Studies on Vitamin E and Selenium Deficiency in Young Pigs
III. Effect on Kinetics of Erythrocyte Production and Destruction

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ABSTRACT

The effect of vitamin E and/or selenium deficiency on the kinetics of erythrocyte production and destruction has been investigated in swine. The plasma iron turnover rate, ^{59}Fe incorporation into newly formed red cells as well as the ^{51}Cr apparent red cell half-life, were not found to be significantly affected by either vitamin E deficiency, selenium deficiency or deficiency of both, as compared to replete animals. The results of this study suggest that vitamin E is not a limiting factor for normal erythropoïesis in young growing pigs. Erythropoïesis appeared, however, to be slightly decreased in selenium deficient pigs and will need to be further investigated.

RÉSUMÉ

Cette expérience visait à étudier l'effet de déficiences simultanées ou non en vitamine E et en sélénium sur la cinétique de la production et de la destruction des hématies, chez le porc. La période biologique du fer plasmatique, l'incorporation du ^{59}Fe dans les hématies nouvellement formées et la demi-vie apparente des hématies marquées au ^{51}Cr ne subirent pas d'altération appréciable, lors de déficiences simultanées ou non en vitamine E et en sélénium, par comparaison avec les sujets témoins. Les résultats de cette expérience laissent supposer qu'une déficience en vitamine E n'affecte pas l'érythropoïèse, chez le porcelet en croissance. Une déficience en sélénium semble cependant causer une légère diminution de l'érythropoïèse; cette observation justifierait une recherche plus approfondie.

INTRODUCTION

Several investigators (14, 25, 28) have reported an anemic condition to accompany vitamin E deficiency in swine but the pathogenesis of the anemia has not been further investigated. Nafstad (25) and Baustad and Nafstad (1) considered the vitamin E deficiency associated anemia to be the result of inadequate erythroid production, based on the results of bone marrow morphological studies conducted in vitamin E deficient pigs. In human infants, the vitamin E responsive anemia of prematurity (21, 23, 30, 31) appears to be hemolytic in nature as evidenced by Ritchie *et al* (34) who found strikingly short erythrocyte survival times by means of $DF^{32}P$ and ^{51}Cr labels. In primates, the vitamin E responsive anemia (5, 6) appears to be mainly the result of ineffective erythropoïesis (3, 8, 33) although the survival of ^{51}Cr tagged red cells was also found to be moderately shortened (22).

The objective of this study was to determine the pathogenesis of the reported vitamin E deficiency associated anemia in swine by means of studying the kinetics of erythrocyte production and destruction. Furthermore, selenium deficiency was also considered as a possible cause of hemolytic

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anemia, since selenium was recently found to be a component of the enzyme glutathione peroxidase in erythrocytes of rats (35), chicks (27), cattle (9) and sheep (29).

MATERIALS AND METHODS

The first group of 24 pigs previously described (10) were used in the present study when they reached nine weeks of age.

Radiochromium (^{51}Cr) and radioiron (^{59}Fe) were used in a combined procedure to study erythrocyte production and destruction (41). For each animal, 15 ml of whole blood was collected in 1.5 ml ACD solution[1] and the red cells spun down at 2000 rpm for ten minutes at 20°C.[2] The supernatant plasma was removed and 7 ml was added to 25 microcuries ^{59}Fe as ferrous citrate[3] (specific activity 12.3 mCi/mg Fe). From the packed red cells, 4 ml were diluted with 10 ml normal saline[4] and labelled with 50 microcuries ^{51}Cr as sodium chromate[5] (specific activity, 105.8 mCi/mg Cr). Both the plasma and red cell isotope mixtures were incubated at 37°C for 30 minutes. Labelled red cells were then washed three times with normal saline to remove excess ^{51}Cr. A small aliquot (0.1 ml) from both the labelled red cells and plasma was kept for each animal as a standard for the calculation of the total radioactivity injected. For each pig, 6 ml of its own ^{59}Fe-labelled plasma and 11 ml of its own ^{51}Cr-labelled red cell suspension in two different 12 ml plastic syringes were injected into the ear veins on day zero (i.e. nine weeks of age).

Ten minutes after the ^{51}Cr-labelled red cell injection, a 3 ml blood sample was collected in EDTA, the PCV determined and 2 ml whole blood transferred into a gamma counting vial. The ^{51}Cr activity in that sample was used for the red cell mass estimation which was obtained by the formula:

$$\frac{^{51}Cr \text{ activity/ml labelled red blood cells x ml injected x PCV corrected for trapped plasma}}{^{51}Cr \text{ activity/ml postinjection sample x 100}}$$

A factor of 0.97 was used to correct for the amount of plasma trapped in the packed cell fraction (38). No corrections were made for F cells factor (37).

At three, six, ten, 15, 30, 60, 120, 180 and 240 minutes after the ^{59}Fe labelled plasma injection, 2 ml blood samples were collected in EDTA, centrifuged at 2000 rpm for ten minutes[6] at 4°C and 1 ml supernatant plasma was transferred into a gamma counting vial. Concurrently with the six minute sample, serum was obtained for iron determination to be used in the iron turnover calculation. The ^{59}Fe activity in plasma was plotted against time on semi-log paper to obtain the iron disappearance curve. The first part of the curve was extrapolated to zero-time using the least squares method to estimate the ^{59}Fe activity present at zero-time, which was used in plasma volume determination. Similarly, using the least squares method, the half-time (T ½) of the second component (C_2) of the iron disappearance curve was calculated and used in the iron turnover calculation. The first component (C_1) was determined by subtracting the extrapolated values of C_2 from the observed values prior to C_2. The plasma volume in ml was obtained by the formula:

$$\frac{^{59}Fe \text{ activity/ml labelled plasma x ml injected}}{^{59}Fe \text{ activity/ml postinjection sample (zero-time)}}$$

The formula given by Huff *et al* (19) was used to calculate the plasma iron turnover in milligrams per kilogram per day:

$$\frac{0.693 \text{ x plasma iron (mg/ml) x plasma volume (ml) x 24}}{^{59}Fe \text{ T } \tfrac{1}{2} \text{ (hours) x body weight (kg)}}$$

Starting on day 1 (24 hours after the isotope injections) and daily thereafter until day 8, a 3 ml blood sample was collected in EDTA from each animal and 2 ml whole blood was transferred into a

[1]Acid Citrate Dextrose Solution, Abbott Laboratories, Toronto, Ontario.

[2]Sorval RC-3 Refrigerated Centrifuge giving R.C.F. of 1120 x gravity.

[3]Ferrous Citrate Fe^{59} Injection, Charles E. Frosst & Co., Toronto, Ontario.

[4]Normal Saline, 0.9%, Abbott Laboratories, Toronto, Ontario.

[5]Sodium Chromate Cr 51 Injection, Charles E. Frosst & Co., Toronto, Ontario.

[6]R.C.F. of 1120 x gravity.

gamma counting vial after a PCV determination which was corrected for trapped plasma. On day 8, the procedure used to label red cells with ^{51}Cr was repeated and a second estimation of the red cell mass was obtained for each pig. This second red cell mass determination was to be used in the correction of the ^{51}Cr activity in red cells for the increase in the red cell mass as the animals grew during the eight day period of study. A linear increase in the red cell mass was assumed between day 0 and day 8.

To estimate the ^{51}Cr apparent red cell half-life, the ^{51}Cr activity present in red cells between day 1 and day 8 was corrected to 1 ml packed red cells and for the increase in the red cell mass. The percentage ^{51}Cr activity left on each day (activity on day 1 being taken as 100%) was plotted against time on semilog paper and the least squares method used to calculate the half-life (T ½).

The following formula was applied to correct activity for increase in red cell mass:

$$RC^{51}Cr\ Ac = RC^{51}Cr\ At \times \frac{RCMt}{RCMo}$$

$RC^{51}Cr$ Ac is red cell ^{51}Cr activity corrected
$RC^{51}Cr$ At is red cell ^{51}Cr activity at time t
RCMt is the calculated red cell mass at time t
RCMo is the red cell mass on day 0

On the basis of the calculated red cell mass and the ^{59}Fe activity present in circulating red cells between day 1 and day 8, the percentage iron utilization was calculated by the formula (4):

$$\frac{^{59}Fe\ activity/ml\ red\ cells \times red\ cell\ mass\ (ml) \times 100}{total\ ^{59}Fe\ activity\ injected}$$

Radioactivity from ^{59}Fe and ^{51}Cr was determined in a nuclear Chicago Auto Gamma Counter[7] equipped with a pulse-height analyser to differentiate the emission from radioiron and radiochromium. The activity of each sample was counted for a fixed period of ten minutes and corrected for background and for cross-over counting in the two channels. All the samples for a given animal were counted on the same day, hence nuclear decay corrections were not applicable.

The statistical analysis of the data was performed by analysis of variance according to the plan previously described (10).

RESULTS

The plasma iron turnover, ^{51}Cr apparent red cell half-life and red cell ^{59}Fe incorporation results are presented in Table I where the F-values derived from the analysis of variance, are given. The data used in the calculation of the plasma iron turnover rates and in the correction of red cell ^{51}Cr activity for increase in red cell mass for determination of red cell half-lives, are given in Table II. Since no significant differences between the four different treatments (+E+Se, +E–Se, –E+Se, –E–Se) were detected by analysis of variance, the mean disappearance curve of ^{59}Fe from plasma (Fig. 1) and ^{51}Cr labelled red cells from circulation (Fig. 2), as well as the mean red cell ^{59}Fe incorporation curve (Fig. 3), were calculated from the individual data of the 24 pigs and plotted with the 95% confidence intervals.

From the individual plasma volume and red cell mass determinations for the 24 pigs, the following mean values (± one

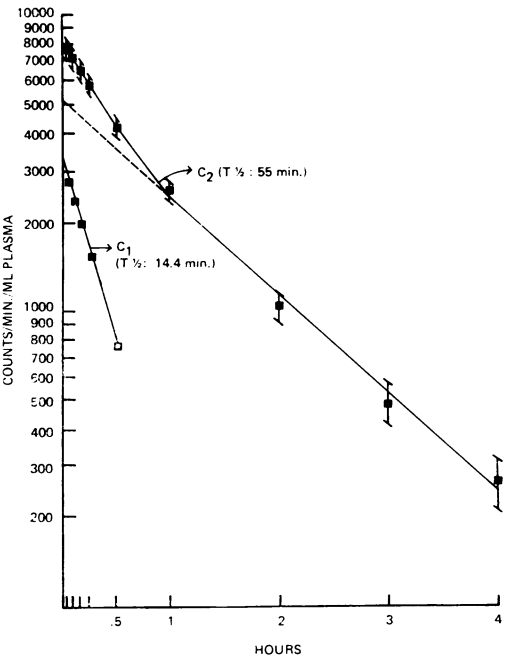

Fig. 1. Mean disappearance of ^{59}Fe from plasma of the 24 pigs (brackets indicate 95% confidence intervals). C_1: First component, C_2: Second component.

[7]Nuclear Chicago, Des Plaines, Illinois.

TABLE I. The Effect of Vitamin E and/or Selenium Deficiency in Swine on the Kinetics of Erythrocyte Production and Destruction

Parameter	Treatment				F-Values	
	+ E + Se	+ E − Se	− E + Se	− E − Se		
	1.52	1.40	1.48	0.87		
	1.07	1.15	1.09	1.56		
Plasma Iron	2.18	1.31	0.97	1.13	Vitamin E	0.10[a]
Turnover Rate	1.17	1.20	1.00	0.83	Selenium	0.55[a]
(mg/kg/day)	1.37	1.31	2.60	2.62	E x Se	0.00[a]
	1.33	1.40	1.80	1.18		
Mean	1.44	1.30	1.49	1.37		
	84.9	73.4	77.6	85.6		
	82.3	81.7	84.0	74.4		
^{59}Fe Incorporation	67.6	73.5	74.3	76.1	Vitamin E	0.03[a]
Into Newly Formed	79.0	78.8	79.2	79.5	Selenium	0.70[a]
Red Cells (%)	84.2	82.6	82.1	81.9	E x Se	0.11[a]
	76.1	80.2	70.7	84.7		
Mean	79.0	80.0	78.0	80.4		
	11.16	10.27	13.59	9.54		
	10.00	10.10	11.08	11.17		
^{51}Cr Apparent Red	10.08	15.84	11.28	13.03	Vitamin E	0.55[a]
Cell Half-Life	13.88	10.96	13.81	14.81	Selenium	0.65[a]
(days)	12.84	10.66	9.45	10.68	E x Se	0.00[a]
	8.52	10.34	8.81	11.02		
Mean	11.08	11.36	11.34	11.71		

[a]Not significant ($F_{0.05}$; 1, 15 degrees of freedom = 4.54)

standard deviation) were calculated:

Plasma volume: 51.0 ± 4.4 ml/kg
Red cell mass: 19.9 ± 1.5 ml/kg
Blood volume: 70.9 ± 4.4 ml/kg
Daily red cell mass increase: 10.0 ± 3.9 ml

The mean (± one standard deviation) body weight at the time of these determinations was 16.3 ± 2.9 kg.

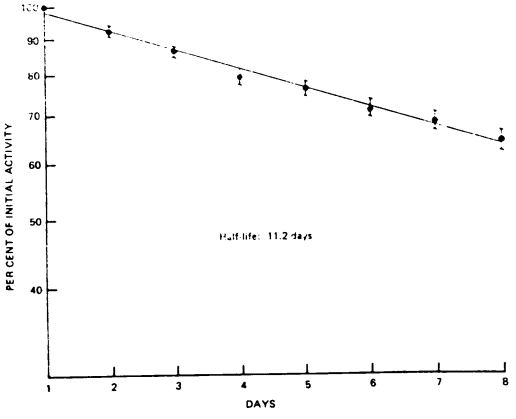

Fig. 2. Mean disappearance of ^{51}Cr-labelled red cells of the 24 pigs (brackets indicate 95% confidence intervals).

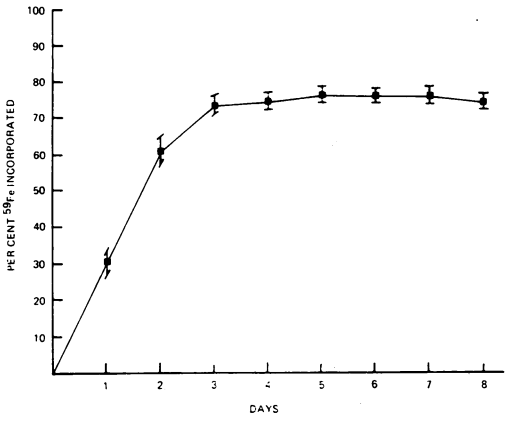

Fig. 3. Mean ^{59}Fe uptake into newly formed erythrocytes of the 24 pigs (brackets indicate 95% confidence intervals).

DISCUSSION

The plasma iron turnover rate, ^{59}Fe incorporation into newly formed red cells as well as the ^{51}Cr apparent red cell half-life, were not found to be significantly affected by either vitamin E deficiency, selenium deficiency or deficiency of both, as compared to replete animals.

TABLE II. Data Used for Plasma Iron Turnover Calculations and for ^{51}Cr Activity Corrections for Red Cell Mass Increase

Treatment	Animal #	Body Weight (kg)	Plasma Volume (ml)	Plasma Iron (times 10^{-5} mg/ml)	^{59}Fe $T_{\frac{1}{2}}$ (min.)	Red Cell Mass on Day 0 (ml)	Red Cell Mass on Day 8 (ml)	Daily Red Cell Mass Increase (ml)
	53	11.3	619.8	123	43.8	241.6	323.3	10.2
	54	13.6	654.5	118	52.8	258.8	314.8	7.0
+ E + Se	56	18.6	947.6	186	43.2	372.5	413.8	5.2
	64	20.4	922.3	123	47.4	438.7	472.9	4.3
	67	23.6	1002.9	162	50.4	567.5	682.5	14.4
	71	12.2	626.9	145	55.8	249.8	281.6	4.0
	12	14.5	807.0	114	45.0	239.8	312.0	7.8
	57	15.4	824.7	105	48.6	287.6	401.9	14.3
+ E − Se	61	15.9	799.5	100	48.6	317.5	392.7	9.4
	59	17.7	840.2	141	55.8	337.2	370.3	4.1
	69	16.3	879.1	155	63.6	350.5	465.9	14.4
	74	16.3	931.6	145	58.8	332.0	462.7	16.3
	51	20.4	1041.6	159	54.6	385.1	468.9	10.5
	55	19.1	945.5	109	49.2	417.1	499.5	10.3
− E + Se	58	19.1	851.3	127	58.8	342.0	456.6	14.3
	66	16.3	740.2	141	63.6	296.2	353.5	7.2
	68	11.3	692.6	161	40.2	210.2	329.1	14.9
	72	13.6	738.6	177	53.4	259.3	342.9	10.4
	60	13.6	662.8	82	46.2	293.3	340.2	5.9
	62	15.0	830.2	136	48.6	304.3	380.5	9.5
− E − Se	63	16.3	795.2	123	52.8	305.2	369.6	8.1
	65	16.8	789.1	109	61.8	312.9	394.2	10.2
	70	14.1	776.3	202	42.6	280.5	377.4	12.1
	73	18.6	963.7	141	61.8	381.7	504.6	15.4

Two exponential rates of clearance (C_1 and C_2) of ^{59}Fe from plasma were observed for each of the 24 pigs and are illustrated by the mean disappearance curve in Fig. 1. The half-time of C_1 and C_2 were respectively 14.4 and 55 minutes. Similarly, Jensen *et al* (20) observed two rates of clearance of ^{59}Fe from plasma of three out of 18 normal growing pigs and demonstrated that the first clearance rate was the result of radioiron uptake by the liver. Since they started to follow the disappearance of ^{59}Fe from plasma only 15 minutes after radioiron injection, they probably missed part of the first clearance rate in the 15 pigs from which they observed a single exponential clearance rate. They (20) reported a mean value of 71.4 minutes for C_2 and 1.11 mg/kg/day for the mean plasma iron turnover as compared to 55.2 minutes and 1.40 mg/kg/day obtained in the present study. More recently, Gipp *et al* (12) reported a half-time of 49.8 minutes for ^{59}Fe disappearance from plasma in control pigs.

It is interesting to note that, although the differences are not statistically significant, the plasma iron turnover rates in selenium deficient animals tend to be smaller than in selenium replete animals (Table I). This slight decrease of the plasma iron turnover rate in selenium deficient pigs, possibly reflects a state of decreased erythropoïesis which goes along with our observation of increased myeloid:erythroid ratios in selenium deficient animals (10).

The mean ^{59}Fe incorporation into newly formed red cells (Fig. 3) indicates that the daily increment of ^{59}Fe labelled red cells began to decrease by the third day following the injection of ^{59}Fe, and further increase in isotope almost ceased after the fourth day. Jensen *et al* (20) reported a similar ^{59}Fe uptake curve suggesting an interval of about four days for erythrocyte production in swine which is similar to man (42) and calf (40).

From the mean disappearance of ^{51}Cr-labelled red cells as illustrated in Fig. 2, the calculated half-life was 11.17 days as compared to a mean (±one standard deviation) of 11.37 ± 1.92 days as calculated from the 24 individual half-lives. The values reported in the literature for ^{51}Cr apparent red cell half-life in growing swine are quite variable. Bush *et al* (2) found a mean half-life of 17 days in normal growing swine which had received ^{51}Cr-labelled

homologous cells and Talbot and Swanson (39) reported the mean 50% survival time of ^{51}Cr-labelled porcine erythrocytes to be 28 days when autologous injections of cells were used and 13.8 days when homologous injections of cells were used.

It is surprising that although vitamin E deficiency causes a defect in red cell antioxidant protection, as evidenced mainly by the increase of red cell lipid peroxides but also to some extent by the increased lytic sensitivity of erythrocytes in vitamin E deficient animals (11), the ^{51}Cr apparent red cell half-lives were not found to be shortened in the chronically vitamin E deficient animals. It is, however, possible that a sudden oxidant attack or a stress factor such as rancid feed will further deplete the antioxidant protection and lead to an acute episode of intravascular hemolysis of the hypersusceptible red cells.

From the present findings of normal kinetics of erythrocyte production and destruction in vitamin E deficient pigs and the absence of a demonstrable anemia (10), it is concluded that vitamin E is not a limiting factor for normal erythropoiesis in growing pigs. The direct cause to effect relationship between vitamin E deficiency and anemia is not, however, a universally accepted fact. The findings of several investigators (13, 15, 32) tend to indicate that the anemia in vitamin E deficient premature human infants is not basically related to vitamin E deficiency. Experimental tocopherol depletion in men (16, 17, 18) was not found to cause anemia and in human malabsorption syndromes associated with subnormal plasma tocopherol levels, anemia is not usually a prominent feature and is difficult to relate to vitamin E deficiency (36). It has been shown in rats that vitamin E functions as a regulator of heme synthesis at one of the rate limiting steps in thte pathway to heme but such a biochemical function of vitamin E does not appear to cause a demonstrable anemia in the vitamin E deficient rats (24, 26). In primates where anemia in relation to vitamin E deficiency has been most thoroughly studied (3, 5, 6, 8, 22, 33), a severe anemia is reported to occur after a variable (five to 30 months) tocopherol deprivation period but the variability of onset is unexplained and seems to be independent of the serum vitamin E concentration because vitamin E may be practically undetectable in blood serum for two years before the onset of disease (7).

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