Hematologic Response of Pyridoxine Deficient Swine to Pyridoxine.* (28571)

G. E. CARTWRIGHT, DORIS KURTH AND M. M. WINTROBE
Department of Medicine, University of Utah College of Medicine, Salt Lake City

Horrigan(1) has described an unidentified erythropoietic substance in liver, which is active in some patients with "pyridoxine-responsive" anemia and which presumably is involved in metabolic pathways of tryptophane and pyridoxine. In our earlier experiments in pyridoxine-deficient swine(2) we noted that the anemia which developed did not respond completely to administration of pyridoxine. Similar observations were made by others in dogs(3). However, at the time of these earlier studies biotin, folic acid and Vit. B_{12} were not available in crystalline form and were not included in the vitamin supplements.

The purpose of this investigation was to determine if the anemia of pyridoxine-deficient swine fed a purified diet supplemented with all of the currently recognized "B" vitamins except pyridoxine would respond completely to administration of pyridoxine. The hematologic values attained following administration of pyridoxine are compared with values in a control group which received the same diet as the deficient animals, except that pyridoxine was given from the beginning of the experiment. The hematologic values obtained in the control group are compared with the values in swine fed the purified diet and supplemented with condensed fish solubles to determine if unknown nutrient factors would produce an additional increase in blood values.

Methods. Ten swine of the Chester-White breed were housed in individual cages and started on the experimental diet at 24 days of age. The basal diet was fed in amounts of 36.4 g (152 calories)/kg of body wt/day and consisted of purified casein (Sheffield devitaminized), 26.1%; sucrose, 57.7%; lard, 11.0%; and swine salt mixture No. 3(4), 5.2%. The following vitamins, in crystalline form, were placed in capsules and administered 3 times a week (mg/kg body wt/day): 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 cyanocobalamin, 0.10. In addition all of the animals received the following supplements: choline chloride, 10/mg/kg/day; Vit. A, 3,000 units/kg/wk; Vit. D, 600 units/kg/wk; Vit. E, 1 mg/kg/wk; and Vit. K, 1 mg/kg/wk.

Pyridoxine deficiency was produced in 4 pigs (16-21 through 16-24) by feeding the above diet without pyridoxine. Three animals (14-14, 14-15, 14-16) were fed the complete diet and served as controls. Three animals (14-11, 14-12, 14-13) were fed the complete purified diet and in addition received condensed fish solubles (Fishel Products Co., Kingsbury, Calif.) in an amount of 5% of the diet.

The hematologic methods have been described(5).

Results. Response to pyridoxine. Oral administration of pyridoxine, 0.2 mg/kg/day, was followed by a reticulocytosis which reached maximal values of 17 to 26% by the 5th day. The volume of packed red cells (V. P. R. C.) increased to a value of 40 to 43 ml/100/ml within 16 to 27 days (Table I). The microcytosis and hypochromia disappeared within 27 days after pyridoxine therapy was begun.

One of the pigs (12-21) was followed for 50 days after the V. P. R. C. reached 40 ml/100 ml. During this period the V. P. R. C. varied between 39 and 43 ml/100 ml. Thus, the hematologic response to pyridoxine was sustained.

After the anemia had responded to pyridoxine, crude oral liver extract (Valentine's liquid extract of liver, N. F.) was given to a further increase in the V. P. R. C. could 3 of the animals for 10 days to determine if

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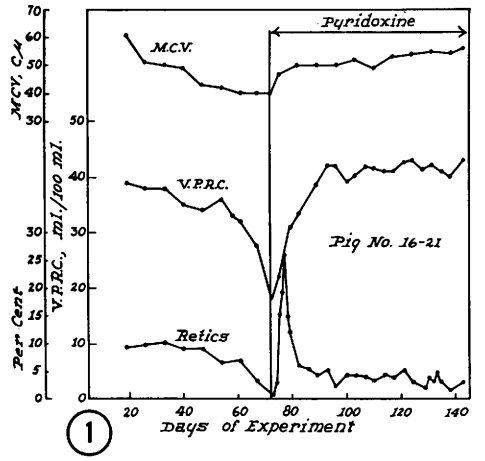

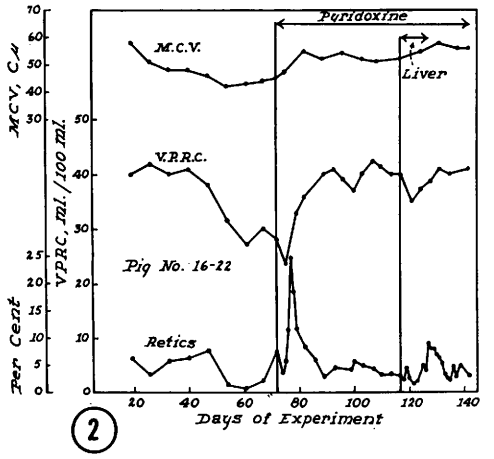

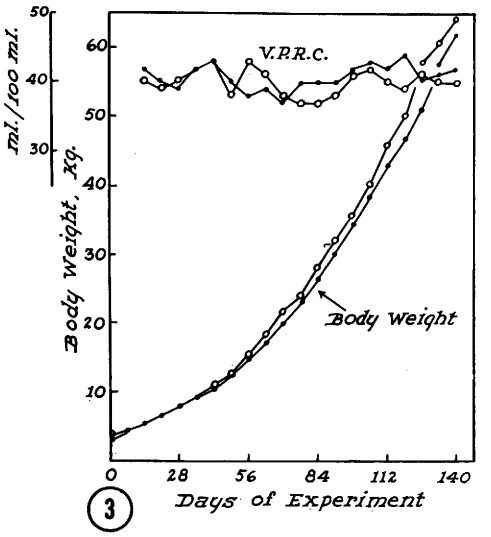

TABLE I. Response of Pyridoxine-Deficient Swine to Pyridoxine.

	V.P.R.C., ml/100 ml	M.C.V., cμ	M.C.H.C., %
Before deficiency	40 (38–42)	56 (51–60)	32 (31–32)
Deficient	23 (18–28)	42 (40–45)	28 (27–29)
After pyridoxine	41 (40–43)	53 (51–54)	33 (30–33)
After crude liver extract	42 (41–42)	55 (53–56)	33 (33–34)

V.P.R.C., volume of packed red cells; M.C.V., mean corpuscular volume; M.C.H.C., mean corpuscular hemoglobin concentration.

be obtained. One of the pigs (16-23) was given 120 ml of the preparation daily; the second (16-24) was given 240 ml daily; the third (16-22) was given 420 ml daily. In the last 2 pigs the amounts of liver extract given produced anorexia and diarrhea.

The V. P. R. C. of pig 16-24 decreased from 40 to 36 ml during the first 4 days of therapy. The reticulocytes increased to a maximum of 6% on the 6th day of therapy and thereafter the V. P. R. C. increased to 42 ml/100 ml. The V. P. R. C. of pig 16-22 decreased from 40 to 35 ml/100 ml by the 4th day of liver therapy. The reticulocytes increased to 9% on the 10th day of therapy and the V. P. R. C. increased to 41 ml/100 ml (Fig. 2). Two weeks after completion of the liver therapy, the mean value for the V. P. R. C. for the 3 pigs was 42 ml/100 ml as compared with a value of 41 ml before the extract was given (Table I).

Control groups. Three animals were supplemented with pyridoxine, 0.2 mg/kg/day,

FIG. 1. Development of anemia in a pyridoxine-deficient pig and response to pyridoxine. M.C.V., mean corpuscular volume; V.P.R.C., volume of packed red cells; Retics, reticulocytes.

FIG. 2. Development of anemia in a pyridoxine-deficient pig and response to pyridoxine. Administration of crude oral liver extract for 10 days was followed by a decline in volume of packed red cells (V.P.R.C.), a reticulocytosis and regeneration to previous level. M.C.V., mean corpuscular volume; V.P.R.C., volume of packed red cells; Retics, reticulocytes.

FIG. 3. Volume of packed red cells (V.P.R.C.) and growth of swine fed the purified diet (○) and 11 water soluble vitamins compared with those of animals fed the same diet and supplemented with condensed fish solubles (●). Curves represent mean values for 3 pigs in each group.

from the beginning of the experiment and were followed at weekly intervals for a period of 140 days. The V. P. R. C. varied between 35 and 43 ml/100 ml (Fig. 3). On the 140th day of the experiment, the mean V. P. R. C. for the 3 animals was 40 ml/100 ml. (39 to 41).

The diet of 3 additional animals was supplemented with pyridoxine, 0.2 mg/kg/day, from the beginning of the experiment. In addition fish solubles were added to the diet as a source of unidentified factors. The V. P. R. C. varied between 37 and 45 ml/100 ml (Fig. 3). On the 140th day of the experiment, the mean V. P. R. C. for the 3 pigs was 42 ml/100 ml (40 to 44). The growth rate for both groups of controls was similar (Fig. 3).

Discussion. In our earlier experiments(2) when swine were fed a casein diet supplemented with thiamine, riboflavin, nicotinic acid, choline, pantothenic acid, inositol and para-aminobenzoic acid but not biotin, folic acid, Vit. B_{12} or pyridoxine, the V. P. R. C. increased slowly to values of 29 to 36 ml/100 ml after administration of pyridoxine. In the present studies biotin, folic acid and Vit. B_{12} were included in the vitamin mixture and the pyridoxine deficient animals responded completely and rapidly to administration of pyridoxine. Addition of crude oral liver extract to the diet did not result in a further increase in the V. P. R. C. Furthermore, the values for the V. P. R. C. attained after administration of pyridoxine were comparable to the values in swine given 11 water soluble vitamins from the beginning of the experiment and to the values observed in swine fed the 11 vitamins and a crude source of unidentified factors (fish solubles). Thus, the requirement of the pig for an unidentified hemopoietic factor was not demonstrated under the conditions of these experiments. This is in contrast to the monkey(6) which apparently requires an unidentified hemopoietic factor present in liver.

These results are in no way at variance with the observations of Horrigan(1) in man. The anemia of pyridoxine-deficient pigs is unquestionably different in its pathogenesis from the "pyridoxine-responsive" anemia in human subjects(7).

Summary. The anemia of pyridoxine-deficient swine fed a purified diet with 10 crystalline water soluble vitamins responded rapidly and completely to administration of pyridoxine. The mean volume of packed red cells (V. P. R. C.) at the beginning of the experiment was 40 ml/100 ml; at time of therapy, 23 ml/100 ml; and after therapy with pyridoxine, 41 ml/100 ml. Administration of crude oral liver extract elicited no further increase in the V. P. R. C. (mean, 42 ml/100 ml). The mean V. P. R. C. of swine receiving the purified diet plus pyridoxine from the beginning of the experiment was 40 ml/100 ml. The mean V. P. R. C. of swine fed a purified diet supplemented with all 11 water soluble vitamins and fish solubles was 42 ml/100 ml. The requirement of the pig for a hemopoietic factor in addition to the 11 known water soluble vitamins was not demonstrated under the conditions of these experiments.

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