STUDIES ON COPPER METABOLISM. XIX

The Kinetics of Iron Metabolism and Erythrocyte Life-Span in Copper-Deficient Swine*

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In previous publications (1–3) from this laboratory the morphological and biochemical characteristics of the anemia associated with a deficiency of copper in swine were described and the similarities between the anemia of copper deficiency and that of iron deficiency were pointed out. Evidence has also been presented that the absorption of iron from the gastrointestinal tract is impaired in copper-deficient pigs and that the anemia can be neither alleviated nor prevented by the parenteral administration of iron. These studies have been interpreted as suggesting that anemia in experimentally produced copper deficiency develops as a consequence of an inability to absorb, mobilize, and utilize iron.

The purpose of the present publication is to describe the results of studies on the plasma iron turnover rate, the red cell iron turnover rate, and the red cell life span as determined with radioiron (Fe^{59}) in three copper-deficient swine. Similar studies in normal swine (4) and in swine with various types of experimentally induced anemia (5) other than copper deficiency have been reported already. Data will also be presented on the *in vivo* life span of chromium-tagged erythrocytes in normal, copper-deficient, and iron-deficient pigs.

Methods

Details concerning the diet, care, and handling of the swine have been described elsewhere (2). The Fe^{59} was supplied[1] as ferric chloride. The methods for the determination of

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[1] Obtained from the Abbott Laboratories on allocation from the United States Atomic Energy Commission.

the plasma iron turnover rate (PITR),[2] the red cell iron incorporation rate (RBC IIR)[3], the red cell iron turnover rate (RBC ITR),[4] the red cell life span as measured with radio-iron, and the technic used in body surface counting have been described in another publication (4).

Radioactive copper (Cu^{64}) was supplied as a solution of cupric acetate.[1] The specimens were counted in a well-type, thallium-activated sodium iodide crystal scintillation counter with an efficiency of 5 per cent for Cu^{64}. Since the half-life of Cu^{64} is 12.8 hours, a record was kept of the exact time at which each count was performed in order to correct the observed activity for radioactive decay.

The procedure described by Read (6) was used in the determination of erythrocyte life span with radioactive chromium. Fifty μc. of radioactive sodium chromate ($Na_2 Cr^{51} O_4$), containing less than 0.5 mg. of carrier chromium, were incubated at room temperature with 50 ml. of blood from the donor pig. After 30 minutes of incubation, 100 mg. of ascorbic acid was added to the blood. The recipient animal was anesthetized with 5 per cent pentobarbital and the Cr^{51}-tagged blood was injected into an ear vein.

Heparinized blood samples were obtained 24 hours after the injection and thereafter three times a week. The volume of packed red cells was determined on each blood sample. Two ml. of blood was pipetted into a cuvette and centrifuged, after which the plasma was removed. The packed red cells were hemolyzed with saponin.

The activity present in each sample was recorded as counts per minute per milliliter of red cells by the appropriate calculation from the volume of packed red cells. The sample obtained 24 hours after the injections was considered to contain 100 per cent of the activity and this day was taken to be day 0. The activity in later samples was plotted as per cent of the activity present at time 0.

Since the animals were growing during this experiment, the concentration of isotope was diluted by the expanding blood volume. In a previous study it was shown that the red cell volume in growing swine increases in a manner that roughly parallels body weight (7). In order to correct for growth, the determined proportion of the Cr^{51} remaining in the circulation was corrected by the use of the following formula (8):

$$\frac{RBC_t^{Act} \times Wt_t}{Wt_0} = RBC_c^{Act}$$

in which

RBC_t^{Act} is the proportion of Cr^{51} remaining at time t.
Wt_t is the body weight at time t.
Wt_0 is the body weight on day 0.
RBC_c^{Act} is the proportion of Cr^{51} remaining after correction for increase in body weight.

RESULTS

Ferrokinetic Studies.—The data are summarized in Tables I and II. Details concerning the 18 normal swine have been published elsewhere (4).

The half-time of disappearance of Fe^{59} from the plasma (T 1/2) averaged 0.28 hours in the copper-deficient as compared to 1.19 hours in the normal animals. The mean plasma iron turnover rate (PITR) was 1.76 mg./kg. day in the deficient animals as compared to 1.11 mg./kg. day in the control swine. The curves of uptake of Fe^{59} into the red cells of the copper-deficient swine

[2] PITR, plasma iron turnover rate.
[3] RBC IIR, red cell iron incorporation rate.
[4] RBC ITR, red cell iron turnover rate.

as compared with the normal animals are shown in Fig. 1. An average of 71 per cent of the injected Fe^{59} was eventually incorporated into the red cells of the deficient animals as compared to an average of 92 per cent in normal swine.

TABLE I

Data from Which the Ferrokinetic Calculations Were Made

Determination	Normal		Copper-deficient			
	Mean	Range	13–82	13–84	13–85	Mean
Body weight, *kg.*	28.8	8.6–97.0	21.0	22.4	21.3	21.6
Growth rate, *kg./day*	0.33	0.10–0.64	0.11	0.15	0.00	0.08
Plasma volume, *ml./kg.*	47.7	33.9–60.3	74.7	60.2	83.7	72.8
RBC volume, *ml./kg.*	30.4	20.0–44.9	14.2	16.5	14.8	15.2
Volume of packed red cells, *ml./100 ml.*	37.6	34.2–47.0	19.0	24.2	16.2	19.8
Hemoglobin, *gm./100 ml.*	12.7	10.7–14.4	4.6	6.2	4.8	5.2
Plasma iron, *μg./100 ml.*	166	95–295	42	34	43	40

TABLE II

Ferrokinetic Data in Normal and in Copper-Deficient Swine

	Normal		Copper-Deficient			
	Mean	Range	13–82	13–84	13–95	Mean
T 1/2*, *hrs.*	1.19	0.72–1.67	0.28	0.20	0.35	0.28
PITR‡ *mg./kg. day*	1.11	0.40–1.66	1.86	1.70	1.71	1.76
Per cent of injected Fe^{59} incorporated into RBC	92	72–100	69	79	65	71
RBC IIR§, *mg./kg. day*	1.01	0.40–1.66	1.28	1.34	1.11	1.24
Iron incorporated due to growth, *mg./kg. day*	0.42	0.03–0.85	0.08	0.11	0.00	0.06
RBC ITR‖, *mg./kg. day*	0.59	0.32–1.03	1.20	1.23	1.11	1.18
RBC life span, *days*	63	34–100	12	14	14	13

* The time at which the concentration of Fe^{59} in the plasma had decreased to half of its initial value.

‡ Plasma iron turnover rate.

§ Red cell iron incorporation rate.

‖ Red cell iron turnover rate.

The red cell iron incorporation rate (RBC IIR) represents the amount of iron entering newly produced red cells. Since the swine were growing, this value is the sum of the amount of iron necessary to keep pace with the expansion of the red cell mass necessitated by growth and the amount necessary for the production of erythrocytes needed to replace those being destroyed. The RBC IIR averaged 1.24 mg./kg. day in the copper-deficient animals as compared to 1.01 mg./kg. day in the control swine. The red cell iron turnover rate

(RBC ITR), which represents the amount of iron necessary to replace that which has been liberated by the destruction of red cells, averaged 1.18 mg./kg. day in the deficient pigs as compared to 0.59 mg./kg. day in the normal animals. Because growth was greater in the control animals than in the deficient swine,

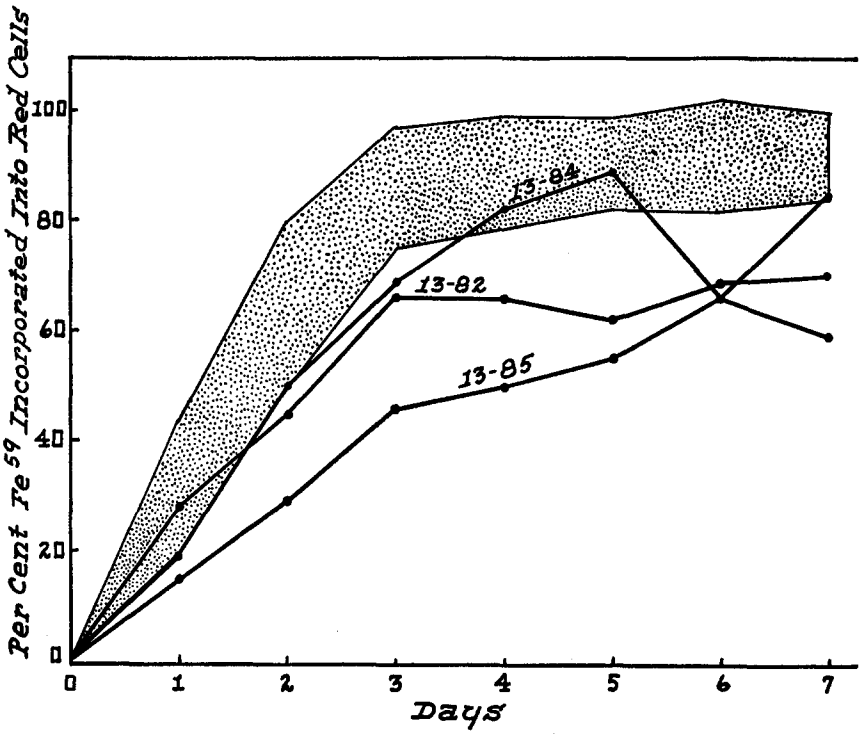

FIG. 1. The uptake of radioactivity into the red cells of three copper-deficient pigs (13-82, 13-84, and 13-85) compared with the uptake of radioactivity in the red cells of normal pigs (shaded area).

The values on the ordinate refer to the per cent of the administered radioactivity found in the erythrocytes.

the value for the RBC ITR in the copper-deficient animals differed more strikingly from the normal than did the RBC IIR.

The average red cell life span calculated on the basis of the ferrokinetic studies was 13 days in the deficient animals as compared to 63 days in the control swine.

Body Surface Activity after the Administration of Radioioron.—Body surface counts were carried out over the liver, spleen, and sacral bone marrow of six control animals (4) and the three copper-deficient pigs. The pattern of uptake of Fe^{59} into the three organs of a representative copper-deficient animal is compared in Fig. 2 with that of a control pig.

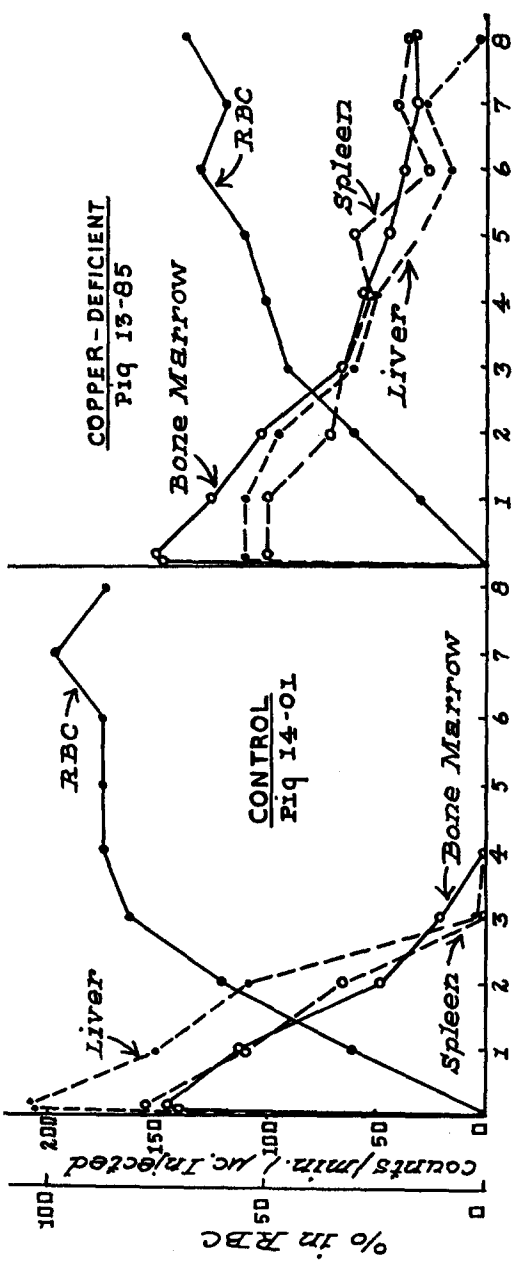

FIG. 2. Uptake of Fe^{59} into red corpuscles and into the liver, spleen, and sacral marrow of a control pig (14-01) and a copper-deficient pig (13-85).

The rate of uptake of iron into the three sites was equally rapid in both groups but the maximum activities over the liver and the spleen differed. The maximum activity detected over the liver of the control pigs ranged from 165 to 410 counts/min. per μc. injected whereas that detected over the liver of the deficient swine ranged from 110 to 130. The maximum activity detected over the spleen of the control and deficient animals ranged from 155 to 360 and 100 to 110, respectively. There was no difference, however, between the two groups in the maximal activity detected over the sacral marrow. The disappearance of the isotope from the organs was slower in the deficient animals than in the control animals. 5 days after the injection no detectable activity was present over

TABLE III

Erythrocyte Half-Life as Determined by Use of Sodium Chromate (Cr^{51})

Group	Donor	Recipient	No. of pigs	Half-life			Significance of difference from group A
				Mean	Range	S.D.	
				days	*days*	*days*	
A	Normal	Normal	4	17.0	11.8–19.8	3.6	
B	Copper-deficient	Copper-deficient	10	9.0	6.8–12.7	1.9	$t = 3.69, P < .01$
C	Normal	Copper deficient	4	15.9	10.9–19.1	3.6	$t = 0.37, P > 0.7$
D	Copper-deficient	Normal	5	13.3	11.2–17.0	2.2	$t = 1.57, P > 0.1$
E	Iron-deficient	Iron-deficient	3	18.8	13.9–21.5	4.2	$t = .49, P > 0.6$

S.D. refers to standard deviation

Comparison of group B with group D reveals that the two groups are different at the 1 per cent level of significance ($t = 3.4$).

the liver, spleen, and sacral marrow of the control pigs but significant activity was still present over the three sites in the copper-deficient pigs.

Erythrocyte Life Span Determined with Radioactive Chromium.—The life span of the erythrocytes was measured by the use of radioactive chromium in a total of 26 pigs. Four normal animals were given labelled cells from normal pigs (group A); ten copper-deficient pigs were given labelled cells obtained from copper-deficient pigs (group B); four copper-deficient pigs were given labelled erythrocytes obtained from normal animals (group C); five normal swine were given labelled cells from copper-deficient swine (group D); and three iron-deficient swine received labelled erythrocytes from iron-deficient swine (group E). In no case did an animal receive its own cells. The results are summarized in Table III.

In Fig. 3, the disappearance curve of radiochromium from the blood of one representative pig of each of the first four groups is plotted on a semi-logarithmic scale. The data closely approximated a straight line in each case. This is to be expected in swine since it has been shown that most of the cells are destroyed

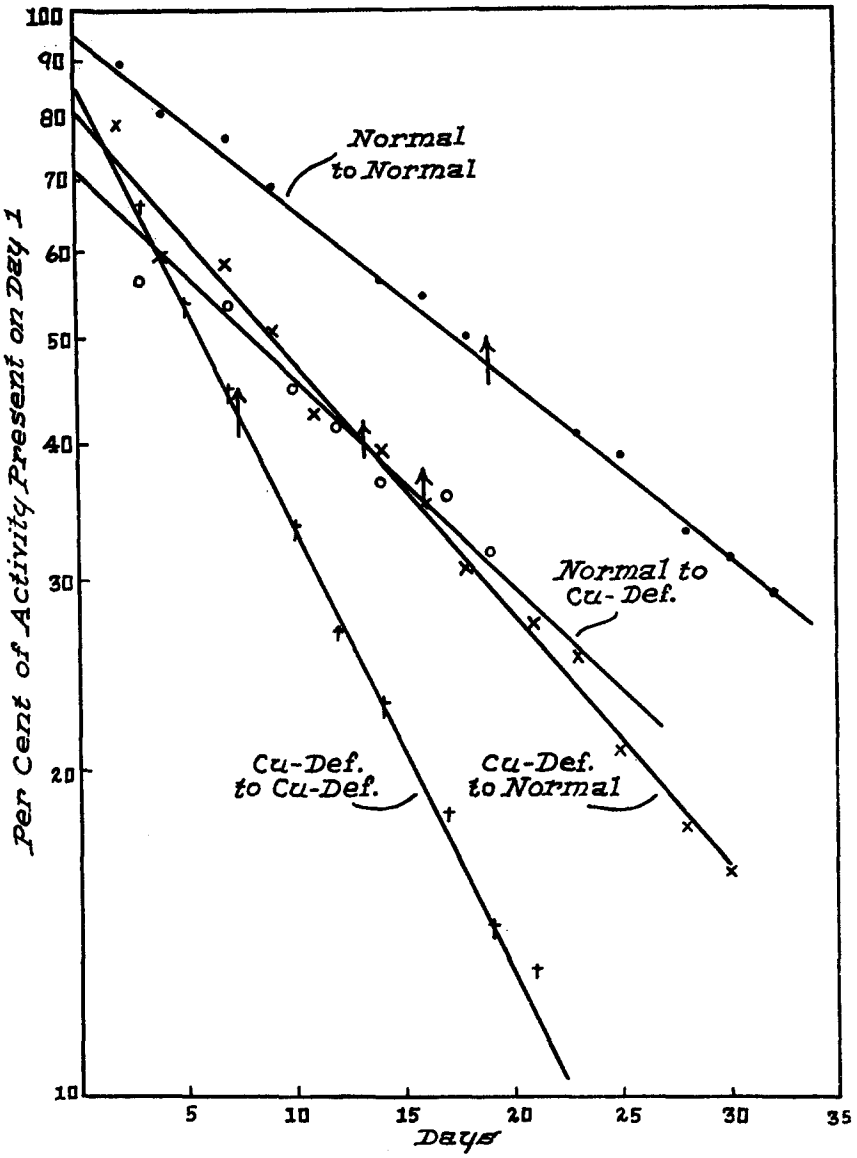

FIG. 3. Disappearance of radioactive chromium from the blood of normal and copper-deficient swine given labelled erythrocytes obtained from normal and copper-deficient pigs. The arrows refer to the time when half of the radioactivity had disappeared from the blood.

in a random manner (8). For practical purposes, the disappearance of radiochromium in swine represents the resultant of two first order functions, cell destruction and chromium elution. The objection presented by Mollison and

Veall (9) to the use of the semi-logarithmic scale in presenting chromium erythrocyte survival data obtained from human subjects does not apply to data obtained from swine.

The mean erythrocyte half-life of normal cells transfused into normal pigs was 17 days. The half-life of erythrocytes from swine deficient in copper, transfused into copper-deficient swine (group B) was significantly shorter. On the other hand, the half-life of red cells from control animals transfused into copper-deficient animals (group C), was normal. When erythrocytes from copper-deficient swine were transfused into normal animals (group D), the half-time survival approached the normal value. When cells obtained from pigs deficient in iron were transfused into iron-deficient pigs (group E), the half-time survival of the cells was normal.

TABLE IV

Uptake of Radiocopper by Erythrocytes Following the Intravenous Administration of 1 mc. of Cupric^{64} Acetate

	Pig A		Pig B		Pig C	
Time Hours	Plasma	RBC	Plasma	RBC	Plasma	RBC
hrs.	*c.p.m./ml.*	*c.p.m./ml.*	*c.p.m./ml.*	*c.p.m./ml.*	*c.p.m./ml.*	*c.p.m./ml.*
0.5	51,931	732	32,642	1,002	24,312	330
1	31,307	589	21,589	778	15,608	157
2	24,015	669	14,466	833	9,845	155
4	21,022	822	11,722	558	7,188	175
6	19,713	908	12,417	713	7,026	224
8			11,193	810	6,115	232
24	20,132	1,727	12,579	2,427	7,118	454
48	19,802	3,040			6,432	943

Uptake of Radiocopper By Erythrocytes.—It has been shown previously (2) that the copper content of red cells from copper-deficient swine is reduced as compared with that of normal red cells. Therefore, the possibility was considered that the improvement in the survival of erythrocytes from copper-deficient pigs when transfused into normal pigs as compared with their survival in the circulation of copper-deficient swine was due to the reconstitution of the copper content of the copper-deficient red cells when exposed to normal plasma.

In order to determine whether copper can be taken up from the plasma by red cells, 1 mc. of radiocopper in the form of cupric^{64} acetate containing 1 mg. of copper was injected intravenously into each of three normal pigs. The amount of radioactivity in the plasma and in the red cells (washed three times with saline) was determined at various time intervals after the injection. As shown in Table IV, appreciable activity was present in the erythrocytes within ½ hour following the administration of radiocopper. Following this the erythrocyte radioactivity tended to decline as the plasma activity declined (10).

However, after 24 to 48 hours, the activity in the cells increased again even though the total activity in the plasma remained constant. This secondary rise may have been due to the appearance in the circulation of cells labelled in the marrow (11).

To determine whether erythrocytes can take up copper from plasma under *in vitro* conditions, 2 μc. of radiocopper (cupric^{64} acetate), containing 2 μg. of copper was added to 50 ml. of blood obtained from each of three normal pigs.

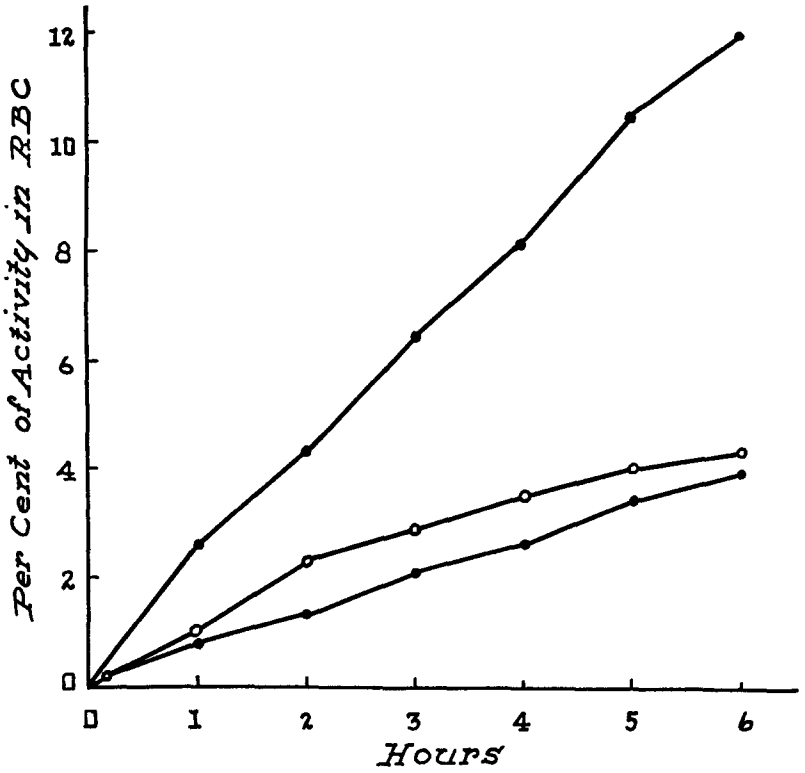

FIG. 4. The rate of uptake of radiocopper into erythrocytes incubated *in vitro* at 37°C. Each curve represents the radioactivity in the red corpuscles of a single pig.

Acid-citrate-dextrose solution was used as an anticoagulant. The blood was incubated at 37°C. and was shaken continuously. Aliquots were removed at various time intervals. The red cells were washed twice with saline and the radioactivity in C.P.M./ml. of packed red cells was determined. The results are expressed as the proportion of the activity added which was taken up by the erythrocytes. In a period of 6 hours, 3.9, 4.3, and 11.9 per cent of the activity was present in the red cells of the three pigs (Fig. 4).

Unfortunately, at the time the radiocopper was available, no animals de-

ficient in copper were on hand so that the rate of uptake of radiocopper into copper-deficient red cells could not be studied.

DISCUSSION

The present studies indicate that the survival time of the erythrocytes of copper-deficient swine is shorter than normal. This observation is not entirely unexpected since we had noted previously (2) that, once anemia begins to appear in copper-deficient animals, it develops so rapidly and becomes so severe that, if the survival time were not decreased, it would have to be postulated that red cell production had ceased completely.

The fact that erythrocytes from a normal pig, when transfused into a copper-deficient pig survived a normal period of time suggests that the shortened survival time is not due to an extracorpuscular abnormality. On the other hand, if the decreased life span were due to an intracorpuscular cause, one would expect that the copper-deficient cells would not survive for a normal period when transfused into a normal recipient. Such was not the case. When cells from a copper-deficient pig were transfused into a normal pig, the survival time approached normal. An explanation for this observation is related to our earlier observation (2) that the copper content of erythrocytes from copper-deficient pigs is reduced below normal. It is possible that copper enters the "copper-deficient" red cells from normal plasma and corrects the intracorpuscular defect. This suggestion is supported by the demonstration of uptake by red corpuscles of radiocopper from plasma.

The studies with radioiron indicate that the rate of hemoglobin production (RBC IIR) in the copper-deficient pigs was only 1.1 to 1.3 times greater than in the normal animals. This value may be a little low owing to the fact that it is difficult to estimate exactly the proportion of isotope incorporated into erythrocytes when the life span of the cells is greatly shortened. Some of the cells are destroyed as rapidly as they are labelled and are discharged into the circulation. This is probably the explanation for the depression of the curve of uptake of Fe^{59} into the red cells of the deficient animals. Rapid destruction of newly labelled cells would also explain the retention of isotope in the tissues of the copper-deficient animals longer than in control animals (Fig. 2) since the isotope was probably returning to the tissues even during the time when it was being incorporated into erythrocytes. However, even if it were assumed that all of the isotope was incorporated into erythrocytes the rate of hemoglobin production would be only 1.9 times normal.

Since we have shown previously (5) that, under the stimulus of phenylhydrazine administration, the daily hemoglobin production of normal pigs can be increased fourfold, it appears from the present data that the ability to produce hemoglobin is greatly impaired in copper-deficient swine. It seems justifiable, therefore, to conclude that anemia develops in the absence of

copper both because of a shortened erythrocyte survival time and limitation of the capacity of the marrow to produce cells.

One explanation for the manner in which copper deficiency causes these alterations is that copper is an essential component of adult red corpuscles and that a certain minimum of copper must be available for the production of red corpuscles as well as for the maintenance of their integrity in the circulation. When the supply of copper is adequate, cells are produced each of which contains approximately 61 μμμg. of copper (2) and such cells survive for a normal period of time. When the supply of copper is the limiting factor in erythropoiesis, fewer cells are produced and those cells which are formed contain a subnormal quantity of copper (about 26 μμμg.). By economizing both on the number of cells produced and the quantity of copper put into them, maximal use of the available supply of copper is made. However, the amount of copper incorporated into each cell is so small that the cells are unable to function normally and their survival time is shortened.

The difference between the plasma iron turnover rate and red cell iron incorporation rate represents the amount of iron being turned over through channels other than the circulating red cells. From these data it can be calculated that the control pigs turned over an average of 0.10 mg. of iron/kg. day and the deficient animals turned over an average of 0.52 mg./kg. day. Thus, since both the plasma iron turnover rate and the tissue iron turnover rate were greater in the copper-deficient than in the control swine, it would seem that our previous postulate (1) that the mobilization of iron is impaired in copper deficiency must be incorrect. However, our earlier conclusion (1) that copper influences the rate of absorption of iron finds no contradiction in the present studies and is supported in more recent studies (12).

SUMMARY

Ferrokinetic studies were performed in three copper-deficient swine and the results have been compared with similar studies in 18 normal pigs. The mean value for the plasma iron turnover rate in the deficient swine was 1.76 mg./kg. day; for the red cell iron incorporation rate, 1.24 mg./kg. day; for the red cell iron turnover rate, 1.18 mg./kg. day; for the red cell life span, 13 days. Corresponding figures in the normal swine were 1.11 mg./kg. day, 1.01 mg./kg. day, 0.59 mg./kg. day and 63 days, respectively.

The red cell life span was measured by the use of radioactive chromium in a total of 26 pigs. The mean erythrocyte half-life of normal cells transfused into normal pigs was 17 days. The mean half-life of erythrocytes from copper-deficient swine transfused into copper-deficient swine was 9 days. The mean half-life of red cells from control animals transfused into copper-deficient swine was 16 days while that of erythrocytes from copper-deficient swine transfused

into normal pigs, was 13 days. The mean half-life of cells from iron-deficient pigs transfused into iron-deficient pigs was 19 days.

It is concluded that copper deficiency anemia results from both a shortened erythrocyte survival time and limited capacity of the bone marrow to produce red cells. It is suggested that copper is an essential component of erythrocytes in swine.

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