that some serum samples have been encountered from which added ferrocyanide cannot be completely recovered either by the Husson technique or the modified procedure. Recovery is complete from most serum samples, but the possibility that there is some binding of ferrocyanide to serum protein *in vivo* has not been ruled out. However, the fact that the ferrocyanide clearance agrees with both inulin and creatinine clearance so closely at high and low plasma concentrations, indicates that the ferrocyanide determined in the serum must approximate very closely that which is filterable at the glomerulus.

Conclusion. Inulin, exogenous and endogenous creatinine, and ferrocyanide ion are all cleared at the same rate by the dog kidney, and probably exclusively by glomerular filtration.

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Vitamin B_{12} and Sub-Optimal Levels of Pantothenic Acid in Chick Nutrition.* (21306)

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The syndrome caused by the lack of pantothenic acid was reported by Norris and Ringrose(1) to be characterized by retarded and rough feather growth, definite, crusty, scab-like lesions in the corners of the mouth, and sometimes a cracking of the epithelium between the toes.

The pantothenic acid requirement for the chick has been placed at 5.5 mg/kg by Bauernfeind *et al.*(2) and at 14.3 mg/kg by Jukes(3). More recently, the National Research Council (4) recommended the use of 11.0 mg/kg of feed. The requirement of the chick for vit. B_{12} has been placed at 11.0 μg/kg by the National Research Council(4).

In view of the reports by Evans *et al.*(5) and Yacowitz *et al.*(6) concerning the effect of vit. B_{12} upon the pantothenic acid content of the liver, it appeared desirable to investigate the effect of vit. B_{12} upon the pantothenic acid metabolism when a sub-optimal amount (based on the National Research Council recommendations of 1950) of pantothenic acid was fed. In addition, the effect of pantothenic acid on B_{12} metabolism was investigated. This was done by making observations on growth, deficiency symptoms, and liver storage of B_{12} and pantothenic acid in 4-week old chicks.

Material and methods. Unsexed New Hampshire chicks were randomly distributed into 8 groups of 12 birds each. The birds were weighed, wing-banded, and placed in electrically heated batteries with raised wire floors. After the initial weighing, the chicks were weighed at weekly intervals during the 4 week experiment. The basal diet consisted of 25% purified soybean protein†, 67% glucose (cere-

* Cerelose was supplied through the courtesy of the Corn Products Refining Co., Argo, Ill. Folic acid was supplied by Lederle Laboratories, Pearl River, N. Y.; biotin by Hoffmann-La Roche, Inc., Nutley, N. J.; and the rest of the B-vitamins by Merck and Co., Rahway, N. J. Methionine was obtained from Dow Chemical Co., Freeport, Texas.

† Drackett 220 obtained from the Drackett Co., Cincinnati, O.

TABLE I. Effect of Vitamin B_{12} and Pantothenic Acid on Growth, Pantothenic Acid Deficiency Symptoms, Mortality and B_{12} and Pantothenic Acid Content of Chick Livers.

Supplement to basal	Avg wt at 4 wk (g)	Severity of deficiency Mouth*	Feet†	% mortality	B_{12} content, mμg/g	PA content, μg/g
PA‡(1.66 mg/kg)	141.0	.88	.00	57.8	66.4	61.7
B_{12} (50 μg/kg)	100.2	1.17	.07	73.6	176.4	62.2
PA (1.66 mg/kg) + B_{12} (50 μg/kg)	130.9	1.17	.25	36.8	152.0	62.4
PA (" ") + B_{12} (100 ")	124.4	1.40	.40	47.5	302.8	65.7
PA (" ") + B_{12} (250 ")	109.0	1.58	.58	36.8	267.8	62.5
PA (3.32 ") + B_{12} (50 ")	204.8	.19	.00	15.8	201.9	62.4
PA (6.64 ") + B_{12} (50 ")	314.0	.00	.00	00.0	239.0	85.4
PA (9.96 ") + B_{12} (50 ")	315.9	.00	.00	15.8	215.5	102.4

* Dermatitis about mouth. † Cracking of epithelial tissue between toes. ‡ Pantothenic acid.

lose), 5% salts IV (Hegsted *et al.*, 7), and 3% refined soybean oil. Methionine and glycine were added to all diets at the levels of 7.5 and 4 g/kg, respectively. In addition, the following were added in the specified number of mg/kg of diet: thiamin hydrochloride, 4; riboflavin, 6; pyridoxine hydrochloride, 4; niacin, 100; folic acid, 2; biotin, 0.2; 2-methyl-1, 4-naphthoquinone, 0.5; and alpha-tocopheryl acetate, 3. Choline was added at the level of 2 g/kg. Vitamins A and D were added at the levels of 10000 I.U. and 2000 I.C.U./kg respectively. The plan of the experiment is shown in Table I. At the end of the 4 week experimental period, the chicks were sacrificed and the livers removed for vitamin determinations. A 2.5 g portion of liver was placed in a graduated cylinder and water added to 50 ml. This was then homogenized in a Waring blendor for 5 minutes. A 10 ml aliquot (500 mg of liver) was removed for vit. B_{12} determinations. A 2 ml aliquot (100 mg of liver) was removed for pantothenic acid determinations. Vit. B_{12} was liberated by incubating the homogenate at 37° C for 24 hours in the presence of pancreatin. The assay was carried out according to the procedure of Skeggs *et al.*(8) with *Lactobacillus leichmannii* 4797 as the test organism. Acid production at the end of 72 hours was used as the index of growth. Pantothenic acid was liberated by incubating for 3 hours at 37° C in the presence of a pH 8.3 buffer, intestinal phosphatase, and pigeon liver enzyme. Pantothenic acid analyses were carried out using *Lactobacillus arabinosus* 8014 according to the method of Skeggs and Wright(9). Acid production at the end of 72 hours was used as an index of growth. The birds were examined at the end of the experiment and scored for dermatitis about the mouth and for cracks in the epithelial tissue between the toes. In order to arrive at some means of expressing these results, the deficiency symptoms were calculated separately. The severity of the deficiency was scored as +, 2+, or 3+ with 3+ indicating the most severe. Each of the +'s was then counted as a unit. The total number of +'s for each symptom was then divided by the total number of birds in that group. The result obtained expresses the degree of severity of each symptom per bird in that group.

Results. Growth. The average weight of the chicks was decreased when B_{12} (50 μg/kg) was added to the low pantothenic acid (1.66 mg/kg) diet (Table I). A further reduction in growth occurred as the level of B_{12} was increased. The severity of deficiency symptoms also increased as the addition of B_{12} was increased (Table I).

The average weight of the birds was increased by increasing the level of pantothenic acid to 3.32 and 6.64 mg/kg respectively. The latter mentioned level was adequate for the prevention of the deficiency symptoms. The soybean protein used in this study supplied 10.5 mg of pantothenic acid/kg of diet (PA content supplied by Drackett Co.). This value plus the 6.64 mg of supplemental pantothenic acid, which was the lowest level that was effective in promoting adequate growth and preventing dermatitis, gives a figure of 17.14 mg/kg. This is only slightly higher than the level reported by Jukes(3).

B_{12} and pantothenic acid content of liver. The B_{12} content of liver was increased by ad-

dition of B_{12} to the diet. The addition of 50 μg of B_{12} to the basal diet containing 1.66 mg of added pantothenic acid per kg increased the B_{12} content from 66.4 to 152.0 mγ/g of liver. The B_{12} storage in the liver was further increased to 302.8 mγ/g by increasing the B_{12} content of the diet to 100 μg/kg. An additional increase in the B_{12} level of the diet did not further increase the B_{12} content of the liver above that obtained with 100 μg of B_{12}. These observations are in agreement with the earlier work of Couch *et al.* (10).

The addition of 1.66 mg of pantothenic acid per kg of diet to the basal diet containing 50 μg of B_{12} had no effect upon the B_{12} level in the liver. The B_{12} content of the liver was increased to 201.9 mγ/g when 3.32 mg of pantothenic acid was added to the diet, and to 239.0 mγ/g when 6.64 mg of pantothenic acid were added. No further effect on the B_{12} in the liver was produced by adding 9.96 mg pantothenic acid per kg of diet. This indicates that pantothenic acid may have had a sparing effect on vit. B_{12}.

The pantothenic acid content of the liver was increased by the addition of 6.64 mg or 9.96 mg of pantothenic acid per kg of diet. The addition of pantothenic acid at these levels increased the total pantothenic acid content of the liver to 85.4 and 102.4 μg/g of liver, respectively. These values are somewhat lower than that found in other experiments where a higher level of supplemental pantothenic acid was fed. All levels of pantothenic acid fed below the 6.64 mg/kg level had a pantothenic acid content in the liver ranging from 61.7 to 65.7 μg/g. The pantothenic acid content of the liver was not influenced by the addition of vit. B_{12} to the diet.

Summary. 1. Growth of birds fed a pantothenic acid-low diet was decreased by addition of vit. B_{12}. The degree of severity of pantothenic acid deficiency symptoms observed was increased by addition of vit. B_{12}. The lowest level of supplemental pantothenic acid that supported growth and prevented deficiency symptoms was 6.64 mg/kg of diet or 17.14 mg/kg when pantothenic acid contributed by the protein is added. When the supplemental pantothenic acid level was increased to 9.96 mg/kg, no additional effect was observed. 2. The B_{12} content of liver was increased by addition of that vitamin to the chick diet and this was proportional to the level of B_{12} added. Additional increase in B_{12} content of the liver was observed when the pantothenic acid level in the diet was increased. This indicates that pantothenic acid may have had a sparing effect on B_{12} when the pantothenic acid content of the diet was adequate. 3. The pantothenic acid content of the liver was influenced only when the level of supplemental pantothenic acid was raised to 6.64 mg/kg or a total of 17.14 mg/kg or higher.

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