Experimental Production of Siderocytes *

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Erythrocytes containing one or more granules that give a positive Prussian blue reaction for iron were apparently recognized by workers in the nineteenth century (1) but not until 1941 was the term "siderocyte" applied to them by Grüneberg (2, 3).

The significance of siderocytes has been a matter of conjecture since their original description. Pappenheimer, Thompson, Parker, and Smith (4) suggested that the granules represent intra-erythrocytic parasites. Case (5) proposed that they are a breakdown product of hemoglobin. In 1947 McFadzean and Davis (6) recognized that many normoblasts in the bone marrow normally contain iron-positive granules and suggested that the blood siderocyte is derived from the marrow sideroblast. In addition, they observed that the number of siderocytes in the blood was increased in many clinical conditions, particularly in hemolytic anemias, after splenectomy. They suggested that siderocytes are defective cells and that the spleen removes them.

Douglas and Dacie in 1953 (7) investigated the incidence of siderocytes and sideroblasts in normal subjects and in a variety of patients with hematologic disorders. They observed that siderocytes were usually absent from the blood in the presence of the spleen but were present in the blood after splenectomy for whatever reason. The greatest

number was found in cases of hemolytic anemia with high reticulocyte counts persisting after splenectomy. They concluded that siderocytes occur normally in the bone marrow during the formation of hemoglobin but that the granule is rapidly metabolized as the erythrocyte matures. They suggested that in the absence of the spleen, the metabolism of the granule within the cell is delayed and siderocytes then appear in the circulation. Crosby in 1957 (1) in a well-designed study demonstrated conclusively that the spleen is somehow able to bring about the dissolution or removal of the siderotic granule without removing or destroying the erythrocytes containing them. He referred to this as the "pitting" function of the spleen (8).

In the last few years considerable attention has been directed to the study of the pathogenesis of a variety of refractory anemias characterized by the presence in excessive numbers of sideroblasts and siderocytes. These have been summarized by Mollin (9). Our own interest in this subject has led us to investigate systematically the factors that influence the level of siderocytes in the circulation of experimental animals. In this paper studies will be presented that deal with the influence of iron stores, splenectomy, a defect in heme synthesis as exemplified by pyridoxine deficiency, rapid blood regeneration induced by phlebotomy, and various combinations of these factors on the circulating level of siderocytes in the blood of swine. These studies were designed to develop experimental animal models by means of which the factors involved in the production and metabolism of this erythrocytic nonheme iron pool might be clearly defined. From these initial studies it appears that two different and unrelated mechanisms exist for the removal of iron from this pool.

Methods

Approximately 165 swine of mixed breed were used in these experiments. The animals were housed in individual cages and started on the experimental diet at 3 to 5 weeks of age.

The basal diet was fed in amounts of 36.4 g (152 calories) per kg of body weight per day and consisted of purified casein (Sheffield devitaminized), 26.1%; sucrose, 57.7%; lard, 11.0%; and swine salt mix, 5.2%. The swine salt mixture was the same as no. 3 of our early experiments (10) except that 0.04 g of ferrous sulfate (7 H₂O) was substituted for iron pyrophosphate. The following vitamins, in crystalline form, were placed in capsules and administered three times a week in milligrams per kilogram body weight per day: thiamine hydrochloride, 0.25; riboflavin, 0.12; nicotinic acid, 1.20; pyridoxine hydrochloride, 0.20; calcium pantothenate, 0.50; inositol, 0.20; p-aminobenzoic acid, 0.10; biotin, 0.10; pteroylglutamic acid, 0.10; and cyanocobalamin, 0.01. In addition all of the animals received the following supplements: choline chloride, 10 mg per kg per day; vitamin A, 3,000 U per kg per week; vitamin D, 600 U per kg per week; vitamin E, 1 mg per kg per week; and vitamin K, 1 mg per kg per week.

Pyridoxine deficiency was produced by omitting pyridoxine from the vitamin mixture. A combination pyridoxine and iron deficiency was produced by omitting pyridoxine from the vitamin mixture and ferrous sulfate from the salt mixture. A more severe deficiency of iron alone was induced by feeding a condensed milk diet supplemented with copper (11). To produce an increase in iron stores, we administered iron dextrin intramuscularly. Phlebotomized animals were bled daily by jugular puncture and were not anesthetized. Iron dextrin was given to these animals in amounts that would more than replace the iron lost through phlebotomy. Phenylhydrazine, 50 mg per kg of body weight, was administered orally five times weekly. Splenectomy was performed under intravenous pentobarbital anesthesia after the animals had received the experimental diet for 2 or 3 weeks.

Cross-transfusion experiments were performed in the following manner. When the donor was a pyridoxine-deficient animal, the femoral artery was exposed and cannulated under anesthesia, and the animal was exsanguinated into vacuum bottles containing acid-citrate-dextrose (ACD) solution. When the donor was a phlebotomized animal, the blood was collected by direct jugular puncture without anesthesia. The blood was then labeled with ⁵¹chromium (12). The recipient was anesthetized and the femoral vein exposed and cannulated. One hundred to 150 ml of blood was removed rapidly from the recipient, and the labeled blood was then pumped in over a period of 3 to 5 minutes.

Thin smears of blood and bone marrow were fixed for 10 minutes in absolute methanol, then stained to demonstrate siderocytes and sideroblasts according to the method of Rath and Finch (13). Reticulum and iron-containing granules were stained in the same preparation by a modification of the method of Douglas and Dacie (7). After initial supravital staining of reticulum with new methylene blue N (14), the smear was stained for iron as indicated above and then counterstained with 0.01% safranin O, freshly diluted from a refrigerated 0.5% stock solution. In performing siderocyte and reticulocyte counts, we examined 1,000 cells. In performing sideroblast counts, we counted 200 normoblasts.

The methods used to determine serum iron (15) and total iron-binding capacity of the serum (16) have been described previously.

Results

Influence of iron stores and splenectomy on the siderocyte count of normal pigs

The blood of six normal pigs was examined for siderocytes at weekly intervals (Table I, group I). Siderocytes were present in low numbers in the blood of each of these. The values given in Table

TABLE I

Influence of iron stores and splenectomy on the siderocyte counts of normal swine*

Group	Description	No. animals	VPRC	Serum iron	TIBC	Per cent saturation TIBC	Retics	Sidero-cytes	Sidero-blasts (bone marrow)
			ml/100 ml	*μg/100 ml*			%	%	%
I	Normal	6	43† (41–45)	109 (75–153)	566 (445–648)	20 (14–25)	2.8 (1.1–4.9)	0.2 (0–0.6)	13 (2–24)
II	Normal + im iron	3	47 (45–49)	102 (67–127)	361 (337–380)	28 (20–33)	2.0 (1.1–3.6)	0.2 (0–0.3)	20 (18–21)
III	Splenectomized	8	42 (39–47)	113 (78–162)	538 (392–605)	21 (13–27)	3.2 (1.3–4.9)	0.9 (0–2.9)	10 (1–26)
IV	Splenectomized + im iron	5	46 (45–49)	116 (86–153)	403 (348–478)	28 (23–32)	2.3 (1.3–3.8)	0.2 (0–0.3)	26 (15–36)
V	Splenectomized, iron deficient	3	18 (16–32)	16 (10–23)	917 (776–1,100)	2 (1–3)	6.6 (4.8–8.9)	0.0	2 (1–3)

* Abbreviations: VPRC = volume of packed red cells; TIBC = total iron-binding capacity of the serum; retics = reticulocytes.

† The values tabulated are the means of the last determinations before the death or sacrifice of each animal; the figures in parentheses refer to observed range.

TABLE II

Differential counts of reticulocytes without siderotic granules (R cells), reticulocytes with siderotic granules (R-S cells), and siderocytes without reticulum (S cells), in the blood of normal, pyridoxine-deficient, and phlebotomized pigs

Group	Description	No. of animals	No. of observations	R cells	R-S cells	S cells
				%*	%	%
I	Normal	11	15	4.8 ± 1.99†	0.5 ± 0.69	0.1 ± 0.12
III	Normal, splenectomized	5	10	4.0 ± 1.52	0.8 ± 1.00	0.2 ± 0.20
VI	Pyridoxine deficient	12	32	0.3 ± 0.49	4.4 ± 2.56	7.4 ± 3.75
VIII	Pyridoxine deficient, splenectomized	3	27	1.7 ± 1.85	4.3 ± 2.31	16.1 ± 9.19
XIII	Phlebotomized	2	44	18.3 ± 3.88	30.9 ± 6.18	0.9 ± 0.78
XIV	Phlebotomized, splenectomized	1	10	18.8 ± 3.84	26.9 ± 3.72	1.0 ± 0.79

* Number of cells per 100 erythrocytes.
† Mean ± 1SD.

I are the last values obtained before the termination of the experiment. Except for one isolated value of 7.6%, the maximal count observed in normal animals throughout the experiment was 0.8%. No tendency was observed for the siderocyte count to increase or decrease between the ages of 1 and 5 months, the age period in which the animals were studied.

To determine the effect of increased body stores of iron on the circulating siderocyte count, we gave three normal animals 15 g of iron by intramuscular injection over an 87-day period (Table I, group II). The reticulum cells in the bone marrow of these animals contained massive amounts of iron. In spite of this, siderocyte values were less than 1.0%, and serum iron values were not greater than in group I.

To investigate the influence of the spleen on the siderocyte count, we splenectomized eight normal animals (Table I, group III) and performed siderocyte counts at weekly intervals for about 2 months. Siderocytes remained within normal limits.

An additional five splenectomized pigs were given from 3 to 15 g of intramuscular iron over a period of 1 to 3 months (Table I, group IV). Again the reticulum cells in the bone marrow were loaded with iron, but increasing iron stores had no influence on the siderocyte count or on the concentration of iron in the serum.

Iron deficiency was produced in three splenectomized pigs (Table I, group V). Siderocytes were consistently absent from the blood. Only
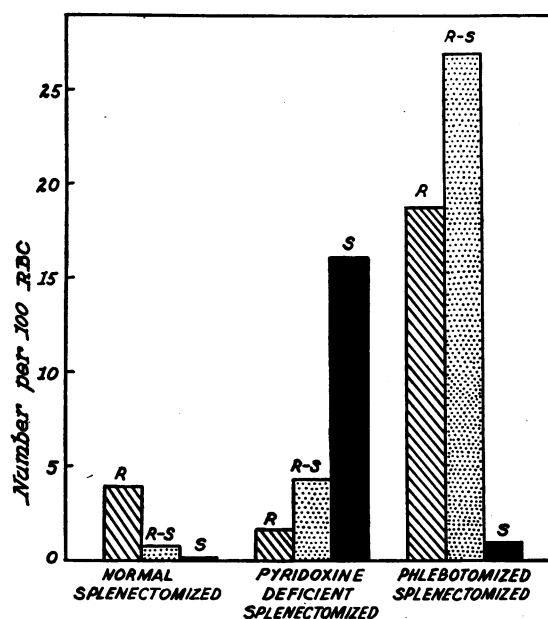

FIG. 1. DISTRIBUTION OF RETICULOCYTES (R CELLS), RETICULATED SIDEROCYTES (R-S CELLS), AND NONRETICULATED SIDEROCYTES (S CELLS) IN THE BLOOD OF NORMAL, PYRIDOXINE-DEFICIENT, AND PHLEBOTOMIZED PIGS AFTER SPLENECTOMY. The values represent the means of groups and are expressed as number of cells per 100 erythrocytes (RBC).

TABLE III

Influence of iron stores and splenectomy on the siderocyte count of pyridoxine-deficient swine

Group	Description	No. ani-mals	VPRC	Serum iron	TIBC	Per cent saturation TIBC	Retics	Sidero-cytes	Sidero-blasts (bone marrow)
			ml/100 ml	*μg/100 ml*			%	%	%
VI	Pyridoxine deficient	5	19* (17–23)	366 (250–505)	366 (240–538)	100 (94–100)	0.9 (0.5–1.8)	1.8 (0.9–4.1)	35 (15–50)
VII	Pyridoxine deficient + im iron	4	23 (20–28)	264 (134–523)	378 (200–595)	70 (39–100)	0.8 (0.4–1.0)	4.8 (2.8–8.2)	27 (12–34)
VIII	Pyridoxine deficient, splenectomized	23	25 (13–36)	384 (258–533)	405 (253–657)	95 (54–100)	4.4 (0.7–13.7)	31.6 (11.5–42.1)	30 (11–45)
IX	Pyridoxine deficient, splenectomized + im iron	9	22 (18–28)	534 (165–860)	443 (208–568)	100 (79–100)	5.6 (1.4–15.4)	37.2 (16.4–54.1)	37 (19–51)
X	Pyridoxine deficient, splenectomized, iron deficient	7	22 (16–32)	171 (25–288)	632 (464–835)	27 (3–59)	6.0 (2.5–9.3)	5.9 (0.3–11.4)	6 (5–8)

* The values tabulated are the means of the last determinations before the death or sacrifice of each animal; the figures in parentheses refer to observed range.

on two occasions throughout the experiment was an occasional (0.1%) siderocyte observed.

In all of the above groups, the siderocytes usually contained a single small round granule with a discrete margin.
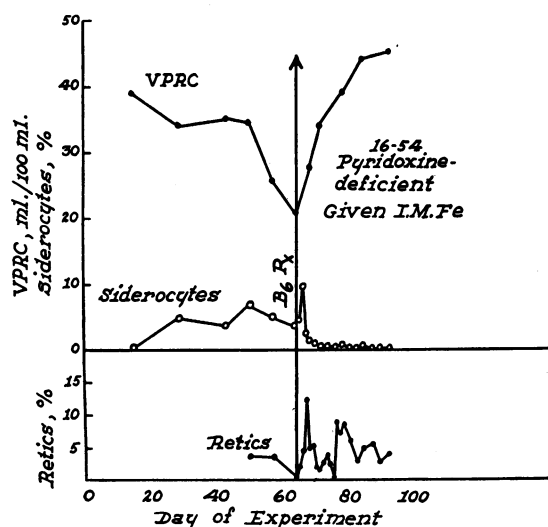

FIG. 2. DEVELOPMENT OF ANEMIA AND SIDEROCYTOSIS IN A PYRIDOXINE-DEFICIENT PIG. This (no. 16–54) animal was given 5 g of iron as iron dextrin by the intramuscular route between the 11th and 63rd days of the experiment. Beginning on the 63rd day of the experiment (B₆ Rx) the animal was given 100 mg of pyridoxine intramuscularly for 3 days, followed by 0.2 mg per kg body weight by mouth daily thereafter. Retics = reticulocytes; VPRC = volume of packed red cells.

In groups I and III, the cells were differentiated into reticulocytes that contained no siderotic granules (R cells), reticulocytes that also contained siderotic granules (R-S cells), and siderocytes that contained no reticulum (S cells). The results were expressed as number of cells per 100 red blood cells (Table II and Figure 1).

The reticulocyte (R cell) was the predominant cell in both groups, and most of the cells that contained siderotic granules were also reticulated (R-S cells).

The proportion of sideroblasts was greater in those animals receiving iron supplementation (Table I, groups II and IV) than in those that did not (groups I and III). In all groups, sideroblasts usually contained only one or two granules. Cells containing more numerous granules were seen in the iron-supplemented pigs. In three of these animals, rare (less than 1%) "ringed" sideroblasts were seen. In the severely iron-deficient animals (Table I, group V) sideroblasts were infrequent and contained only a single small granule.

Influence of a defect in heme synthesis on the siderocyte count

Pyridoxine deficiency. Pyridoxal phosphate is required for the first step in the biosynthesis of heme, the condensation of glycine and succinyl-coenzyme A to form Δ-aminolevulinic acid (17);

consequently a severe impairment in heme synthesis exists in pyridoxine-deficient pigs (18). Associated with the defect in heme synthesis, the absorption of iron is increased (19, 20), the serum iron increases, transferrin becomes saturated, tissue iron stores are increased, and severe microcytic hypochromic anemia supervenes (21). Pyridoxine deficiency was studied to ascertain the effect of such a defect in heme synthesis on the siderocyte count.

A slight but significant siderocytosis was observed in pyridoxine-deficient pigs (Table III, group VI) as compared with normal animals (Table I). The maximal siderocyte counts observed in these animals throughout the course of the experiment ranged from 5.7 to 17.4%. Siderocytes began to increase after 2 to 4 weeks of pyridoxine deprivation. The increase either immediately preceded or coincided with the development of anemia. Siderocyte counts throughout the course of the deficiency in one animal are shown in Figure 2. The serum iron was elevated and the transferrin was saturated in all members of this group. The administration of intramuscular iron in amounts of 5 g was associated with a further increase in the siderocyte count (Table III, group VII) to a mean value of 4.8% as compared with 1.8%. Splenectomy (Table III, groups VIII and IX) was followed by a striking and uniform increase in the siderocyte count. Values as great as 50% were observed in splenectomized animals. Siderocyte counts throughout the course of the deficiency in a splenectomized iron-loaded pig are shown in Figure 3. Omission of iron from the salt mixture of pyridoxine-deficient, splenectomized pigs (Table III, group X) in great part prevented the development of siderocytosis.

The morphology of the siderocytes in pyridoxine-deficient animals was quite different from the morphology of the siderocytes seen in normal pigs. The granules appeared primarily in microcytic, extremely hypochromic cells and were situated in the rim of hemoglobin at the periphery of the cell. Early in the course of the deficiency the granules were often delicate and filamentous; later, though they were more dense and more easily visualized, their shapes were usually irregular and their margins blurred.

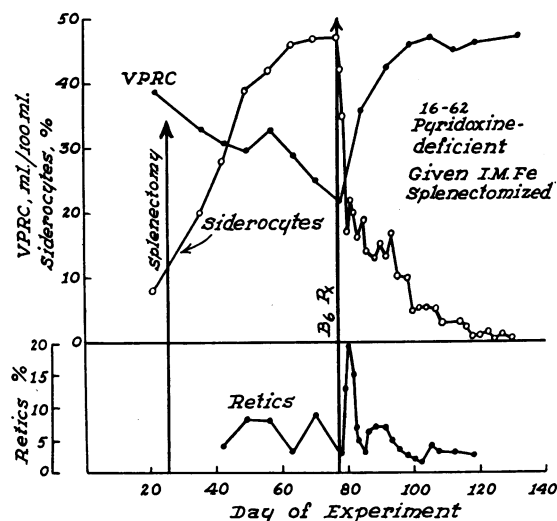Sideroblasts were more numerous in pyridoxine-deficient animals (Table III, groups VI to IX)

FIG. 3. DEVELOPMENT OF ANEMIA AND SIDEROCYTOSIS IN A SPLENECTOMIZED PYRIDOXINE-DEFICIENT PIG. This animal was given 6 g of iron as iron dextrin by the intramuscular route between the 11th and 77th days of the experiment. Beginning on the 77th day of the experiment (B_6Rx) the animal was given 100 mg of pyridoxine intramuscularly for 3 days followed by 0.2 mg per kg body weight by mouth daily thereafter.

than in normal pigs (Table I). In addition, the sideroblasts in these animals contained more and somewhat larger granules than in normal pigs. However, ringed sideroblasts were rarely seen. The proportion of sideroblasts in animals deficient in both pyridoxine and iron (Table III, group X) was reduced.

In 12 animals from groups VI to IX, bone marrow aspirations were performed on the day of therapy with pyridoxine and were repeated 3 to 7 weeks later after correction of the anemia. In these animals, sideroblasts decreased from a mean of 31% (range 12 to 51%) to 11% (range 2 to 31%).

Relation of siderocytes to reticulocytes. In pyridoxine deficiency (groups VI and VIII), the predominant cell was a nonreticulated siderocyte (S cell) (Table II and Figure 1), and siderocytes greatly outnumbered reticulocytes. Thus, the pyridoxine-deficient pigs differed from normal pigs (Figure 1). In normal pigs (groups I and III, Table II) the predominant cell was the reticulocyte (R cell).

Rate of disappearance of siderocytes after pyridoxine therapy. Seven spleen-intact animals were treated with pyridoxine, and the siderocyte count

was followed daily. The siderocyte count increased for the first 2 days, then rapidly declined to control values in 3 to 8 days (mean, 7 days), as illustrated in Figure 2. In seven splenectomized animals treated with pyridoxine, the siderocytes declined slowly, and siderocyte values of 2% or less were not reached for 28 to 42 days (mean, 34 days). The initial rapid decrease in siderocyte count (Figure 3) was due apparently to dilution of the circulating siderocytes by newly formed red cells.

Rate of disappearance of siderocytes transfused into normal, splenectomized, and splenectomized iron-deficient pigs. Siderocytes from pyridoxine-deficient pigs were labeled with ^{51}Cr and transfused into normal spleen-intact and normal splenectomized pigs to evaluate further the role of the spleen in regulating the disappearance of the siderotic granules and to study the fate of the cells containing these granules. Blood from pyridoxine-deficient, splenectomized pigs containing large numbers of siderocytes was transfused into 10
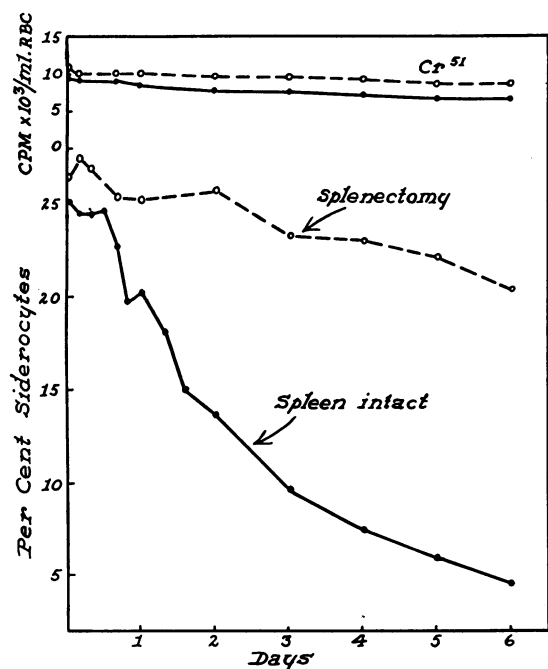

Fig. 4. Rate of disappearance of siderocytes from pyridoxine-deficient pigs transfused into normal spleen-intact and splenectomized pigs. The curves for spleen-intact animals represent mean values for nine animals. The curves for splenectomized animals represent mean values for four animals.

spleen-intact normal recipients and six splenectomized normal recipients.

The siderocytes disappeared from the blood of the spleen-intact recipients considerably more rapidly than from the circulation of the splenectomized recipients (Figure 4). The mean half-time disappearance of the siderocytes from the circulation of the 10 spleen-intact recipients was 1.8 days with a range of from 1 to 2 days. The mean half-time disappearance from the circulation of the six splenectomized recipients was 15.9 days with a range of from 12 to 25 days. The mean half-time disappearance of the ^{51}Cr label was 11.6 days (7 to 15 days) and 17.6 days (14 to 25 days), respectively, in the two groups. In both groups the rate of disappearance of ^{51}Cr-labeled cells was within the normal limits described previously (22, 23). These values give a relative but not a true estimate of the rate of cell loss (24) since chromium elutes from the cell, but it is apparent that in spleen-intact animals the disappearance of siderotic granules is considerably more rapid than the disappearance of the cells themselves, in confirmation of the observation of Crosby (1).

If the iron in the siderotic granules could be transferred directly from the siderocyte to the unsaturated transferrin in the plasma of the recipient, then the apparent rate of loss of transfused siderocytes might be expected to be more rapid from the circulation of splenectomized iron-deficient pigs than from the circulation of splenectomized normal recipients. To investigate this possibility high siderocyte blood from pyridoxine-deficient pigs was transfused into two iron-deficient splenectomized pigs. The serum iron of one animal at the time of the transfusion was 23 μg per 100 ml with a total iron-binding capacity (TIBC) of 767 μg per 100 ml. The serum iron of the second animal was 10 μg per 100 ml with a TIBC of 1,102 μg per 100 ml. The rate of disappearance of siderocytes in these animals was the same as in the normal splenectomized recipients, the half-time values being 16 and 12 days.

Rate of disappearance of siderocytes during in vitro incubation. Blood with large numbers of siderocytes was obtained from pyridoxine-deficient, splenectomized pigs and incubated with constant shaking at 37° C for 72 hours. A decrease in the siderocyte count of only 10% was observed (Figure 5). Thus, under these conditions, little or

no utilization or dispersion of siderotic granules occurred.

Influence of rapid blood regeneration on the siderocyte count

Phenylhydrazine. To determine if increased erythrocyte production is associated with siderocytosis, we gave phenylhydrazine to one spleen-intact and two splenectomized pigs (Table IV, groups XI and XII).

There was a striking increase in siderocytes. The siderocytosis was at least as great in the spleen-intact animal as in the two splenectomized pigs.

The erythrocytes in the blood smear were greatly distorted as a result of the phenylhydrazine, and the considerable amount of cellular debris made it difficult to count siderocytes accurately. For this reason no further studies were done on animals given phenylhydrazine.

Phlebotomy. When 20% or more of the blood volume was removed daily from pigs, a considerable reticulocytosis and siderocytosis developed (Table IV, groups XIII and XIV).

As in the animals given phenylhydrazine, and in contradistinction to pyridoxine-deficient animals, the siderocytosis was as great in spleen-intact pigs (group XIII) as in splenectomized pigs (group XIV). The siderocytes were very macrocytic and well filled with hemoglobin in both groups. The granules were usually multiple, round, and with discrete margins.

The development of anemia, reticulocytosis, and siderocytosis in one spleen-intact (no. 17–35)
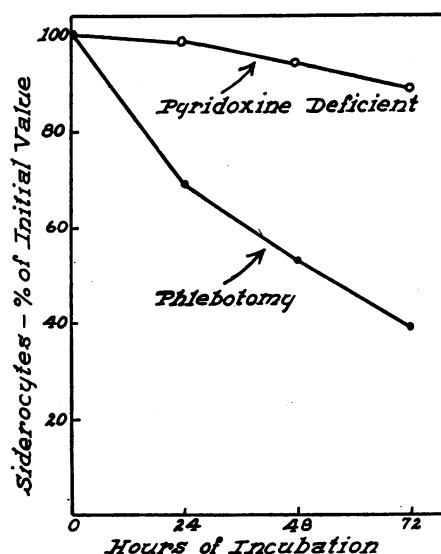

FIG. 5. RATE OF DISAPPEARANCE OF SIDEROCYTES INCU-
BATED IN VITRO. Blood from pyridoxine-deficient and from phlebotomized pigs was defibrinated, and 0.5 ml was transferred to 4-ml screw cap vials and incubated with shaking at 37° C. At the indicated intervals and at zero time, smears were made for reticulocyte and sidero-cyte counting, and incubation was not resumed for that specimen. All procedures were carried out in duplicate and under sterile conditions. Only minimal hemolysis was present at 72 hours.

and in one splenectomized (no. 17–38) pig is shown in detail in Figure 6. The close correlation between the reticulocyte and siderocyte values is apparent. The degree of siderocytosis (30 to 50%) in the spleen-intact animal was as great as in the splenectomized pig (30 to 40%). When phlebotomy was stopped, the siderocytosis declined rapidly and the siderocyte count reached values of

TABLE IV

Influence of phenylhydrazine and of phlebotomy on the siderocyte count of intact and splenectomized swine

Group	Description	No. animals	VPRC	Serum iron	TIBC	Per cent saturation TIBC	Retics	Sidero-cytes	Sidero-blasts (bone marrow)
			ml/100 ml	*µg/100 ml*			%	%	%
XI	Phenylhydrazine	1	30*	306	621	49	66.1	39.2	
XII	Phenylhydrazine, splenectomized	2	27	245	655	37	49.3	41.5	
XIII	Phlebotomized	5	26 (21–32)	213 (101–393)	510 (415–645)	45 (16–85)	45.0 (28–57)	32.8 (21–52)	35.0 (24–56)
XIV	Phlebotomized, splenectomized	6	26 (22–30)	126 (54–237)	490 (356–653)	27 (13–49)	37.6 (27–53)	19.6 (8–39)	22.0 (19–26)

* The values tabulated are the means of the last determinations before discontinuing phlebotomy or phenylhydrazine administration; the figures in parentheses refer to observed range.
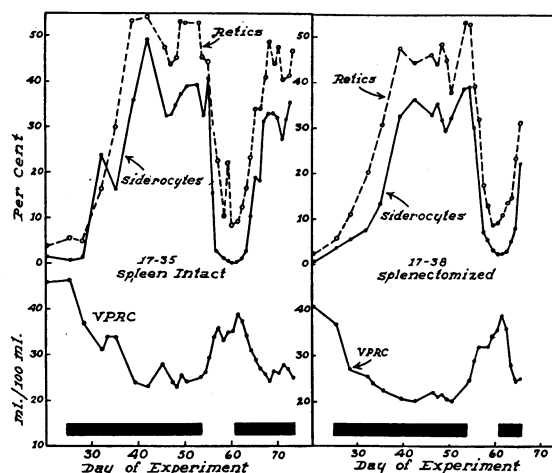
FIG. 6. DEVELOPMENT OF ANEMIA, RETICULOCYTOSIS, AND SIDEROCYTOSIS IN ONE SPLEEN-INTACT AND ONE SPLENECTOMIZED PIG DURING PHLEBOTOMY. Phlebotomy was performed daily during the period indicated by the solid bar at the bottom of the figure. The volume of blood withdrawn daily equaled 20 to 25% of the estimated blood volume.

3% or less within 4 days in both animals. The rate of disappearance of siderocytes, like the rate of formation, appeared to be the same in splenectomized as in spleen-intact pigs. This was in contradistinction to the siderocytosis in pyridoxine deficiency (Table III and Figures 2, 3, and 4).

Relation of siderocytes to reticulocytes. The close parallelism between siderocyte and reticulocyte counts (Figure 6) suggests that most of the
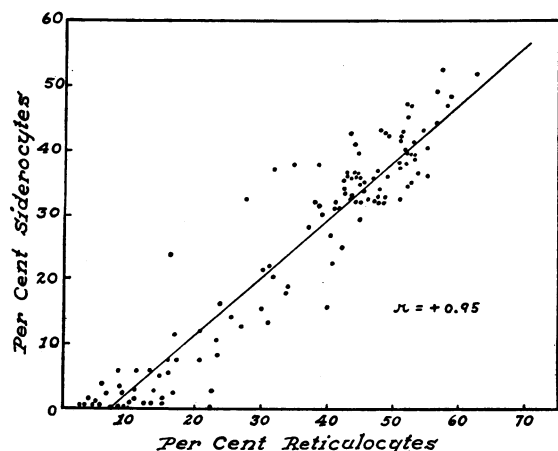

FIG. 7. CORRELATION BETWEEN RETICULOCYTES AND SIDEROCYTES IN THE BLOOD OF PHLEBOTOMIZED PIGS. Each point represents simultaneous reticulocyte and siderocyte counts before, during, and after phlebotomy. The correlation coefficient of + 0.95 is highly significant (p < 0.001).

reticulocytes contained siderotic granules (R-S cells) and that few nonreticulated cells contained such granules. The correlation coefficient between the reticulocyte and siderocyte counts was + 0.95 (Figure 7). By actual differential count of reticulocytes (R cells), reticulated siderocytes (R-S cells), and siderocytes (S cells) the predominant cell after phlebotomy was a reticulated siderocyte (R-S cell), and very few nonreticulated siderocytes (S cells) were present (Table II). Thus, these animals differed from both normal pigs and pigs with a defect in heme synthesis (Figure 1). In
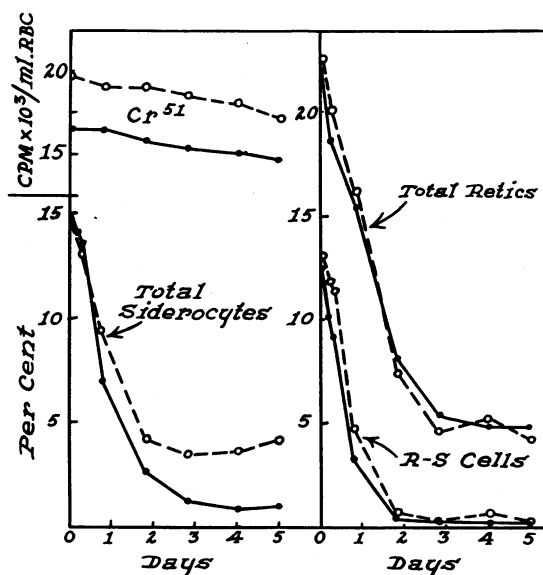

FIG. 8. RATE OF DISAPPEARANCE OF SIDEROCYTES FROM PHLEBOTOMIZED PIGS TRANSFUSED INTO NORMAL SPLEEN-INTACT (SOLID LINE) AND SPLENECTOMIZED PIGS (BROKEN LINE). Each curve represents mean values for four pigs. "Total siderocytes" refers to reticulated siderocytes plus nonreticulated siderocytes. "Total retics" refers to reticulocytes plus reticulated-siderocytes. The curve labeled "R-S cells" refers to reticulated siderocytes.

normal pigs reticulocytes (R cells) predominated; in pyridoxine-deficient pigs siderocytes without reticulum (S cells) were the most prevalent.

Rate of disappearance of siderocytes transfused into normal and splenectomized pigs. Blood from vigorously phlebotomized pigs containing large numbers of siderocytes was labeled with ^{51}Cr and transfused into four normal and four splenectomized pigs.

As was the case in recipients of blood from pyridoxine-deficient pigs, the half-time of the erythrocytes as determined with chromium labeling was

within normal limits in both groups of recipients (Figure 8). Initially, siderocytes disappeared rapidly and at the same rate from the circulation of both types of recipients. However, beginning about 24 hours after the transfusion, the rate of loss of siderocytes was slower in the splenectomized pigs. The possibility was considered that the smaller population of nonreticulated siderocytes (S cells) was removed at a slower rate in the splenectomized pigs than was the larger population of reticulated siderocytes (R-S cells). When the data for only R-S cells were plotted (Figure 8, right), the disappearance curves for R-S cells in spleen-intact and splenectomized pigs were essentially the same and resembled the rate of disappearance of all reticulocytes (R + R-S cells). The half-time disappearance for the R-S cells in all eight pigs was about 10 hours.

Rate of disappearance of siderocytes during in vitro incubation. When blood from a phlebotomized pig was incubated at 37° C for 72 hours, the siderocyte count decreased to 40% of the initial value (Figure 5). By comparison, only 10% of the siderocytes from pyridoxine-deficient pigs (S cells) disappeared when incubated *in vitro* under similar conditions.

Discussion

In the studies reported here greatly increased numbers of circulating siderocytes (> 30%) were observed only *a*) in animals with rapid blood regeneration, reticulocytosis, and adequate iron stores and *b*) in splenectomized pyridoxine-deficient animals with increased iron stores. In spleen-intact pyridoxine-deficient animals with greatly increased iron stores, siderocyte values of 3 to 8% were observed. Animals without pyridoxine deficiency or rapid blood regeneration (reticulocytosis) failed to develop a significant siderocytosis (> 3%) even in the presence of increased iron stores and in the absence of the spleen.

The siderocytes present in the circulation of the pyridoxine-deficient pigs were predominantly siderocytes that were not reticulated (S cells). These cells were increased to a greater degree in the absence of the spleen than when the spleen was present. After therapy with pyridoxine they were removed slowly from the circulation of splenectomized pigs and at a rate that probably approximated the rate of senescent loss of the cells. When the S cells were transfused into normal

spleen-intact and splenectomized pigs, they were removed more rapidly from the circulation of the former than the latter. No evidence could be obtained that these granules could be dispersed or metabolized to a significant degree within the erythrocyte itself either *in vivo* or *in vitro*. All of these observations suggest that siderotic granules present in red cells in the absence of reticulum can be dispersed, metabolized, or removed only in the presence of the spleen.

On the other hand, the siderocytes present in the circulation of pigs after vigorous phlebotomy were predominantly reticulated siderocytes (R-S cells). The number of these cells was as great in spleen-intact pigs as in splenectomized pigs. When phlebotomy was stopped abruptly, they disappeared rapidly from the circulation and as rapidly from the circulation of spleen-intact pigs as from splenectomized pigs. When the R-S cells were transfused into spleen-intact and into splenectomized pigs, they again disappeared at a rapid rate ($t_\frac{1}{2}$ of 10 hours) and irrespective of the presence or absence of the spleen. Finally, when R-S cells were incubated *in vitro,* more than half of the siderotic granules disappeared within 72 hours without evidence of hemolysis of the cells. All of these observations suggest that in the presence of reticulum, the siderotic granule is metabolized within the cell so that it becomes no longer visible. The spleen plays no role in this process. The retention of a few siderocytes in the circulation of splenectomized pigs can be explained by the presence of a small population of S cells.

The observations concerning the different behavior of these two different types of siderocytes are summarized in Table V. From these observations a few provisional comments may be made concerning the origin, metabolism, fate, and significance of siderocytes in the pig.

In the normal pig 10 to 20% of the normoblasts in the bone marrow contain one or more visible siderotic granules (sideroblasts). In normal man, 30 to 50% of the normoblasts contain such granules (6, 7, 25). By electron microscopy, it has been noted that all normoblasts in the marrow of normal individuals and the great majority of reticulocytes have visible iron in the form of ferritin molecules (26). Thus, siderotic granules of some size are a normal finding in marrow normoblasts and reticulocytes until hemoglobinization has been completed. Therefore, it would seem

TABLE V

Summary of the characteristics of the two different types of siderocytes

		Type of siderocyte	
Characteristic		Nonreticulated siderocytes (S cells)	Reticulated siderocytes (R-S cells)
Predominant in		Pyridoxine deficiency	Rapid blood regeneration
Influence of splenectomy on circulating numbers		Increase	None
Time, in days, of disappearance after therapy or cessation of phlebotomy	Spleen intact	7	4
	Splenectomized	34	4
Half-time disappearance, in days, when transfused into	Spleen intact	2	0.4
	Splenectomized	16	0.4
Rate of disappearance *in vitro*; per cent decrease in 72 hours		10	60

logical to assume that circulating siderocytes derive from marrow sideroblasts. Under normal circumstances, hemoglobinization is completed in the final reticulocyte stages in the marrow, and in the circulation only a few reticulocytes and a very rare mature erythrocyte still contain such a granule. In the normal pig less than 1% (mean, 0.2%) of all circulating cells contain siderotic granules, and most of these are within reticulocytes (R-S cells).

When the rate of erythropoiesis is increased, as after severe blood loss, terminal divisions in the bone marrow normoblasts are eliminated (27), and cells (reticulocytes) in which hemoglobin synthesis has not been completed enter the circulation. Reticulocytes, being endowed with mitochondria and polysomes (28, 29), are capable of completing hemoglobin synthesis after leaving the marrow (29). After severe blood loss the many siderotic granules that appear in the circulation are predominantly (97%) within reticulocytes (R-S cells). Thus, intracellular metabolism of the siderotic granule occurs as hemoglobin synthesis is completed. This process is independent of and unrelated to the spleen. Whether the spleen is incapable of removing or dispersing the granules in the presence of reticulum or whether intracellular metabolism proceeds so rapidly in the presence of reticulum that the spleen does not have time to carry out this function is not apparent from our studies.

It should be noted that the number of R-S cells that appear in the circulation will depend upon the availability of iron stores. When these are limited, the proportion of siderocytes will be reduced accordingly.

When hemoglobin synthesis is limited as a re-

sult of a heme synthesis defect, iron not used is apparently stored in the granules. In this case maturation of the reticulum proceeds unaccompanied by hemoglobin synthesis with the result that cells containing siderotic granules but no reticulum (S cells) appear in the circulation. The spleen is capable of eliminating the granules from these cells without destroying the cells themselves. Whether the spleen does this by pitting the granule as suggested by Crosby (8), or whether the spleen in some fashion disperses the iron within the cell so that it is no longer visible, or disperses and then removes the iron by a metabolic process, remains to be determined. With the experimental models developed it should be possible to resolve this question.

Bessis and Breton-Gorius (26, 30), by the use of electron microscopy, have observed that erythrocytic iron may be located either in the cytoplasm as ferritin aggregates or in the mitochondria as ferruginous micelles. These workers, as well as Harris (31), have noted that in certain hypochromic hypersideremic anemias which are partially responsive to pyridoxine that the iron is deposited in sideroblasts primarily in mitochondria. It would be interesting to know whether or not the iron in the reticulated siderocytes observed in phlebotomized pigs is in the same anatomical compartment and in the same ultrastructural form as the iron in the nonreticulated siderocytes (pyridoxine deficiency). Unfortunately, these questions are not answered by the light microscopy studies reported here. Electron microscopic studies are now in progress.

The data obtained in pigs differ in several respects from data reported in man. Whether this

is because of differences in the metabolism of siderocytes in the two species or because of limitations in the data available in man can be elucidated only by further investigations.

Douglas and Dacie (7) observed that siderocytosis developed in man after splenectomy from whatever cause. In 11 presumably hematologically normal subjects, a mean siderocytosis of 4% was observed after splenectomy. In the normal pig or the iron-loaded pig, splenectomy was not associated with a significant increase in siderocytes.

Only a slight degree of siderocytosis was observed by Douglas and Dacie (7) in most patients with hemolytic anemia (hereditary spherocytosis, atypical congenital hemolytic anemia, and acquired hemolytic anemia) before splenectomy. After splenectomy, the siderocyte count increased appreciably in most cases. Presumably the rate of erythropoiesis was greater in these cases before splenectomy than after, although details concerning individual cases are not presented. In the pigs with extremely rapid blood regeneration and adequate iron stores, the siderocyte count (R-S cells) was greatly increased in the spleen-intact animals and was not increased further in splenectomized pigs.

Since complete hemoglobinization and loss of reticulum apparently occur in some R-S cells without complete utilization of all of the available iron, some R-S cells are converted to S cells in the circulation. Therefore, it might be expected that the siderocyte count would temporarily remain elevated in some patients with hemolytic anemia after splenectomy, even though the rate of red cell production returned to normal. However, to interpret the data adequately in patients in light of the observations in the pigs, it would be necessary to know if the siderocytosis was transient or persistent, what the proportion of R, S, and R-S cells was, whether or not iron stores were adequate, and whether or not a defect in hemoglobin synthesis existed in addition to increased red cell production. Future investigations concerned with the significance of siderocytosis in man should include, insofar as possible, such measurements.

Summary

An increase in the number of circulating siderocytes was observed in swine with a defect in heme synthesis induced by a deficiency of pyridoxine and in swine with rapid blood regeneration induced by phlebotomy. The siderocytes produced in these two conditions differed in several important respects.

In pyridoxine deficiency, the siderotic granules were contained mostly within nonreticulated erythrocytes. The number of circulating siderocytes was greater in splenectomized pigs than in spleen-intact animals. When the nonreticulated siderocytes were transfused into normal recipients, they disappeared more rapidly from the circulation of spleen-intact pigs than from the circulation of splenectomized pigs. When the nonreticulated siderocytes were incubated in vitro, no significant disappearance of granules was noted.

After phlebotomy, the siderotic granules were contained within reticulocytes. The degree of siderocytosis was as great in spleen-intact animals as in splenectomized animals. When the reticulated siderocytes were transfused into normal recipients, they disappeared as rapidly from the circulation of splenectomized pigs as from spleen-intact pigs. When reticulated siderocytes were incubated in vitro, most of the granules disappeared within 72 hours.

It is proposed that in both conditions the circulating siderocyte is derived from the marrow sideroblast. In the presence of a defect in heme synthesis, the sideroblast is unable to incorporate all of the iron into hemoglobin as the cell matures, and siderocytes are released into the circulation. The granule in the siderocyte is then dispersed or removed by the spleen. When erythropoiesis is greatly increased in the absence of a defect in heme synthesis, terminal maturation divisions of marrow normoblasts are eliminated. Hemoglobin synthesis is incomplete in the reticulocytes so produced, and the cell carries with it not only the iron granule but also mitochondria and polysomes and is capable of completing hemoglobin synthesis while circulating. During this process the iron granule is metabolized by the cell and the disappearance of the granule is independent of the spleen.

Acknowledgments

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