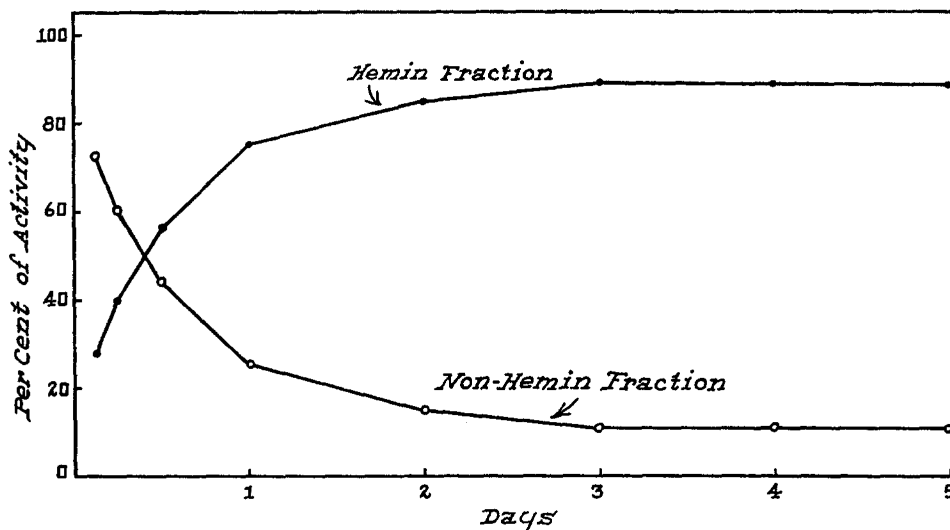

FIG. 5. Distribution of red cell Fe^{59} between hemin and non-hemin fractions as related to time.

Since the pigs were growing rapidly, the blood volume on day 8 was chosen arbitrarily for use in the calculation of the per cent of injected Fe^{59} incorporated into red cells. Blood samples were counted each day until a constant percentage of injected isotope was present for 3 successive days.

In order to determine whether the activity demonstrated in hemolyzed red cells actually represented iron incorporated into hemoglobin, heme was isolated and crystallized from the whole blood samples of 3 of the pigs. The percentage of injected Fe^{59} in heme as compared with that in the non-hemin fraction of red cells is presented in Fig. 5. Non-heme Fe^{59} accounted for approximately 10 per cent of the activity present after the first 48 hours of the study.

The curve of uptake of Fe^{59} by the red cells is shown in Fig. 6. The increase in isotope concentration in the red cells proceeded in an almost linear fashion

for the first 3 days, and then the rate of increase fell off rapidly so that plateau concentrations were attained by the 4th day. Although hemin was crystallized and counted from the blood samples of only 3 of the 18 pigs, these data indicate that a smaller percentage of the Fe^{59} in the red cells was actually a part of the heme moiety during the first 24 hours than thereafter (Fig. 5). Thus, if the isotope concentration of heme is plotted against time, a curve of uptake of Fe^{59} with a suggestion of sigmoid shape results (Fig. 6).

Red Cell Iron Incorporation Rate (*RBC IIR*) *and Red Cell Iron Turnover Rate* (*RBC ITR*).—The RBC IIR expresses the rate of incorporation of iron

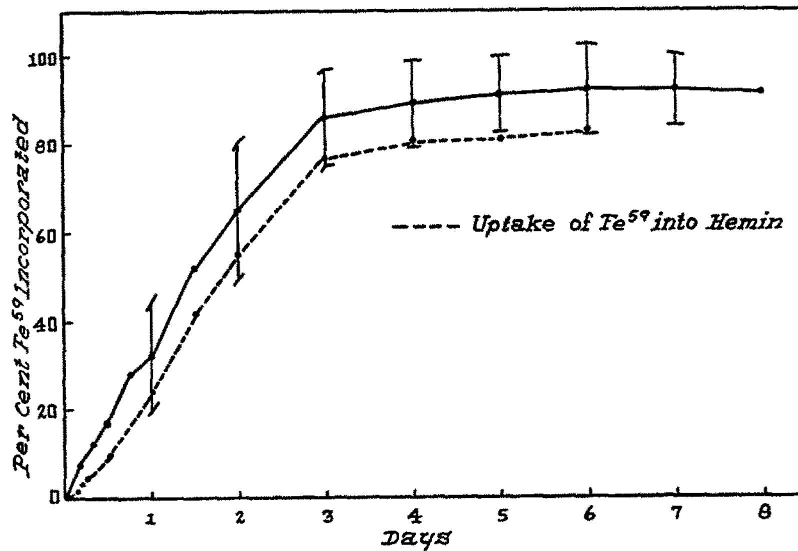

FIG. 6. Uptake of Fe^{59} into red cells (mean $\pm$ 1σ) compared to uptake into hemin (mean curve).

into the red cells each day. In a steady state, the same amount of iron is incorporated into new red cells as is released by red cells that are destroyed. Young swine, however, are not in a steady state but must produce more hemoglobin than they destroy in order to keep pace with their rapidly expanding red cell mass. As a consequence it was necessary to subtract from the RBC IIR the amount of extra iron incorporated into hemoglobin because of the expanding red cell mass. By this subtraction the variable of growth was eliminated so that the difference represented the amount of iron released by cells being destroyed, or the amount of iron that would have been incorporated into new red cells had the pig been in a steady state (RBC ITR). The amount of iron incorporated daily into new red cells in order to keep pace with growth was determined by the following formula:

$$\frac{(\text{TBV day 8} - \text{TBV day 0}) \times \text{Hb} \times 3.4}{8}$$

in which TBV day 8 is the blood volume in milliliters on day 8, and TBV day 0 is the blood volume in ml. on day 0, Hb is the hemoglobin concentration in grams per milliliter of blood, 3.4 is the number of milligrams of iron per gram of hemoglobin, and 8 is the number of days in which the animal grew during the ferrokinetic study

The amount of iron incorporated into new erythrocytes to keep pace with growth in the 18 normal animals is tabulated in Table II. The mean value was 0.42 mg. per kg. day.

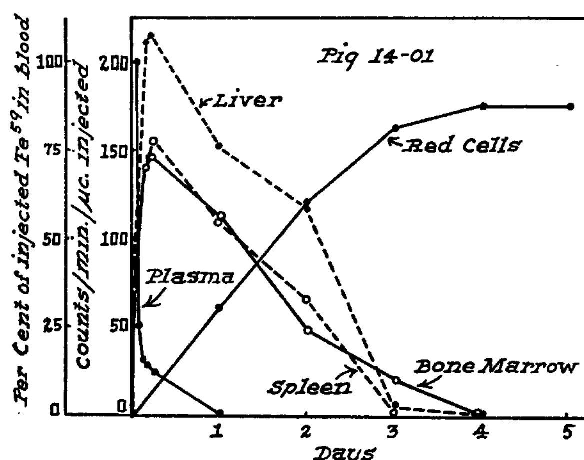

Fig. 7. Body-surface counts obtained at liver, spleen, and sacral bone marrow sites, compared with counts in plasma and red cells.

The RBC IIR, which describes the amount of iron incorporated into all new red cells each day, is shown in Table II. The mean value was 1.01 mg. per kg. day. The RBC ITR represents the amount of iron liberated from red cells that are destroyed each day or the amount of iron that would have been incorporated into new cells had the pigs been in a steady state. The mean value for the RBC ITR was 0.59 mg. per kg. day.

Red Cell Life Span.—The red cell life span was determined by the formulas described previously. The total body hemoglobin was determined on the basis of the blood volume on day 8.

The red cell life spans of the 18 normal swine are shown in Table II. The mean value was 63 ± 16 days.

In Vivo Counting.—The pattern of movement of Fe^{59} obtained by *in vivo* counting over the liver, spleen, and sacral bone marrow of a representative normal pig, compared to the disappearance of isotope from the plasma and its subsequent reappearance in the erythrocytes, is shown in Fig. 7. The

highest peak of activity was at the liver site, with the spleen and bone marrow having lesser peaks. The pattern of movement of the isotope was similar over the three sites counted. Fe^{59} entered the three organs at a rate which approximated that of clearance from the plasma. It was delivered from the respective organs at about the same rate as it appeared in the erythrocytes. All of the isotope had disappeared from the liver, spleen, and bone marrow by the time that 88 per cent of the injected dose appeared in the erythrocytes.

DISCUSSION

In the calculations of the ferrokinetic data, several assumptions must be made. The first assumption is that the animal is in a steady state with respect to hemoglobin synthesis and destruction. Due to the rapid growth of the young swine, this assumption was not valid and hence it was necessary to correct for the amount of iron required to expand the red cell mass. A second assumption is that the iron which is incorporated into the red cells enters the bone marrow directly and is not first recircuited through another organ. That some recircuiting probably occurs is suggested by the observation that 20 per cent of the injected radioiron entered the liver in the pig which was subjected to serial liver biopsies, whereas an average of 92 per cent of the isotope was eventually incorporated into the red cells of the normal swine. If a similar proportion of the radioiron entered the livers of the latter animals, it is apparent that some of the isotope which was eventually incorporated into the red cells had been recircuited through the liver. This may explain the presence of more than one rate for the disappearance of iron from the plasma. A third assumption is that there is no turnover of hemoglobin iron prior to the death of the cell. Data obtained from *in vitro* studies suggest that this assumption is valid (21). A fourth assumption is that all of the radioiron incorporated into the erythrocytes is incorporated into heme. Our observations indicate that about 90 per cent of the red cell iron is present in heme. Another assumption is that the curve of Fe^{59} uptake into the erythrocytes is indicative of the total amount of iron which enters the red cells. It has been shown by the use of glycine-2-C^{14} (22) that in growing swine random destruction accounts for the destruction of more erythrocytes than an age-dependent process. On this ground it may be assumed that random destruction was operating on the cells containing Fe^{59} and the curve of uptake must have been depressed and delayed slightly by this process.

Because these assumptions are not altogether valid and because of the errors inherent in each of the many determinations that enter into the calculation of ferrokinetic data, it is apparent that the turnover rates and erythrocyte life span determined by this method in a growing pig cannot be highly precise. The red cell life span as determined by the ferrokinetic method is primarily of interest because it serves as a check on the validity of the as-

sumptions and measurements that enter into the determination of the turnover rates. The variation from 34 to 100 days (Table II) is probably excessive and may be due to the multiplication of errors detailed previously. The mean value of 63 days, however, agrees well with the life span of 62 days determined in a previous study in which the fate of C^{14}-labelled heme was followed (22). It seems likely, therefore, that at least in normal swine the ferrokinetic method is satisfactory as a means of determining the turnover rate of iron through the plasma and red cells.

The shape of the curve of uptake of Fe^{59} into the erythrocytes and heme of the 18 swine (Fig. 6) is puzzling. The incorporation of isotope into the red cells proceeded in a linear fashion for the first 3 days. This would imply that Fe^{59} entering the bone marrow from the plasma was incorporated in equal quantities into all cells destined to be released during the next 3 days. Austoni (23) showed by the use of the radioautographic technique in rats that, following the intraperitoneal administration of $Fe^{59}Cl_3$, the greatest quantity of isotope is present in the polychromatophilic normoblasts, with slightly smaller amounts in the basophilic normoblasts and orthochromatic normoblasts, respectively. Both red corpuscles and pronormoblasts contain very little isotope. If the incorporation of Fe^{59} occurs in a similar manner in swine, the curve of uptake of Fe^{59} should have been sigmoid in shape. The area of steepest increment should have occurred at the time of release into the blood of red cells which had been polychromatophilic normoblasts at the time when the Fe^{59} was injected. Some of the uptake into the red cells in the early hours of the experiment was probably due to incorporation of radioiron into reticulocytes. This is suggested by the fact that a greater proportion of non-heme Fe^{59} was present in the red cells during the first 24 hours than on subsequent days (Fig. 5). When the non-heme radioiron is subtracted from the total red cell radioactivity, however, the curve is still not truly sigmoid in shape (Fig. 6). The reason for this early rapid incorporation is not apparent.

The daily increment of uptake of Fe^{59} into the erythrocytes began to decrease by the 3rd day following the injection of Fe^{59}, and further incorporation of isotope ceased after the 4th day. If Austoni's observation (23) that the pronormoblast is capable of incorporating small amounts of Fe^{59} is applicable also to swine, the curve of uptake of Fe^{59} suggests that an interval of about 4 days is necessary for the pronormoblast to mature into an erythrocyte.

These data provide a basis for speculation about the size of the labile bone marrow iron pool in growing swine. Since the red cell iron incorporation rate each day is known, and a period of 4 days was required for maximal incorporation of Fe^{59} into the erythrocytes, it is reasonable to speculate that the labile bone marrow pool was four times the RBC ITR, or approximately 68 mg. in an average pig weighing 28.8 kg.

The curves of Fe^{59} activity obtained as a result of body surface counting are surprising in that the highest peak of activity was observed over the liver site. The data obtained from the analysis of serial liver biopsies indicate that a maximum of 15 to 20 per cent of the injected Fe^{59} actually entered the liver tissue (Fig. 4). Since about 92 per cent of the injected isotope ultimately appeared in the erythrocytes, one would expect the bone marrow peak to be at least 4 to 5 times greater than that of the liver. A possible explanation for the greater height of the liver curve is that it was necessary to count this organ through rib bone marrow, and since this tissue was closer to the counter, a large portion of the recorded activity at this site may have been due to Fe^{59} in rib marrow. The same possibility would apply to counts over the spleen site.

SUMMARY

Plasma and red cell iron turnover rates were determined in 18 normal growing swine by the use of tracer doses of Fe^{59}. Body surface counting was performed on a representative group of animals following the injection of the isotope.

The mean half-time of plasma iron disappearance was 1.19 $\pm$ 0.26 hours. Two exponential rates of disappearance of Fe^{59} from the plasma were observed in 15 of the pigs and a single rate was observed in the other 3. The mean plasma iron turnover rate was 1.11 $\pm$ 0.34 mg./kg. day. The average maximum incorporation of Fe^{59} into the erythrocytes was 92 $\pm$ 9 per cent. The mean red cell iron incorporation rate was 1.10 mg./kg. day, but an average of 0.42 mg./kg. day of this was calculated as being due to increasing red cell mass incidental to body growth, so that the true mean red cell iron turnover rate was 0.59 $\pm$ 0.19 mg./kg. day. The average "apparent" red cell life span was 63 $\pm$ 16 days. This is in agreement with the red cell life span of 62 days determined previously with glycine-2-C^{14}.

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