

plasma was employed) and fraction I + III-3 associated with the high molecular weight antihemophilic globulin and cold insoluble globulin and a small amount of plasminogen.

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Effect of Cortisone and of Aureomycin on Baby Pigs Fed a Vitamin B₁₂-Deficient Diet. (18991)

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Using baby pigs as experimental animals, a vit. B₁₂ deficiency has been produced on a "synthetic milk" diet by Neumann *et al.*(1). According to Johnson and Neumann(2), administration of crystalline vit. B₁₂ to this diet will completely alleviate the deficiency symptoms. Stokstad and Jukes(3) reported in some experiments a "sparing effect" of aureomycin upon the vit. B₁₂ requirement of chicks. Lichtman, Ginsberg, and Watson(4) administered aureomycin to pernicious anemia patients and reported a hematological improvement. Cravioto-Munoz *et al.*(5) state that aureomycin can replace vit. B₁₂ in the purified diet of the rat. No work has appeared on the relation of cortisone to nutritional vit. B₁₂ deficiency.

The present study was conducted to determine the effect of aureomycin and of cortisone when administered to baby pigs fed a vit. B₁₂-deficient ration.

Experimental. Six Chester White pigs (Exp. I) and 5 Poland China pigs (Exp. II) were removed from their dams at 48 hours of age and individually fed *ad libitum*. The

alpha-protein "synthetic milk" ration fed was essentially the same as reported in the vit. B₁₂ studies of Neumann *et al.*(6). In addition, 2% of sulfathalidine was added per liter of milk. The method of feeding and care of the animals was similar to that reported previously by Johnson *et al.*(7). In Exp. I two groups were compared. Group I received the basal ration plus intramuscular injections of crystalline vit. B₁₂ at a level of 0.8 µg/kg body wt/day. Group II received the basal ration plus 100 mg of aureomycin per kg of dry matter consumed. After 49 days on this diet they were given 30 µg of vit. B₁₂ by intramuscular injection for 3 successive days and remained on the experiment 14 days after therapy. The 5 pigs in Exp. II received the basal ration for 39 days. At that time they showed signs of vit. B₁₂ deficiency, including a very low reticulocyte count. One pig was continued on the basal ration as a control, while the other pigs were divided into 2 groups. Group IV received 20 mg of cortisone daily by intramuscular injection, and Group V received in their diet 100 mg of aureomycin per kg of dry matter consumed.

Blood samples were taken daily during the treatment period, and reticulocyte counts were determined. The thyroids and tongues of the pigs in Groups IV and V, Exp. II, were removed at autopsy for histological examination.

1. Neumann, A. L., Krider, J. L., and Johnson, B. Connor, *PROC. SOC. EXP. BIOL. AND MED.*, 1948, v69, 513.

2. Johnson, B. Connor, and Neumann, A. L., *J. Biol. Chem.*, 1949, v178, 1001.

3. Stokstad, E. L. R., and Jukes, T. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1951, v76, 73.

4. Lichtman, H., Ginsberg, V., and Watson, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v74, 884.

5. Cravioto-Munoz, J., Poncher, H. G., and Waisman, Harry A., *PROC. SOC. EXP. BIOL. AND MED.*, 1951, v77, 18.

6. Neumann, A. L., Thiersch, J. B., Krider, J. L., James, Marian F., and Johnson, B. Connor, *J. Animal Sci.*, 1950, v9, 83.

7. Johnson, B. Connor, James, M. F., and Krider, J. L., *J. Animal Sci.*, 1948, v7, 486.

TABLE I. Performance of Baby Pigs Given Aureomycin or Vitamin B₁₂.

	Group I Basal + B ₁₂ (7 wk)	Group II Basal + 100 mg aureo- mycin/kg dry matter consumed	
		Before therapy (7 wk)	On therapy (2 wk)
No. of pigs	4	2	
Avg initial wt, kg	1.33	1.56	
" final " "	13.54	6.23	14.45
" daily gain, "	.250*	.095	.550
Dry matter con- sumed (kg/ kg gain)	1.26*	1.74	1.04

* Statistically significant, $P < .01$, over Group II at 7 wk.

Results and discussion. Table I gives a summary of the total performance of the 2 groups of pigs in Exp. I. The pigs in Group II which received vit. B₁₂ injections during the treatment period showed a very marked reticulocytosis (Table III), a 4-fold increase in food consumption, and a significantly improved growth rate. The exceptionally rapid recovery of these pigs after vit. B₁₂ therapy

indicates that an uncomplicated, severe vit. B₁₂ deficiency did exist.

The average daily gains of the 3 groups in Exp. II are summarized in Table II. Twenty mg of cortisone daily had no effect in alleviating the deficiency symptoms of the pigs in Group IV. In fact, cortisone enhanced the deficiency state, the pigs dying after 8-10 days of cortisone injections. Urinary vit. B₁₂ excretion averaged 62 mγ per day following 38 days on the deficient diet and immediately prior to cortisone treatment. After 3 days on cortisone, it had increased to 85 mγ per day. This increase in B₁₂ excretion, which is presumably a sequel to increased protein catabolism induced by cortisone, is probably a reason for the aggravating effect of cortisone on the B₁₂ deficiency. Group V (Exp. II) differed from Group II of Exp. I in that the aureomycin was not added to the basal ration of Group V until a deficiency state existed. The addition of aureomycin at this time caused no reticulocyte response.

The reticulocyte counts for the various pigs of both experiments are given in Table III.

TABLE II. Response of Vitamin B₁₂-Deficient Pigs to Cortisone and Aureomycin.

	Group III	Group IV	Group V
No. of pigs	1	2	2
Avg initial wt, kg	1.65	1.725	1.785
" daily gain, " (depletion period)	.213	.163	.190
Avg daily gain, kg (treatment period)	no treatment	cortisone given	aureomycin fed
	.083	.037	.141

TABLE III. Reticulocytes per 100 Red Blood Cells.

Days on test	Group II (Exp. 1) aureomycin in diet		Group III (Exp. 2) Basal, 1c	Group IV (Exp. 2) basal		Group V (Exp. 2) basal	
	1c	2c		3c	5c	2c	4c
14	—	—	5.2	8.1	5.4	6.9	7
28	3.5	.6	3.4	2.4	1.4	1.7	1.5
35	—	—	1.5	.7	1.2	1.2	1.6
39	—	—	—	.5	1.2	1.2	1.6
42	1.9	2.1					
49	1.1	.5					
Days after treatment	Vit B ₁₂ treatment		No treatment	Cortisone treatment		Aureomycin treatment	
1	2.1	1.6	.2	1.1	2.3	.3	.3
3	6.2	3.9	.1	1.3	3.6	1.3	1.4
5	5	5.7	.6	.5	1	.2	.3
7	6.6	7.4	0	.5	0	.8	0
9	6.6	12.2		—	.1	.5	0
11		8.2		—	—	.1	.1
13	—	5.2	.1	—	—	.2	0

Hemoglobin and red cell counts were also made at the beginning of the treatment period. The red blood cell counts were essentially normal, but the hemoglobin values were low (avg. 10.6 g %).

Examination of the thyroids and tongues of the pigs in Groups IV and V indicated hypertrophy of these organs as reported previously (8). In addition, in certain areas of the thyroids the follicles were abnormally small and contained little colloid.

Summary. A vit. B₁₂ deficiency has been produced in baby pigs fed an alpha-protein "synthetic milk" diet. The addition of aureomycin to this diet, either before or after the

deficiency state existed, did not give a beneficial response. The injection of 20 mg of cortisone daily into vit. B₁₂-deficient pigs enhanced the deficiency state rather than having any curative effect.

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8. Johnson, B. Connor, Neumann, A. L., Nesheim, R. O., James, Marian F., Krider, J. L., Dana, Ann S., and Thiersch, J. B., *J. Lab. and Clinical Med.*, 1950, v36, 537.

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Blood and Plasma Volume and Thiocyanate Space of Normal Chicks.* (18992)

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(Introduced by F. J. Stare.)

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The blood and plasma volumes and thiocyanates space of humans, dogs and rats have been investigated by various authors but no data appear to have been collected on chicks. In connection with studies on high salt diets which will be published subsequently we have determined the volumes of these fluid spaces in normal White Leghorn cockerels of various ages. The ease with which intra-jugular injections may be made in this species may make it a useful species for such studies.

Methods. The methods used have been previously described by Wang and Hegsted (1) in studies with rats. A mixture of Evans Blue Dye and ammonium thiocyanate solution

was injected into the jugular vein of the chick under light ether anesthesia. Ten minutes later a blood sample was taken with a heparinized syringe by heart puncture. The hematocrit was determined with the small Van Allen tubes and the thiocyanate and Evans blue concentrations in the blood determined as previously described. From 0.2 to 0.3 ml of solution containing 5 mg of dye and 40 mg of thiocyanate per ml was used depending upon the size of the chick. The concentrations in the solution injected were determined by analysis after the addition of a known amount to a control sample of chick blood. Preliminary studies showed that ten minutes after injection was sufficient time to yield essentially stable blood concentrations. When several chicks were to be studied, it was convenient to inject 4 chicks at approximately 2½ minute intervals and bleed them during

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1. Wang, C. F., and Hegsted, D. M., *Am. J. Physiol.*, 1949, v156, 227.