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A Green Pigment-Producing Compound in Urine of Pyridoxine-Deficient Dogs.

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Lepkovsky and Nielsen¹ reported that pyridoxine-deficient rats excrete an unidentified compound which can be converted to a green pigment by ferric ammonium sulphate. Administration of pyridoxine to the deficient rat stops the excretion of the precursor of the green pigment in a matter of hours.

To see if pyridoxine deficient dogs excrete a similar compound these studies were instituted.

Methods. The basic diet consisted of the following: Washed casein 41.4 g, sucrose 29.6 g, Crisco 25.7 g, bone ash 2.0 g, and salt mixture No. 185² 1.3 g. The adult mongrel dogs were allowed to eat as much of the diet as desired. The dogs received daily by dropper: thiamine chloride and riboflavin 60 μ g per kilo; nicotinic acid, 2 mg per kilo; calcium pantothenate, 0.6 g per kilo, and choline, 10 mg per kilo, in addition to vitamins A and D.

Red blood cell counts, hemoglobin determinations (Evelyn), and white blood counts were made at weekly intervals. Hematocrit measurements were made by use of Wintrobe tubes. Daily 24-hour urine specimens were tested for the green pigment-producing substance. To filtered urine made alkaline by addition of ammonium hydroxide, ferric ammonium sulphate was added to bring out the color.

Results. A small amount of green pigment-producing compound could be demonstrated in urine after the 23rd day on a diet in one dog, and after 25 days in the other. The amount was small compared to that in the urine of pyridoxine-deficient rats, but it could be concentrated chromatographically¹ and found to behave similarly to the substance in the rat's urine. The compound did not increase during the period of study, although in one dog the red blood cell count, hemoglobin, and hematocrit dropped from 4.5 million, 10.8 g and 31 cc to 1.83 million, 2.9 g and 9 cc, respectively, by the 107th day of the diet. In the other dog the red blood cell count, hemoglobin,

¹ Lepkovsky, Samuel, and Nielsen, Elmer, *J. Biol. Chem.*, in press.

² McCollum, E. G., and Simmonds, N., *J. Biol. Chem.*, 1918, **33**, 55.

and hematocrit dropped from 6.8 million, 15.3 g and 47 cc to 2.13 million, 4.2 g and 11.5 cc before pyridoxine was administered on the 116th day.

Pyridoxine, 60 μ g daily by mouth, was followed by a rise of reticulocytes to 14.4% and a rapid rise in red blood cells and hemoglobin. After 18 days of therapy the red blood cell count was 4.7 million, hemoglobin 11.5 g, and hematocrit 42 cc. The green pigment-producing compound disappeared from the urine within 48 hours.

Pyridoxine-deficient rats whose urine yields large amounts of green pigment-producing substance do not develop such profound anemia as the dogs. Fifteen pyridoxine-deficient rats had hemoglobin values varying from 11.4 to 14.2 g per 100 cc, averaging 13.0 g. The hemoglobin of similar pyridoxine-fed rats varied from 15.2 to 16.5 g, averaging 15.8 g per 100 cc.

Conclusions. 1. Pyridoxine-deficient dogs excrete small amounts of a compound which can be converted to a green pigment by ferric ammonium sulphate. 2. Pyridoxine-deficient rats do not develop as profound anemia as the deficient dogs.

We wish to thank Mrs. D. Parsons for making the hemoglobin determinations and red cell counts on the rats.

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Preservation of Avian Malaria Parasites by Low-Temperature Freezing.*

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Since malaria plasmodia cannot be readily cultivated it has been necessary to maintain them in laboratory animals wherever they are used for research or classroom material, and hence Coggeshall's¹ demonstration that malaria parasites of the monkey could be preserved for at least 70 days[†] in the frozen state seemed to have much potential importance. For laboratory use the avian malarias are even more widely employed, and so experiments were undertaken to apply this technic to them.

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¹ Coggeshall, L. T., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **42**, 499.

[†] Later extended to 151 days. Horsfall, F. L., *J. Bact.*, 1940, **40**, 559.