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Anemia
caloric deprivation
erythropoiesis
infantile marasmus
malnutrition

Hematologic Studies of Severe Undernutrition of Infancy

I. The Anemia of Prolonged Caloric Deprivation in the Pig

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Extract

These studies were designed to define alterations in erythropoiesis that resulted from prolonged and controlled caloric deprivation. The caloric deprivation in the animal model chosen simulated that experienced by human infants receiving caloric intakes so restricted that infantile marasmus results.

The model chosen was the young piglet receiving a diet that would support normal growth when given in adequate amounts. The intake of this diet was so restricted as to stabilize the weight of the animals at 5.0 to 7.0 kg from one to ten months of age.

The studies of erythropoiesis in control piglets revealed a relatively high reticulocyte count associated with rapid growth and expansion of the red cell mass during the early months of life. The hematocrit and the hemoglobin levels increased with age, although there was a slight decrease in the total red cell mass per unit of body weight with increasing age.

Iron kinetic studies were performed in normal growing pigs (table V). The mean values for plasma iron turnover (mg Fe/100 ml whole blood/24 h) were 2.07 at one month of age, 1.54 at two months of age, and 1.06 at three months of age. The mean percentage of injected Fe^{59} that appeared in the circulating erythrocytes seven days after injection was 95 at one month, 82 at two months, and 76 at three months of age. These findings are consistent with the presence of a decreasing rate of erythropoiesis and hemoglobin synthesis accompanying the relative decrease in growth rate with progressive age.

Animals maintained on a very restricted caloric intake were found to have immediate alterations in erythropoiesis (table VI). Plasma iron turnover in these animals was 2.26 mg on the second day following dietary restriction and only 1.28 mg when measured four days after dietary restriction. After ten days, the value was reduced to 0.88 mg. A prompt decrease in urinary erythropoietin excretion was detected during this time and persisted throughout the period of observation while the animals were on the restricted diet (fig. 3). The appearance of iron in circulating erythrocytes seven days following the injection of Fe^{59} was found to be 100% when iron was given two days following dietary restriction. When the isotope was given four days following initiation of the restricted diet, 86.5% appeared in the circulating erythrocytes at seven days. A further decrease to 52% was observed in a study initiated ten days after dietary restriction.

The alterations in erythropoiesis demonstrated by iron kinetic studies were also reflected by a decrease in the reticulocyte count and by an increase in the myeloid:erythroid ratio in bone marrow examined serially.

The measurement of the Cr^{51} total erythrocyte mass prior to dietary restriction was 24.8 ml/kg (fig. 8). After thirty days on restricted caloric intake, the mean value was 25.8 ml/kg. Erythropoiesis, although decreased, continued at a rate sufficient to maintain a constant red cell mass during this immediate period of caloric restriction.

Prolonged caloric deprivation produced a decline in hemoglobin levels and hematocrit in the experimental pigs (fig. 7). The reticulocyte count fell from the level of 5.6 to 1 % or less during the period of dietary restriction and was maintained at the low level of 1 % throughout the period of observation. Cr^{51} erythrocyte mass, which was maintained at a constant level during the first thirty days of dietary restriction, showed thereafter a progressive fall throughout the period of undernutrition and paralleled the fall in hematocrit (table VIII). Levels of protein, folate, and iron in serum were maintained within the normal range throughout the period of caloric deprivation.

Studies performed in pigs after five to seven months of dietary restriction found plasma iron turnover to be lower than that observed in the control animals and essentially the same as that observed after ten days of diet restriction. After six months of diet restriction, iron utilization was reduced to a maximum utilization of 31 to 64% (fig. 9).

After a period of seven to eight months of dietary restriction, five animals were offered diet *ad libitum*. Within three to eight days, a prompt increase in erythropoietin excretion was seen; the reticulocyte count increased and, by six days after *ad libitum* feedings, had reached levels characteristic of normal, rapidly growing pigs. An increase in plasma iron turnover and per cent utilization of iron was measured ten days after initation of *ad libitum* feedings, and the myeloid: erythroid ratio reflected an increase in erythroid precursors soon after starting *ad libitum* feedings.

The hematocrit decreased promptly after initiation of the *ad libitum* diet. The lowest level was reached in five days. The Cr^{51} total red cell mass had increased during the period of falling hematocrit, and the total plasma volume had also greatly increased at this time. The early fall in hematocrit was thus due to the increase in the plasma volume, rather than to any decrease in the circulating erythrocyte mass.

Speculation

The availability of an animal model of prolonged caloric deprivation uncomplicated by infection, parasitism, or blood loss has demonstrated that caloric deprivation *per se* will result in a modest reduction in hematocrit and hemoglobin concentration that is paralleled by a reduction in the total circulating red cell mass. Such animals fail to demonstrate a deficiency of specific nutrients essential for normal erythropoiesis. The finding by others that caloric deprivation may be associated with a hypometabolic state suggests that anemia accompanying caloric deprivation may not be a primary consequence of inadequate nutrition but, rather, a reflection of an adaptive reduction in hemoglobin concentration in the face of a reduced demand for oxygen transport.

Introduction

Alterations in hematopoiesis caused by caloric deprivation in infancy are difficult to assess by clinical studies. Malnourished infants commonly suffer from a variety of complicating conditions such as infections, parasitic infestations, and blood loss. In addition, deficiencies of specific nutrients needed for hematopoiesis, particularly iron and folic acid, may be encountered. When anemia is present in these infants, it often results from multiple causes.

The primary deficiency in the severely malnourished infant may be of calories (marasmus) or protein (kwashiorkor). Definition of specific hematological consequences of calorie and protein deficiency would provide an understanding of the basic hematologic status upon which the influence of other deficiencies or complications may be superimposed.

Infants suffering from relatively uncomplicated marasmus may have little or no anemia [23, 27]. A normocytic anemia found in infants with kwashiorkor is attributed by several authors [1, 3] to protein defi-

ciency but, as previously mentioned, it is impossible in such patients to separate the changes resulting from protein deficiency *per se* from those caused by infection or other specific nutritional deficiencies.

The consequences of acute starvation or protein deficiency have been extensively studied in animals. Acute starvation or protein deficiency in rodents results in a rapid decrease in erythropoiesis [4, 11]. This change is thought to be the result of decreased stimulation of erythropoiesis and can be prevented or reversed by injections of erythropoietin [9, 24, 25]. Less information is available on the consequences of prolonged caloric or protein deprivation, situations more comparable with those seen in malnourished infants.

In the studies to be reported, an attempt has been made to define the anemia of uncomplicated prolonged caloric deprivation in the growing animal. The pig was chosen because it is of adequate size to be studied in early life during a period of normal rapid growth. MCCANCE *et al.* [18] have previously shown that this animal can be maintained for a period of many months on a calorie-deficient diet.

Material and Methods

Twenty-nine pigs from five litters of mixed Yorkshire and large English Black stock were used. Seven animals were selected from three groups of littermates to serve as controls. Three of the control animals were sacrificed at one month of age. The remaining four were utilized for control studies for a period of nine months. The animals were farrowed on a farm, kept with the sow for twelve days, and then weaned and transferred to the University of Washington Vivarium. At the vivarium, all piglets were given a single dose of 600 mg of piperazine citrate. No evidence of parasitism was observed during these experiments. The pigs were not castrated.

A commercial pelleted pig diet known to contain mineral and vitamin supplements adequate to meet the requirements of growing pigs was used to feed both the experimental and control animals throughout these experiments [29]. The composition of two samples of diet from two lots analyzed in our laboratory is shown in table I.

The diet of the animals was controlled from the time of weaning and transfer to the vivarium. The first feedings were limited to warmed, raw cow's milk given four times daily *ad libitum*. The pelleted commercial pig diet was added to the milk in increasing amounts, beginning on the third day. The milk was replaced with water after seven to ten days. The diet was offered *ad libitum* until approximately 30 days of age, when the experimental dietary restriction was begun.

The pigs weighed 5.0 to 7.0 kg at the time of initiation of dietary restriction. The diet was managed to prevent weight gain. Observations on the first experimental group demonstrated that the weight at 30 days of age could be stabilized with an intake of 35 g of the diet given three times daily (8:00 a.m., 12:00 noon, and 4:00 p.m.). All pigs in the experimental groups were weighed daily, and occasional adjustments were made in feeding amounts to minimize any fluctuations in weight. The control pigs consumed the same diet *ad libitum* throughout the period of study.

All animals received 150 mg of iron given intramuscularly as iron dextran [30] at three days of age. The rapidly growing control animals received supplemental oral iron, which provided 100 mg of elemental iron per day from 30 to 150 days of age.

All experimental procedures were performed in the temperature-controlled room in which the animals were housed. Experience with anesthesia and tranquilizing drugs early in the studies indicated that these agents could produce abrupt and unpredicted changes in hematocrit [8]. Since it was found that all procedures could be performed without the use of anesthesia or sedation, none was used. All infusions were given into the ear veins; venipunctures were performed using the jugular, anterior vena cava, or proximal brachial vein, and bone marrow was aspirated from the sternum or rib. The animals were restrained in a dorsal, recumbent position for all procedures.

Peripheral blood cell counts were performed according to accepted manual techniques, using venous blood with heparin or potassium ethylenediamine tetraacetate (K-EDTA) as an anticoagulant. All of the observed reticulocyte levels were corrected for hematocrit variation to a hematocrit of 43.

Iron concentration and the iron-binding capacity of serum or plasma were measured according to the method of FISCHER and PRICE [10]. Total serum protein concentrations were measured by the Biuret reaction, and the albumin concentration was determined

Table I. Composition of diet[1]

Moisture, %	10.3	11.2
Protein (N×6.25), %	20.7	20.3
Fat (petroleum ether extract), %	4.5	4.6
Fiber, %	2.2	2.5
Ash, %	4.8	4.8
Nitrogen-free extract, %	57.5	56.6
Iron, mg/kg	74.8	74.8
Kilocalories/100 g	415	410

[1] Analysis reported involved two separate lots of the same basic diet.

by a salt fractionation procedure [14]. Serum folate activity was measured using the L. Casei assay method performed by Bio-Science Laboratories, Van Nuys, California.

Total Erythrocyte Mass and Plasma Volume

The total circulating erythrocyte mass was measured by labeling autologous erythrocytes with Cr^{51} [21]. Eight ml of venous blood was mixed with 2.0 ml of ACD solution. Five-tenths to 25.0 μc of Cr^{51} was added to the blood, and the mixture was incubated at room temperature for 60 minutes. The incubated cells were washed with normal saline, resuspended, and quantitatively injected into the animals. Plasma volume was determined using radioiodinated human serum albumin (RI^{131} SA) [26].

The I^{131} total plasma volume and the Cr^{51} RBC mass were measured eleven times in seven control animals. It was possible with these data to establish a correction factor of 0.9 to be utilized when the venous hematocrit was used to determine the total body hematocrit. This factor was used to calculate the total blood volume from the measured total red cell mass.

Changes in the venous hematocrit occurred during measurement of the red cell mass. This has been observed by others [8]. In certain animals, expansion of plasma volume occurs because of the restraint and stress of the study procedure. For this reason, in these experiments, total blood volume was calculated from the Cr^{51} red cell mass, using the hematocrit initially determined in the resting animals and the correction factor defined above.

Iron Kinetic Studies

Autologous plasma was incubated with Fe^{59} of high specific activity in the form of ferric chloride and buffered with sodium citrate prior to mixing with plasma. The mixture was incubated for 15 minutes at room temperature, and following removal of an aliquot for use as a counting standard, was injected quantitatively into the ear vein. Venous blood specimens were removed at 5, 10, 20, 30, 45, and 60 minutes. Additional specimens were taken at approximately 30-minute intervals until well beyond the anticipated t ½ of the circulating isotope, usually for two to three hours. One milliliter aliquots of plasma were used for counting. The plasma iron level was determined in alternate samples. Venous blood specimens were removed at 12-hour intervals or more often for the next three days, and then daily for seven to ten days. One milliliter aliquots of the whole blood of these specimens were hemolyzed with saponin and counted. All specimens were counted for Fe^{59} activity in a well-type scintillation counter long enough to have a calculated counting error of less than 3 %.

The Fe^{59} t ½ was determined from the plasma clearance data plotted on semilog paper. In most of the studies, a break in the curve occurred at some point between 30 and 45 minutes after the injection. Similar curves had been published by JENSEN *et al.* [15] in a study of iron kinetics in swine. In a series of experiments designed to identify the factors responsible for the changing rate of clearance of plasma iron, these authors found early concentration of the iron isotope in the liver. After this rapid removal of isotope by the liver, there was equilibration with the plasma pool, and the second slope of the curve was postulated to represent the clearance of iron from plasma by bone marrow [15]. Therefore, in the present studies, the Fe^{59} t ½ in plasma has been calculated using the second slope of the plasma clearance curve. In eight of the forty-four studies performed to date, a curvilinear plot was obtained, and a second slope of the curve could only be approximated (fig. 1). It is likely that the clearance of iron from plasma in the pig is influenced by more than the hepatic and bone marrow pools and may represent interaction of additional iron pools.

Although interpretation with confidence of iron clearance curves in the pig will require further experi-

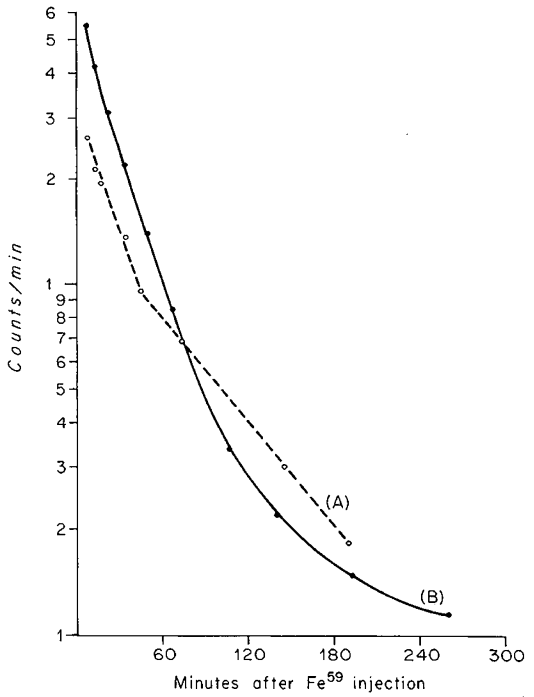

Fig. 1. Plasma Fe^{59} clearance in the pig. Two distinct slopes can be defined in the majority of studies (36 of 44) as shown in curve A. The curvilinear plot of curve B is encountered less frequently (see text).

Table II. Comparison of calculated red cell iron turnover and plasma iron turnover[1]

Pig No.	Age (months)	Weight (kg)	Growth rate (kg/24 h)	Erythrocyte mass (ml/kg)	RBCIT[2] (mg/24 h)	PIT (mg/24h)[3] Slope 2	PIT (mg/24h)[3] Slope 1
18	2	21.4	0.70	20.7	22.18	19.89	42.64
19	2	21.2	0.50	19.0	16.99	21.58	51.05
23	2	19.6	0.60	20.8	20.19	19.61	37.43
26	2	21.7	0.75	19.2	21.64	26.27	52.84
18	3	42.3	0.70	19.7	28.07	22.28	67.01
19	3	43.2	0.70	18.9	28.12	24.35	42.58
23	3	37.8	0.60	19.3	24.87	29.65	38.31
26	3	46.8	0.90	21.7	38.19	41.85	60.69

[1] See text for method of calculation.

[2] Red blood cell iron turnover, calculated using erythrocyte mass, growth rate, and a red cell life span of 70 days [26].

[3] Plasma iron turnover, calculated using the formula:

$$\text{PIT mg/24 h} = \frac{0.693 \times \text{plasma Fe } (\mu\text{g}/100\text{ ml}) \times \text{plasma volume (ml)} \times 1440}{\text{t ½ (min)} \times 100{,}000}$$

mentation, this study attempted to evaluate the validity of determining plasma iron turnover from the curves described above. A theoretical value for erythrocyte iron turnover in the control animals was calculated at 60 and 90 days of age, using the available data on the total red cell mass, the rate of growth, and the measured life span of the circulating erythrocytes [28]. These calculated theoretical values were compared with values for plasma iron turnover determined from the observed Fe^{59} plasma clearance (table II). The values for plasma iron turnover calculated from the initial slope were one and a half to two and a half times greater than the theoretical values for erythrocyte iron turnover. The values calculated from the second slope of the plasma iron clearance curves approximated the theoretical values. These data agree with the concept of JENSEN *et al.* [15] that the second slope of the clearance curve represents iron clearance from the plasma primarily for erythropoiesis. Therefore, plasma iron turnover values were calculated using the t ½ value of the second slope of the clearance curve and the following formula:

$$\text{Plasma Fe turnover (mg of Fe/100 ml whole blood/24 h)} = \frac{\text{Plasma Fe } (\mu\text{g}/100\text{ ml}) \text{ (100–Hct.)}}{\text{t ½ (min) } 100}$$

The utilization of radio-iron for erythrocyte production was calculated as follows:

$$\text{\% Fe utilization} = \frac{\text{Circulating activity (cpm) in 1.0 ml whole blood} \times \text{blood volume} \times 100}{\text{Total administered activity (cpm)}}$$

Values for iron utilization in all experiments were arbitrarily expressed as the values observed on day seven after Fe^{59} injection. Corrections were made in these results for changes in total blood volume in those animals experiencing rapid growth during the period of study.

The mean transit time of marrow iron is calculated as the period from the time of 50 % clearance from the plasma to the time of reappearance in circulating whole blood of 50 % of the iron found in whole blood seven days after the injection [7].

Erythropoietin

Quantitative, 24-hour collections of urine were obtained for erythropoietin determination. The bladder was emptied by suprapubic aspiration, and the animals were then restrained in a standing position in Pavlov stands. Only male pigs were used so that a funnel could be taped over the penis and connected to tubing leading to a polyethylene bottle surrounded by dry ice. The collection was completed at the end of twenty-four hours by performing a second suprapubic aspiration of the bladder contents. Erythropoietin assays were done using the polycythemic mouse [19] and expressed as units of erythropoietin standard B/24 hours.

Results

Control Pigs

Under the conditions of these observations, the control animals grew rapidly and remained free of disease. The progressive changes in weight are listed in table III, and the results of the studies of the four control animals are listed in tables III, IV, and V.

Normal progressive increases in hematocrit and hemoglobin concentration were observed during the period of rapid growth [20]. Hematocrit values of approximately 45 to 50 % and hemoglobin concentrations of 14.0 to 16.0 g/100 ml were reached by eight months of age.

The results of serum iron and saturation of iron-binding protein measurements, bone marrow sideroblast counts, and erythrocyte indices indicated that the iron supplements given to these animals were such that iron was not limiting erythropoiesis (table III). The values reported are similar to those previously reported for iron-supplemented pigs [5].

The data from determinations of Cr^{51} red cell mass and the calculated plasma volume are listed in table IV. The Cr^{51} total erythrocyte mass showed a minimal decrease per unit of body weight during the sixty-day period of these observations. A more marked and progressive decrease in plasma volume occurred. This decrease resulted in a reduction in total blood volume per kg of body weight. This change is similar to that previously reported by HANSARD [13] and by BUSH *et al.* [8].

Reticulocyte counts showed higher values in the early months of life when rapid growth of the pig is associated with expansion of red cell mass; lower values were seen as growth rate decreased (table III). Reticulocyte counts stabilized at approximately 2 % after four months of age.

The data from iron kinetic studies are recorded in table V. The mean value for plasma iron turnover, mg Fe/100 ml whole blood/24 h, was found to be 2.07, 1.54, and 1.06 at 1, 2, and 3 months of age, respectively. The mean percentage of the injected dose of Fe^{59} that was present in circulating erythrocytes seven days after injection was 95, 82, and 76 at 1, 2, and 3 months of age, respectively. The mean marrow transit time at these same ages was 22.7, 28, and 28.3 hours. These findings agree with the decreasing rates of erythropoiesis and hemoglobin synthesis associated with the relative decrease in growth rate with progressive age.

Undernourished Pigs

Animals maintained on restricted caloric intake developed changes in physical appearance and activity similar to those described by MCCANE [18]. The goal of dietary management was to stabilize weight at 5 to 7 kg, which was the weight of the pigs at thirty days of age. Over the period of several months of dietary restriction, the animals experienced an average gain in weight of 1.5 kg (table VII). Maintenance on the restricted diet was characterized by loss of body fat, slight linear growth, and a disproportionate increase in head size.

The undernourished animals demonstrated a progressive decrease in motor activity, exhibiting active behavior during the first two months on the diet, but after having received the restricted diet for six to eight months, were inactive during most of the day. Body temperature was perceptibly lower in these animals than in normal animals [16]. Under the conditions of these experiments, all of the animals remained free from infection or infestation. The skin remained intact and a good appetite was maintained, even after many months of dietary restriction. The general appearance of the undernourished pigs is shown in figure 2.

Immediate Effects of Diet Restriction

Immediate alterations in erythropoiesis resulting from the limitation of caloric intake were demonstrated

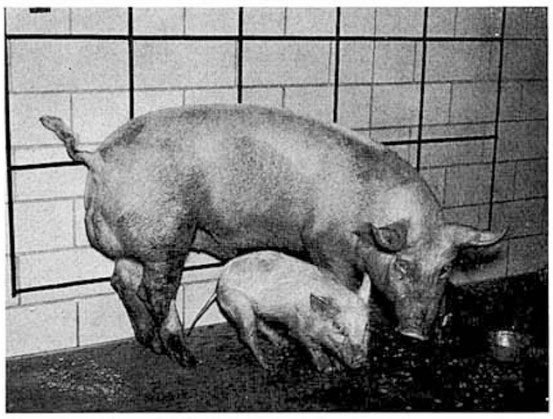

Fig. 2. Littermate pigs four months of age. The smaller animal had received the calorie-restricted diet since 30 days of age.

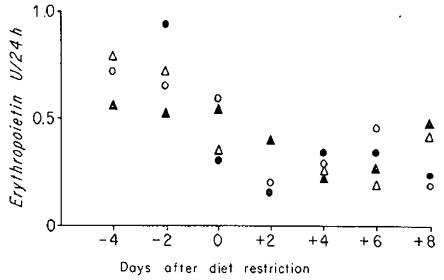

Fig. 3. Erythropoietin excretion in 4 pigs before and immediately after caloric restriction.

Table III. Hematologic observations of four control pigs[1]

Age (days)[2]	30	60	90	120	150	180	240	270
Weight (kg)	7.2	21.6	42.9	60.1		102.8		149.7
	6.5–8.0	20.1–22.4	38.0–47.2	53.2–65.8		86.2–124.7		124.0–185.0
Hemoglobin (g/100 ml)	11.1	10.8	12.5	13.3	13.4	13.8	15.1	15.1
	10.5–12.4	8.9–12.3	12.0–13.1	12.9–14.5	12.8–14.0	12.9–14.7	14.3–16.0	14.5–15.8
Hematocrit (%)	35.5	38.6	40.9	42.5	41.9	44.8	48.4	46.0
	32.5–38.0	35.0–42.0	39.0–43.5	41.0–46.0	41.0–44.5	41.5–47.0	45.5–51.0	44.0–48.5
MCHC (%)	30.0	30.5	30.6	31.3	31.9	30.9	31.0	32.8
	29.0–31.0	30.0–31.0	30.0–31.0	30.5–31.5	31.5–32.0	30.0–31.5	30.0–31.5	32.0–33.5
MCH ($\mu\mu$g)	21.8	21.0	20.8	20.5	20.0(3)	21.7(3)	21.5	22.5
	20–23	19–23	20–21	19–23	19–21	19–23	20–23	21–24
MCV (μl^3)	70.0	67.0	68.8	65.0	62.7(3)	69.0(3)	68.0	68.3
	65–74	64–72	64–71	60–72	60–65	61–73	62–73	64–71
Reticulocytes (%)	4.8	3.9	2.4	2.3	2.0	2.1	1.9	1.9
	4.2–5.8	3.3–4.6	1.5–3.1	1.9–2.7	1.8–2.1	1.9–2.3	1.8–2.0	1.5–2.2
Serum iron (μg/100 ml)	139.3	150.5	133.5	97.8	148.3	115.5	168.3	150.8
	95–195	124–176	105–178	80–111	111–184	103–124	148–197	133–180
Total iron-binding capacity	734.0	876.0	722.5	705.5	776.0	674.5	651.0	635.0
(μg/100 ml)	692–780	856–920	642–800	666–754	736–812	666–692	604–688	584–660
% saturation of iron-binding capacity	18.9	17.1	18.4	13.8	19.0	17.1	25.8	23.8
	13.7–25.0	14.4–20.3	15.3–24.5	12.0–16.1	15.1–22.7	15.5–18.1	22.3–30.3	20.3–27.3
Serum folate ($\mu\mu$g/ml)						8.2		7.0
						6.6–9.0		5.6–7.6
Total proteins (g/100 ml)	4.7	5.3	5.9	6.2	6.1	6.3	7.2	6.8
	4.4–5.0	5.0–5.4	5.6–6.0	5.7–6.7	5.8–6.4	6.0–6.6	6.5–7.9	6.5–7.0
Albumin (g/100 ml)	2.2	2.6	2.8(2)	2.6(3)	2.6(2)	2.5	2.4	2.9
	2.0–2.5	2.4–2.7	2.7–2.9	2.6–2.7	2.4–2.7	2.1–2.7	1.9–3.0	2.7–3.1

[1] All values listed represent the mean and the range of observations on four animals except when designated otherwise by numbers in parentheses.
[2] All of the results for a stated age are results of study performed at that age or the closest study within ± 15 days of the designated age.

by iron kinetic studies performed at 2, 4, and 10 days following initiation of dietary restriction and by measurement of erythropoietin excretion during this period. The immediate changes in erythropoietin excretion are shown in figure 3. A prompt decrease in erythropoietin excretion was observed, and excretion was maintained at a reduced level during this immediate period of observation (table VI).

The calculated values for plasma iron turnover of 2.26 mg/100 ml of whole blood/24 h on the second day following dietary restriction was similar to values in animals studied prior to any alteration of the diet

Table IV. Cr^{51} erythrocyte mass and calculated plasma and blood volume in control pigs[1]

Age (days)	30	60	90
Weight (kg)	6.0	20.9	42.5
	5.7–6.4	19.6–21.7	37.8–46.8
Venous hematocrit (%)	37.0	35.5	40.9
	35.0–38.0	29.5–41.0	39.0–43.5
Cr^{51} erythrocyte mass (ml/kg)	21.9	19.9	19.9
	21.0–22.9	19.0–20.8	18.9–21.7
Plasma volume (ml/kg)	43.9	43.1	34.3
	40.4–47.2	35.4–53.1	30.6–40.1
Blood volume (ml/kg)	65.8	63.0	54.2
	61.4–68.9	56.1–72.3	50.3–61.8

[1] The values listed represent the mean and the range of observations on 3 pigs at 30 days and 4 pigs at 60 and again at 90 days of age.

Table V. Iron kinetic studies in control pigs[1]

Age (days)	30	60	90
Weight (kg)	6.0	20.9	42.5
	5.7–6.4	19.6–21.7	37.8–46.8
Growth rate (kg/day)	0.24	0.64	0.73
	0.20–0.30	0.50–0.75	0.60–0.90
Plasma iron (μg/100 ml)	108.7	176.3	126.3
	94–121	141–230	83–165
t ½ Fe^{59} (min)	38.0	76.0	73.0
	35–42	70–98	57–87
Plasma iron turnover (mg/100 ml whole blood/24 h)	2.07	1.54	1.06
	1.90–2.37	1.47–1.65	0.91–1.22
Marrow transit time (h)	22.7	28.0	28.3
	18–26	25–30	26–32
Maximum % utilization by 7 days	95.0	82.5	75.8
	92–100	75–89	67–83

[1] The values listed represent the mean and the range of observations on 3 pigs at 30 days and 4 pigs at 60 and again at 90 days of age.

Table VI. Iron kinetic studies in calorie-deprived pigs[1]

Days after caloric restriction	0 (3)	2 (1)	4 (2)	10 (1)	162 (4)	200 (4)	–	–
Days after diet *ad libitum*	–	–	–	–	–	–	10 (1)	60 (3)
Weight (kg)	6.0	6.0	6.0	5.7	6.8	6.9	8.9	42.7
	5.7–6.4		5.7–6.3		5.8–7.6	6.3–8.3		38.0–48.0
Growth rate (kg/day)	0.23	0.0	0.0	0.0	0.0	0.0	0.40	0.60
	0.2–0.3							0.4–0.8
Plasma iron (μg/100 ml)	108.7	188.0	165.5	129.0	119.5	173.0	130.0	69.0
	94–121		160–171		102–146	107–269		50–89
Fe^{59} t ½ (min)	38	54	82.5	94	107.5	135.3	30.0	38.7
	35–42		82–83		88–118	88–220		23–48
Plasma iron turnover	2.07	2.26	1.28	0.88	0.76	0.87	3.03	1.26
(mg/100 ml whole blood/24 h)	1.90–2.37		1.28–1.29		0.61–0.85	0.80–1.01		0.97–1.46
Marrow transit time (h)	22.7	25	34.5	44	–	52.5	19.0	–
	18–26		33–36			36–66		
Maximum % utilization by 7 days	95.0	100	86.5	52	–	50	83	–
	92–100		83–90			31–64		

(day 0). Plasma iron turnover on day four was reduced to 1.28 mg/100 ml of whole blood/24 h. By day ten, there was a further decrease to 0.88 mg, a value similar to that found after five to seven months of restricted dietary intake.

The pattern of iron utilization for erythrocyte production is shown in figure 4. The appearance of iron in circulating erythrocytes seven days following injection of the isotope was 100 % when iron was given two days following dietary restriction. When given four

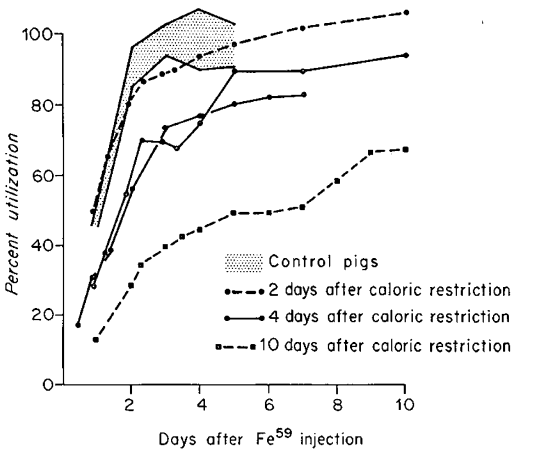

Fig. 4. Iron utilization for erythrocyte production in 3 control pigs 30 days of age and in other pigs 2, 4, and 10 days after caloric restriction.

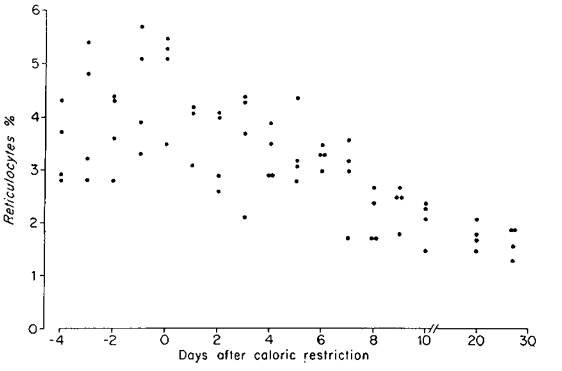

Fig. 5. Reticulocyte count in 4 pigs before and immediately after caloric restriction.

Note table VI:

[1] The values listed represent the mean and range of observations when more than one animal was studied. The number of animals studied at each day is listed in parentheses.

days following dietary restriction, 86.5 % of the isotope was in the circulating erythrocytes at seven days. A further reduction to 52 % was observed in the one study initiated ten days after dietary restriction. The transit time of marrow iron was progressively prolonged during this period (table VI). A value of 24 hours was observed after two days on the restricted diet, and in the two studies performed four days after dietary restriction, the transit time was 34.5 hours. It was 44 hours in the study initiated after ten days on the restricted diet.

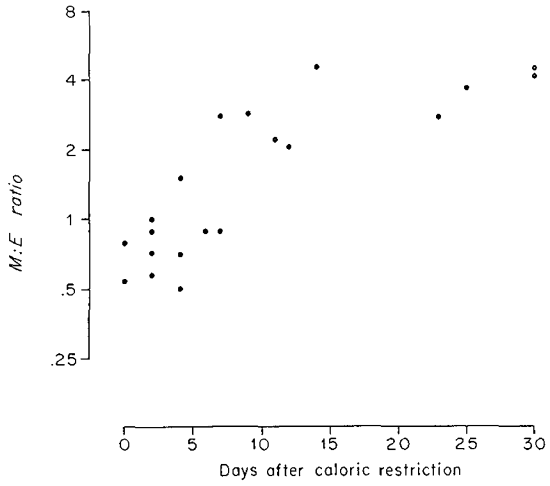

Fig. 6. The myeloid : erythroid ratio in pigs immediately after caloric restriction.

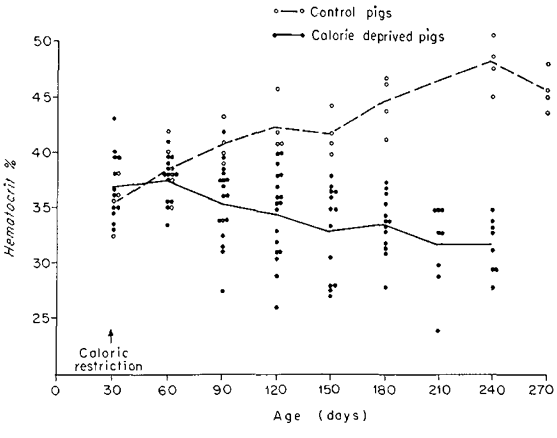

Fig. 7. The hematocrit in control and calorie-deprived pigs. All the values for a stated age are results of study performed at that age, or the closest study within ±15 days of the designated age. The lines unite the mean values at each age.

The alterations in erythropoiesis demonstrated by the iron kinetic studies were reflected in a decrease in the reticulocyte count (fig. 5). Reticulocyte counts were maintained at the high normal levels for the first few days following dietary restriction; by eight to ten days, there was a fall to approximately 2 %. The myeloid : erythroid ratio was increased by ten days following dietary restriction and was similar to that found throughout the period of restricted dietary intake (fig. 6).

The mean value of Cr^{51} total erythrocyte mass prior to dietary restriction was 24.8 ml/kg; after thirty days of restricted caloric intake, a mean value of 25.8 ml/kg was observed (fig. 8). These findings indicated a continuance of erythropoiesis following caloric restriction. Erythropoiesis, although decreased, continued at a rate sufficient to maintain a constant red cell mass in the nongrowing animals during this period.

Hemoglobin and Hematocrit. A slight increase in hemoglobin and hematocrit was observed during the first thirty days on the restricted diet (table VII and fig. 7). After this time, a decline in hemoglobin and hematocrit was observed. These undernourished pigs did not experience the progressive increase in hemoglobin to a level of 14.0 to 16.0 g/100 ml, which was observed in normal, well-nourished animals at eight months of age.

Erythrocyte Indices. During the first thirty days of restricted dietary intake, the mean corpuscular hemoglobin concentration increased to a level higher than that of the controls; this increase was maintained throughout the period of observation. The mean corpuscular volume declined to values somewhat less than those observed in the control animals. A similar change occurred in the mean corpuscular hemoglobin (table VII).

Reticulocytes. The value of 5.6 % reticulocytes is characteristic of a period of rapid growth prior to initiation of dietary restriction. Mean reticulocyte counts of 1 % or less were constant during the period of restricted dietary intake. There was no progressive change in reticulocyte count, nor were there any significant increases or decreases in reticulocyte numbers observed during the period of dietary restriction (table VII).

Serum Iron and Serum Iron-Binding Capacity. Serum iron and percent saturation of the iron-binding protein increased during the two months immediately following initiation of the restricted diet. These values were maintained within the range of normal throughout the observation period (table VII).

Serum Folate Activity. Serum folate activity, measured at seven months of age after six months of dietary restriction, was slightly higher in the restricted animals than in the control animals (table VII).

Serum Proteins. Serum albumin levels were similar to those in the control animals throughout the period of

Table VII. Hematologic observations in calorie-deprived pigs[1]

Age (days)[2]	30	60	90	120	150	180	210	240
Days after caloric restriction	0	30	60	90	120	150	180	210
Weight (kg)	5.7 (18)	6.4 (18)	6.2 (18)	6.3	6.4 (15)	6.9 (12)	7.2 (11)	7.2 (8)
	5.0–6.8	5.0–8.6	4.9–8.0	5.3–8.2	5.2–8.7	5.6–8.7	5.5–8.7	5.4–9.0
Hemoglobin (g/100 ml)	11.1 (12)	12.3 (10)	11.8 (13)	11.3 (18)	10.7 (13)	11.0 (12)	10.5 (8)	10.1 (8)
	10.1–13.3	11.3–13.5	8.9–12.8	8.6–13.1	8.6–12.4	9.4–12.2	8.2–11.7	8.4–11.7
Hematocrit (%)	37.0 (12)	37.7 (15)	35.5 (17)	34.6 (18)	33.0 (13)	33.7 (13)	31.8 (8)	31.8 (8)
	33.0–43.0	33.5–41.0	27.5–42.0	26.0–40.0	27.0–38.0	38.0–37.5	24.0–35.0	28.0–35.0
MCHC (%)	30.4 (12)	33.0 (10)	32.9 (13)	32.6 (18)	33.2 (13)	32.5 (12)	33.3 (8)	33.0 (8)
	28.0–32.0	32.0–34.0	32.0–34.0	30.0–34.0	31.5–34.0	31.0–34.0	32.0–35.0	32.0–34.0
MCH ($\mu\mu$g)	20.5 (12)	17.5 (8)	18.8 (12)	18.6 (18)	20.2 (13)	20.4 (10)	18.8 (4)	19.0 (4)
	17.0–25.0	16.0–20.0	16.0–21.0	16.0–23.0	16.0–25.0	18.0–23.0	17.0–20.0	18.0–21.0
MCV (μl^3)	67.1 (12)	52.4 (8)	57.0 (12)	57.1 (18)	60.8 (13)	62.6 (10)	54.5 (4)	56.8 (4)
	52.0–78.0	49.0–61.0	47.0–63.0	47.0–69.0	49.0–74.0	54.0–70.0	49.0–59.0	53.0–61.0
Reticulocytes (%-corr.)	5.6 (8)	0.7 (15)	0.4 (17)	0.6 (18)	1.0 (12)	0.8 (13)	0.9 (8)	0.2 (4)
	2.8–7.6	0.0–2.1	0.1–2.1	0.1–1.9	0.3–2.2	0.3–1.5	0.4–1.4	0.1–0.4
Serum iron (μg/100 ml)	126.2 (8)	171.2 (6)	174.5 (13)	156.8 (6)	156.0 (10)	154.0 (11)	189.0 (3)	166.6 (8)
	63–184	160–202	144–210	88–218	110–226	98–210	167–213	108–220
Total iron-binding capacity	763.8 (8)	731.0 (6)	650.8 (12)	708.3 (6)	739.6 (10)	794.7 (11)	798.7 (3)	795.5 (8)
	630–894	652–774	418–820	494–860	442–1322	656–1026	720–866	604–952
% saturation of iron-binding capacity	16.9 (8)	23.6 (6)	28.0 (12)	22.0 (6)	21.8 (10)	19.4 (11)	23.7 (3)	20.8 (8)
	7.8–26.9	20.8–28.0	18.6–41.6	17.8–28.7	17.0–32.8	14.4–26.3	23.2–24.6	17.9–27.5
Serum folate ($\mu\mu$g/ml)							10.0 (4)	
							9.0–11.0	
Total proteins (g/100 ml)	5.2 (7)	5.5 (12)	5.4 (13)	5.3 (10)	5.7 (10)	5.7 (11)	5.6 (9)	6.0 (3)
	4.5–6.3	5.0–6.0	4.6–6.0	4.8–5.6	5.4–6.2	4.8–6.4	4.8–6.6	5.9–6.0
Albumin (g/100 ml)	2.7 (7)	2.9 (11)	2.6 (11)	2.7 (10)	2.4 (10)	2.5 (11)	2.6 (9)	2.3 (2)
	2.1–3.3	2.6–3.2	2.1–3.1	2.4–3.0	1.9–2.6	1.9–3.1	1.7–3.4	2.0–2.6

[1] All values listed represent the mean and the range of observations on the number of undernourished pigs indicated by the numbers in parentheses.
[2] All of the results for a stated age are results of study performed at that age or the closest study within ± 15 days of the designated age.

Table VIII. Cr^{51} erythrocyte mass, plasma volume, and total blood volume in calorie-deprived pigs[1]

Age (days)	26 (4)	58 (4)	95 (4)	124 (4)	181–200 (5)	215 (4)	256 (3)
Days after caloric restriction	0	28	65	94	151–170	185	226
Weight (kg)	5.6	6.0	6.2	6.0	6.6	6.8	6.9
	5.3–5.9	5.7–6.5	6.0–6.6	5.2–7.4	5.8–7.6	6.2–8.2	6.3–7.9
Venous hematocrit (%)	38.0	39.4	38.6	38.1	34.4	33.9	30.2
	36.0–40.0	38.0–41.0	37.5–39.0	35.5–40.0	31.0–37.5	32.0–35.5	25.5–34.0
Cr^{51} erythrocyte mass (ml/kg)	24.8	25.8	24.2	20.7	18.2	16.5	14.0
	21.3–28.3	24.2–27.9	22.9–25.1	19.7–22.2	16.4–20.6	15.8–17.3	10.5–15.9
Plasma volume (ml/kg)	47.6	47.1	45.5	39.7	40.5	37.6	37.3
	43.5–52.0	43.9–49.1	44.6–46.4	38.3–42.0	37.6–42.3	36.3–39.1	35.3–41.1
Blood volume (ml/kg)	72.4	72.9	69.7	60.4	58.7	54.1	51.4
	64.8–78.6	68.1–75.6	67.9–71.5	58.2–61.7	56.0–61.0	52.3–55.2	45.8–57.0

[1] The values listed represent the mean and the range of observations at the ages indicated. The number of pigs studied is shown in parentheses.

study. The concentration of serum globulins was less than that in the control animals after ninety days of caloric restriction (table VII).

Cr^{51} RBC Mass. The results of determinations of erythrocyte mass and blood volume in undernourished pigs are listed in table VIII and shown in figure 8. Four animals were studied at 26, 58, 95, and 124 days of age. Different animals maintained on the restricted diet were studied at 180, 200, 215, and 256 days of age. The erythrocyte mass values showed a slight increase at the end of the first month of dietary restriction and then a progressive fall throughout the period of undernutrition. This decrease paralleled the fall in hematocrit.

It can be observed from the values in table VIII that although the changes in hematocrit paralleled those of the red cell mass, a decrease in the total plasma volume occurred during the period of prolonged undernutrition. This reduction resulted in fall of the hematocrit, which was less than that of the total red cell mass.

Iron Kinetic Studies. Iron kinetic studies were performed after approximately five and seven months of dietary restriction. The data from these studies are listed in table VI. Plasma iron turnover at the time of these studies was essentially the same as that observed after ten days of diet restriction. The values were quite similar in all of the animals studied, even though there were considerable variations in plasma iron concentrations.

Iron utilization was studied in four animals after six months of diet restriction. A maximum utilization of 31.0 to 64.0 % of the injected iron was observed in these undernourished animals (fig. 9). The mean transit time of marrow iron, 52.5 hours, was prolonged when compared with findings in the rapidly growing control animals.

Urinary Erythropoietin. Urinary excretion of erythropoietin was assayed in four pigs after seven months of caloric restriction. Erythropoietin activity could not be detected in urine samples collected for 24 or 48 hours. Pooled collections of urine from three pigs, representing a collection period of 110 hours, contained 0.18 units of erythropoietin activity.

Effects of ad libitum *Caloric Intakes by Undernourished Pigs*

After a period of dietary restriction of eight months in two pigs and of seven months in three additional pigs, the animals were offered the previously described diet in amounts exceeding that which would be normally consumed. This *ad libitum* feeding was continued throughout the remaining period of observation. The undernourished animals took increasing amounts of food eagerly, and no adverse effects from the increased food intake were observed. The pigs promptly manifested increased motor activity. All of the undernou-

Table IX. Hematologic observations of calorie-deprived pigs following *ad libitum* caloric intake[1]

Days after diet *ad libitum*[2]	0	30	60	90	150	210
Weight (kg)	7.2 (5)	21.8 (5)	42.6 (5)	63.3 (4)	108.7 (2)	–
	6.5–7.9	18.2–25.7	38.6–48.2	60.1–65.5	103.5–114.0	
Hemoglobin (g/100 ml)	10.2 (5)	11.0 (5)	12.1 (5)	13.4 (4)	13.8 (2)	15.5 (2)
	7.7–11.3	10.5–11.6	11.3–13.5	12.8–14.5	13.3–14.3	14.9–16.2
Hematocrit (%)	31.7 (5)	34.6 (5)	39.5 (5)	42.6 (4)	43.2 (2)	49.2 (2)
	24.0–36.0	32.5–37.5	37.5–42.5	39.5–47.0	41.5–45.0	48.0–50.5
MCHC (%)	32.2 (5)	31.8 (5)	30.7 (5)	31.6 (4)	32.0 (2)	31.5 (2)
	31.0–35.0	30.5–32.5	29.0–32.0	31.0–32.5	32.0–32.0	31.0–32.0
Reticulocytes (%-corr.)	1.7 (5)	5.2 (5)	3.1 (5)	2.1 (4)	2.6 (2)	1.4 (2)
	1.4–2.1	4.5–6.3	2.3–4.0	1.8–2.8	2.0–3.2	1.1–1.7
Serum iron (μg/100 ml)	176.8 (5)	100.8 (5)	95.6 (5)	110.0 (4)	105.5 (2)	136.0 (2)
	167–183	63–172	55–156	94–135	92–119	105–167
Total iron-binding capacity (μg/100 ml)	819.2 (5)	654.0 (5)	628.0 (5)	665.5 (4)	619.0 (2)	696.0 (2)
	694–952	634–666	484–762	620–762	610–628	666–726
% saturation of iron-binding capacity	21.8 (5)	15.4 (5)	14.9 (5)	16.5 (4)	17.0 (2)	19.8 (2)
	19.0–26.3	9.5–26.7	11.0–20.4	15.1–17.9	14.6–19.5	14.5–25.1
Total proteins (g/100 ml)	6.1 (5)	5.5 (5)	5.5 (5)	6.5 (4)	6.5 (2)	7.0 (2)
	5.7–6.4	5.2–5.7	5.2–6.0	6.2–6.8	6.4–6.6	6.6–7.5
Albumin (g/100 ml)	2.4 (5)	2.5 (5)	2.5 (5)	2.6 (4)	3.3 (2)	3.0 (2)
	1.7–2.8	2.1–2.8	2.3–2.9	2.1–2.9	3.0–3.7	2.9–3.1

[1] All values listed represent the mean and the range of observations on the number of undernourished pigs indicated by the number in parentheses.

[2] All of the results for a stated day after *ad libitum* feeding are of studies performed at that day or the closest study within ± 15 days of the designated day. Studies listed on day 0 were performed on that day or within 15 days previously.

rished pigs experienced a prompt and progressive gain in weight when offered the diet *ad libitum* (table IX). The overall gain in body weight paralleled to a striking degree the weight gain experienced by one-month-old control animals.

Erythropoietin. Erythropoietin excretion in urine was measured in three animals between three and eight days and between fifty-five and sixty days following *ad libitum* feedings. Each animal was studied twice during each of these periods. There was a readily detectable increase in erythropoietin excretion within three to eight days of *ad libitum* feedings over that found while the animals were receiving the restricted diet (fig. 10).

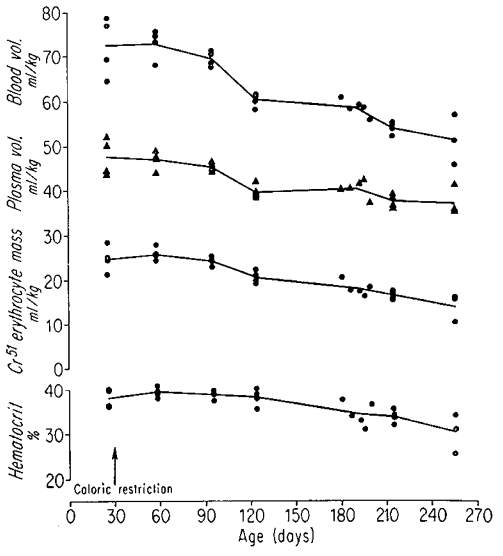

Fig. 8. The Cr^{51} RBC mass, hematocrit, and calculated total blood volume and plasma volume in calorie-deprived pigs. The lines unite the mean values at each age.

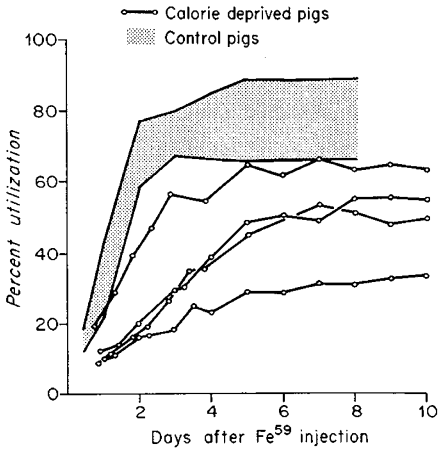

Fig. 9. Iron utilization for erythrocyte production in 4 pigs after 6 months of caloric deprivation and the range of 8 observations of 4 control pigs at 2 and 3 months of age.

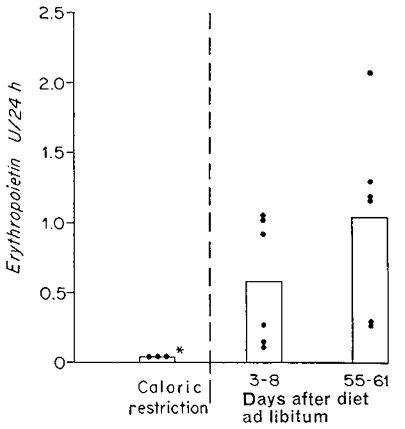

Fig. 10. Urinary erythropoietin excretion following *ad libitum* caloric intake. The columns represent the mean values. * = pooled urine of 3 pigs representing a collection period of 110 hours. Mean erythropoietin activity is 0.04 U/24 h.

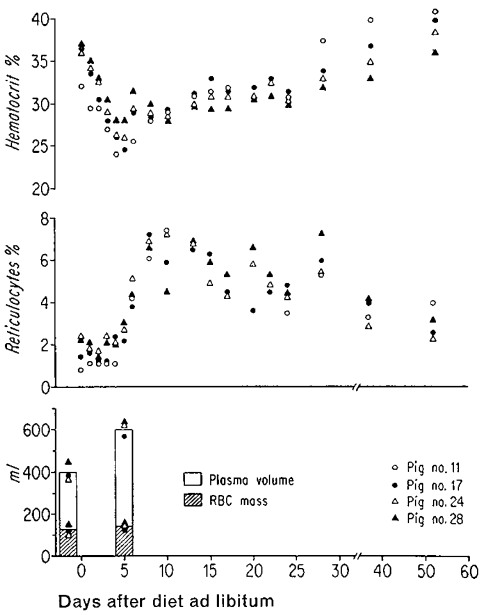

Fig. 11. Hematocrit, reticulocyte count, total erythrocyte mass and the calculated plasma volume in calorie-restricted pigs following *ad libitum* caloric intakes.

Reticulocyte Count. The reticulocyte count was increased six days following *ad libitum* feedings and reached the highest levels at approximately ten days (fig. 11). The relatively high reticulocyte count that is normally found in rapidly growing pigs was maintained for several weeks and showed a gradual decline, paralleling the decreasing rate of growth (table IX). This finding is observed in normal pigs.

Bone Marrow. An increase in erythroid precursors was found in bone marrow soon after initiation of *ad libitum* feedings. There were no cytologic abnormalities observed in marrow during this period of active erythropoiesis.

Iron Kinetics. The results of one iron kinetic study performed at ten days and of three studies performed at sixty days after initiation of the *ad libitum* diet are shown in table VI. The study carried out at ten days showed increases in plasma iron turnover and percent utilization of iron and a decrease in the transit time of marrow iron.

Hematocrit, Hemoglobin, and Total RBC Mass. The hematocrit showed a distinct fall soon after initiation of the *ad libitum* diet. The lowest level was reached in five days and was followed by a progressive increase (fig. 11). After several months, the hemoglobin and hematocrit values reached levels similar to those found in control pigs (table IX). The total red cell mass was determined five days after initiation of the *ad libitum* diet, the time when hematocrit values were at the lowest level (fig. 11). The red cell mass at that time had increased slightly when compared with the value found prior to the change in diet. The total plasma volume was greatly increased at this time. The early fall in hematocrit was thus due to an increase in plasma volume, rather than to any decrease in the circulating erythrocyte mass.

Serum Iron. The rapid growth and active erythropoiesis that occurred after initiating feeding *ad libitum* was associated with a fall in the serum iron concentrations (table IX). Oral iron supplementation was provided during this period, and serum iron was maintained at normal levels during the subsequent period of rapid growth.

Serum Folate Activity. Serum folate activity was measured in four animals before the *ad libitum* diet was offered. The mean value of folate activity at this time was 10.6 $\mu\mu$g/100 ml (range 9.0 to 11.0 $\mu\mu$g/100 ml). The rapid growth and more active erythropoiesis following increased food intakes were accompanied by a decrease in folate activity to a mean of 6.3 $\mu\mu$g/100 ml (range 5.4 to 7.0 $\mu\mu$g/100 ml), when measured after fifteen days of the *ad libitum* diet.

Serum Protein. Total serum protein and serum albumin concentrations did not change significantly during the early period of *ad libitum* diet intake, and, after 90 days, increased slightly (table IX).

Discussion

These studies were designed to define alterations in hematopoiesis resulting from prolonged and controlled caloric deprivation. The prompt reduction in erythropoiesis, which immediately followed caloric restriction, was similar to that which has been described in acute starvation and in short-term protein deficiency in rodents [4, 9, 11, 24]. The degree of reduction in erythropoiesis in such experiments is related to the severity of dietary restriction. When restricted to a degree that results in weight loss, rats show essentially complete cessation of erythropoiesis with a progressive reduction in red cell mass [24]. The pigs in the present study were kept at a relatively constant weight after caloric restriction and were able to maintain a constant red cell mass during the first two months. Therefore, the initial decrease in erythropoiesis was no greater than the decrease in growth rate.

After approximately thirty to sixty days of dietary restriction, the pigs in this study experienced a progressive reduction in total red cell mass. This reduction resulted in a failure to achieve a normal hemoglobin concentration; a definite anemia was present after four to six months of caloric deprivation. Since there was a decrease in the total plasma volume during this same period, the deficit in the total red cell mass was not accurately reflected by the hematocrit determinations.

The anemia observed in the animals after four to six months of caloric deprivation was normochromic and somewhat microcytic. The circulating life span of the erythrocyte has been shown not to be shortened in these pigs [28]. The serial study of the reticulocyte count, iron kinetics, and the myeloid:erythroid ratio in bone marrow showed that the decreased hemoglobin concentration was not accompanied by any compensatory increase in erythropoiesis. It is concluded from these observations that the anemia observed in the pig after prolonged caloric deprivation resulted from a decreased production of erythrocytes and hemoglobin.

Specific nutritional deficiencies compromising erythropoiesis may be encountered in infants who receive severely restricted caloric intake. In the present study, however, it is unlikely that specific nutritional deficiencies were responsible for the anemia observed in the undernourished animals, inasmuch as dietary management resulted in a reduction of intake of vitamins and trace minerals proportional to the reduction in calories and resultant growth. Adequate levels of serum iron and serum folate activity were present throughout the period of dietary restriction. No cytological abnormalities, such as megalobastosis or ring sideroblasts, were observed in bone marrow to suggest the presence of specific nutritional deficiencies that would alter erythropoiesis. Quantitative analysis of

the diet for concentrations of all essential nutrients was not practical with the large amount of diet used over the many months of these observations. It would hardly be possible to accurately estimate the nutritional requirements for the calorically deprived, nongrowing, young animals that were used in these experiments. Further experiments are being designed to define the role of any specific nutritional deficiency causing the anemia observed here.

If a specific nutritional deficiency is not the cause for anemia in the calorically deprived animal, decreased erythrocyte production could result from a lack of stimulation of erythropoiesis. The low concentrations of erythropoietin found in the urine of the undernourished animals support this hypothesis.

The response of the undernourished animals to the *ad libitum* intake of the diet after six to eight months of caloric undernutrition may be compared with the clinical response of undernourished infants to nutritional rehabilitation. The pigs observed in this study were free of any complications at the time that nutritional rehabilitation was initiated and demonstrated an immediate gain in body weight. Increased excretion of erythropoietin in urine was detected within three to eight days of *ad libitum* dietary intake. This was followed by a prompt increase in erythropoiesis. The fall in hematocrit that occurred in these animals soon after the beginning of nutritional rehabilitation has been frequently observed in malnourished infants during recovery. Opinions differ as to whether this fall in hematocrit is a manifestation of hemodilution or the result of compromised erythropoiesis caused by the significant nutritional deficiencies that become manifest upon the initiation of growth in the recovering undernourished subject [1, 3, 16]. Measurement of total red cell mass and calculation of plasma volume during this period demonstrated that, in the pigs, the hematocrit fall was a consequence of a prompt increase in the circulating plasma volume in the presence of a slight increase in total red cell mass.

The rapid growth response of undernourished pigs, when fed a diet *ad libitum*, is similar to that seen in normal pigs of similar size [17]. This period of rapid growth is associated with a high degree of vulnerability to development of specific nutritional deficiencies that are frequently encountered in undernourished infants receiving high caloric intakes during periods of nutritional rehabilitation [12]. It is evident from the experience with the pigs in this study that adequately supplemented diets can support rehabilitative growth performance without development of specific deficiency states.

The hematologic findings in rehabilitated pigs were similar to those seen in younger animals of similar weight. A higher reticulocyte count associated with growth was observed, and hemoglobin levels progressively increased to reach the levels of 14 to 16 g/100 ml observed in control animals. Apparently there was no permanent impairment of erythropoiesis despite the many months of caloric deprivation experienced by the animals.

The low levels of urinary erythropoietin observed in the undernourished animals in the presence of anemia could be the result of an inability of such animals to synthesize this protein because of prolonged nutritional deprivation. Although this possibility cannot be excluded, the maintenance of stable levels of total plasma protein and the prompt response in erythropoietin excretion when the animals were fed *ad libitum* make it unlikely. Probably erythropoietin excretion does not increase in response to a low hemoglobin concentration, because such concentrations of hemoglobin may be sufficient to meet the needs of oxygen transport in animals under conditions of restricted caloric intake. It has been suggested by McCance *et al.* [19], who have reported decreased oxygen consumption and lowered body temperature in pigs managed similarly, that starved animals may have decreased tissue oxygen need.

Similar observations have been reported in severely marasmic infants. Mönckeberg *et al.* have demonstrated decreased oxygen consumption [22], reduced basal metabolic rate, reduced I^{131} uptake by the thyroid gland, and low BEI levels [6] in such infants. It would thus appear that caloric undernutrition can induce a hypometabolic state with a resultant decrease in tissue oxygen needs, a condition that results in decreased stimulation of erythropoiesis. Anemia that develops in the face of caloric deprivation would then appear to be similar to that seen in other hypometabolic states, such as hypothyroidism and hypopituitarism.

Summary

The effects of prolonged caloric deprivation on erythropoiesis have been studied in the young pig. Animals were maintained on a restricted diet that prohibited growth for periods as long as ten months. Erythropoiesis was evaluated with serial measurement of hematocrit, hemoglobin mass and concentration, reticulocyte counts, iron kinetic studies, and urinary erythropoietin excretion.

Immediately after being fed the restricted diet, the young pigs exhibited a distinct reduction in erythropoiesis. A gradual decline in hematocrit and hemoglobin concentrations developed after several weeks of caloric restriction. These changes were accompanied by proportional reductions in total erythrocyte mass

and plasma iron turnover. Excretion of erythropoietin in urine was markedly reduced.

No evidence of specific nutritional deficiencies developed in the diet-restricted animals. It is postulated that the reduced hemoglobin mass may represent an adaptive response to reduced needs for oxygen transport in the undernourished animal that is not a result of nutritional deficiency.

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