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Hematologic Manifestations of Lysine Deficiency in Swine.* (23919)

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Lysine deficiency has been produced experimentally in rats and swine by feeding diets deficient in lysine(1), by feeding diets containing "deaminized casein" as a source of protein(2,3), and by feeding diets low in lysine to which hexahomoserine (6-hydroxy-DL-norleucine), a lysine antagonist, was added (4-7). Under all of these conditions anemia was observed. In rats Smith and Stohlman (3) found a macrocytic megaloblastic type of anemia, whereas Gillespie, Neuberger and Webster(1) reported that the anemia was hypochromic. The morphologic characteristics of the anemia in swine have not been recorded(6).

The purpose of this report is to describe the hematologic manifestations of lysine deficiency in swine.

Methods. Twenty-three swine of the Chester-White breed were housed in individual cages and were started on the experimental diet at 3 weeks of age. The diet consisted of yellow corn, 71.1%; cottonseed meal, 25%; salt mixture 3.8%; terramycin feed supplement (Bi-Con TM 10), 0.1%; and a B vitamin mixture, 0.02%. The diet was fed in an amount of 35 g/kg of body weight/day. In addition, all animals received 10 mg of choline chloride/kg of body weight daily, and 3000 units of Vit. A, 600 units of Vit. D, 1 mg of Vit. E, and 1 mg of Vit. K/kg of body weight by mouth each week. The salt mixture consisted of calcium carbonate, 1.5 g; calcium phosphate, 1.4 g; sodium chloride, 0.5 g; iron citrate, 0.3 g; manganese sulfate, 0.04 g; zinc chloride, 0.03 g; copper carbonate, 0.005 g; and cobalt carbonate, 0.0006 g. The B vitamin mixture consisted of the following (mg): thiamine hydrochloride, 0.25; riboflavin, 0.12; nicotinic acid, 1.20; pyridoxine hydrochloride, 0.20; calcium pantothenate, 0.50; inositol, 0.20; para-aminobenzoic acid, 0.10; biotin, 0.10; pteroylglutamic acid, 0.10; and cobalamine, 0.01. Five pigs were given hexahomoserine (6-hydroxy-DL-norleucine) after they had been taking the experimental diet for 112 days. The hexahomoserine was added to the diet in an amount of 0.5% of the diet. The control animals were fed a casein, sucrose, and lard diet which has been described previously(8). Plasma iron was determined by the method of Hamilton, *et al.*(9). Plasma copper was determined by the method of Gubler, *et al.*(10). Serum protein estimations were performed by a modification(11) of the method described by Weichselbaum(12).

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TABLE I. Summary of Observations.

Determination	Control group	Corn diet	Corn diet + hexahomoserine
No. animals	18	18	5
Red cell count, $\times 10^6$ per mm^3	7.96± .74 (6.5– 9.1)	6.31± .74 (4.4– 7.2)	3.90 (2.8– 4.7)
Hemoglobin, g %	14.6 ± .80 (13.6– 16.9)	10.5 ± 1.42 (7.4– 12.2)	6.8 (4.9– 8.5)
Vol packed red cells, ml/100 ml	45 ± 2.7 (40 – 50)	31 ± 3.2 (23 – 36)	21 (15 – 27)
Mean corpuscular vol, μ^3	56 ± 4.1 (47 – 63)	50 ± 3.5 (46 – 57)	54 (52 – 58)
Mean corpuscular hemoglobin, $\mu\mu g$	18 ± 1.9 (15 – 21)	17 ± 1.5 (14 – 20)	17 (17 – 18)
Mean corpusc. hemoglobin conc., %	33 ± 1.6 (30 – 35)	33 ± 1.5 (31 – 35)	32 (31 – 33)
Reticulocytes, %	1.1 ± .72 (0 – 6)	2.8 ± 1.58 (1 – 8)	.9 (0 – 2)
Leukocyte count, $\times 10^3/mm^3$	13.8 ± 6.18 (11 – 26)	15.3 ± 7.17 (8 – 33)	9.3 (6 – 12)
Plasma iron, μg %	169 ±38.8 (102 –480)	160 ±34.5 (116 –212)	156 (61 –290)
Plasma copper, μg %	206 ±26.3 (113 –288)	148 ±16.2 (126 –177)	157 (84 –185)
Total serum protein, g %	5.49± .17 (5.3– 6.0)	4.74± .31 (4.2– 5.3)	3.82 (3.3– 4.2)
Serum albumin, g %	2.92± .30 (2.4– 3.2)	1.63± .42 (1.1– 2.1)	1.40 (1.3– 1.5)

Values refer to mean ± 1 stand. dev. Figures in parentheses refer to range.

Hemoglobin was determined as cyanmethemoglobin by the use of a photometer.

Results. The hematologic observations are summarized in Table I. All of the animals were anemic after 4 weeks on the corn diet. The anemia was mild in degree and constant in severity with little tendency to progress with time (Fig. 1 and 2). The morphology of the erythrocytes was within normal limits, as were the reticulocyte, leukocyte and plasma iron values. A moderate but significant degree of hypocupremia ($t = 4.7$; $P<.001$) and hypoproteinemia ($t = 6.9$; $P<.001$) were present. The hypoproteinemia was due to a reduction in serum albumin.

Bone marrow differential counts on smears obtained from aspirated material were within normal limits. Histologic sections of sternal, rib, vertebral and femoral bone marrow, liver, lymph node, spleen, heart and skeletal muscle were prepared from 3 untreated pigs and from one pig treated with lysine. Examination of the bone marrow sections revealed normal cellularity. No significant histologic abnormalities were noted in the other tissues examined.

Six of the anemic pigs were treated with casein by substituting purified casein for the 25% cottonseed meal in the diet. In all 6 of the pigs this dietary alteration was followed by reticulocytosis (6 to 18%) and alleviation

TABLE II. Hematologic Response to Administration of Casein and Lysine.

	Before therapy			After therapy			
				Retic. max		V.P.R.C.	
Pig. No.	V.P.R.C., ml/100 ml	Retic., %	Therapy	Day	%	Day	ml/100 ml
15–17	30	2	Casein	6	6	45	45
19	24	3	"	4	10	46	46
32	33	2	"	5	18	20	46
33	35	2	"	4	18	12	44
34	33	1	"	5	16	12	43
35	34	2	"	6	18	12	43
36	31	2	Lysine	3	12	14	39
37	34	2	"	4	12	11	39

V.P.R.C. refers to vol of packed red cells. Retic. max refers to reticulocyte maximum.

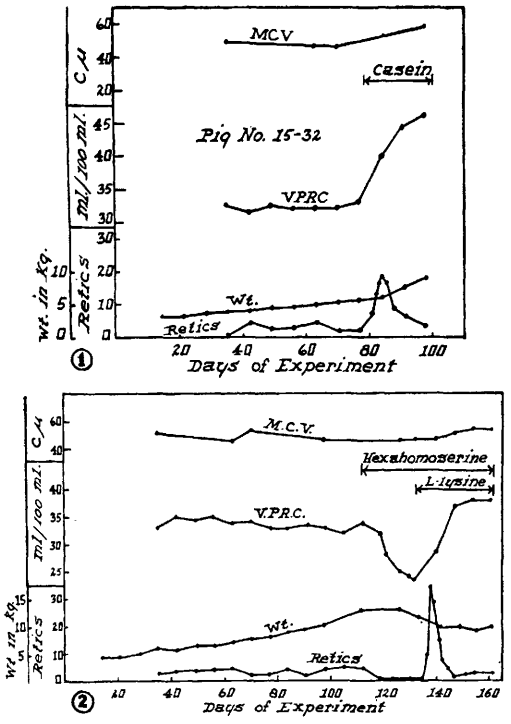

FIG. 1. Hematologic response to substitution of casein for cottonseed meal.

FIG. 2. Development of severe anemia following administration of hexahomoserine and response to administration of L-lysine.

M.C.V., mean corpuscular vol; V.P.R.C., vol of packed red cells; Wt, body wt; retics, reticulocytes.

of the anemia (Table II). The hematologic response of one such pig is shown in detail in Fig. 1.

Two pigs were treated with lysine. Two g of L-lysine were added to each 100 g of diet. In both instances this was followed by reticulocytosis and an increase in the volume of packed red cells (V.P.R.C.) (Table II). However, in neither of the 2 pigs did the V.P.R.C. reach levels as high as following substitution of the cottonseed meal with casein.

Five pigs were given hexahomoserine after they had been taking the corn diet for 112 days. In all 5 pigs a severe normocytic, normochromic anemia and a mild to moderate degree of leukopenia developed (Table I). The plasma iron levels remained within normal limits.

Histologic sections of the tissues were prepared from 4 of the severely anemic animals given hexahomoserine. The bone marrow revealed normal cellularity. No significant abnormalities were noted in the other tissues examined. Bone marrow differential counts on thin smears obtained from aspirated material were within normal limits.

One of the pigs given hexahomoserine was treated with lysine. Two g of L-lysine were added to each 100 g of diet and the hexahomoserine was continued. Administration of lysine was followed by reticulocytosis (maximum, 35%) and complete alleviation of the anemia (Fig. 2).

Discussion. It is evident from the data presented that lysine is essential for normal erythropoiesis in swine. The animals fed the corn diet developed anemia and, when hexahomoserine was added, the volume of packed red cells decreased rapidly. Addition of lysine to the diet, either in the presence or absence of hexahomoserine, was followed by reticulocytosis and rapid alleviation of the anemia.

The anemia observed was normocytic and normochromic and was unaccompanied by reticulocytosis and is thus similar morphologically to that observed in protein-deficient (13), tryptophane-deficient(14), and in niacin-deficient(15) swine.

Chronic protein-deficiency in pigs is accompanied by a moderate degree of hypocupremia and hypoferremia(13). Both of these elements are bound to specific proteins under normal conditions. Copper is present in plasma as a copper protein, ceruloplasmin. Iron is bound to the iron-transport protein, transferrin. It is reasonable to suppose that, in protein deficiency, hypocupremia and hypoferremia are the consequence of impaired synthesis of the specific binding proteins. In the animals deficient in lysine the degree of hypocupremia was comparable to that observed in protein-deficiency. From this it would appear that lysine is essential for ceruloplasmin synthesis. The fact that hypoferremia was not present in the lysine-deficient animals suggests that lysine is not of critical importance in synthesis of transferrin.

The earlier studies on the morphology of red cells in lysine deficient rats were limited to an estimation of the color index. Gillespie *et al.*(1) reported that the color index was

decreased; Smith and Stohlman(3) found the index to be increased. This discrepancy may have been due to inaccuracies in hemoglobinometry. However, the latter workers also observed a great variety of cellular abnormalities in the blood, such as anisocytosis, poikilocytosis, polychromatophilia, Howell-Jolly bodies, nucleated red cells, myeloblasts and myelocytes. Such abnormalities were not observed in the blood of the anemic swine. In the rats the anemia was accompanied by a reticulocytosis of 5 to 25%. The explanation for these differences between the 2 species is not obvious.

Summary. 1. Twenty-three swine were fed a diet deficient in lysine. In addition, 5 swine were given hexahomoserine (6-hydroxy-DL-norleucine), a lysine antagonist. 2. All animals developed a normocytic, normochromic anemia, which was accompanied by hypoalbuminemia and hypocupremia. No alterations from the normal were noted in the plasma iron concentration or in bone marrow morphology. The anemia responded rapidly to administration of lysine. 3. The data presented indicate that lysine is essential for normal erythropoiesis in swine.

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