

blood became stabilized, the diet was changed to one which regularly caused black tongue. A slow but steady fall in blood levels resulted, accompanied at first by an increased number of circulating reticulocytes, followed by slight increase in the blood levels. As the experiment continued, however, anemia became severe and the reticulocytes stabilized at low levels. Goldberger⁴ had shown that liver extract was capable of preventing and curing the symptoms of experimental black tongue. Accordingly the effect of administering liver extract to dogs with the indol-and-deficient-diet anemia was tested. A sharp rise in reticulocytes and improvement in blood levels resulted in every instance in spite of continued indol feeding.

Although ample control experiments had shown that no such anemia ever occurred under the experimental conditions due to the diet feeding alone, further experiments were made to control this factor. Dogs fed diets of milk exclusively showed a similar susceptibility to indol. This was equally true of animals which refused food and became voluntarily malnourished.

Pathological studies showed a very marked extension of active marrow during the anemic phase. The marrow throughout was solid, deep red, gelatinous tissue which showed on histological examination an increased cellularity and a predominance of immature forms.

Summary and Conclusion. Orally administered indol is causative of anemia in dogs fed a diet which is causative of black tongue. The same amount of indol does not cause considerable anemia in dogs fed a normal diet. Liver extract is preventive and curative of the anemia.

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Effect of Diet on Susceptibility of Canine Hematopoietic System to Damage by Amidopyrine.

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The association of amidopyrine administration with the occurrence of acute agranulocytosis of human beings has been described by Kracke and Parker,¹ Madison and Squier,² and many others.

⁴ Goldberger, J., and Sebrell, W. H., *Pub. Health Rep.*, 1930, **45**, 3064.

¹ Kracke, R. R., and Parker, F. P., *J. Lab. Clin. Med.*, 1933-34, **19**, 799.

² Madison, F. W., and Squier, T. L., *J. Am. Med. Assn.*, 1934, **102**, 755.

The attempts to induce hematological disorders in normal animals by the administration of amidopyrine have not been uniformly successful.

Miller and Rhoads³ reported leukopenia in the terminal phase of acute canine black-tongue and Day⁴ reported a leukopenia in monkeys fed diets low in their content of vitamin B₂ (G). This observation suggested that the combination of amidopyrine administration with the feeding of a deficient diet might be productive of abnormal hematopoiesis. Such an experiment has been made but paradoxically the effect observed was anemia rather than leukopenia.

The black-tongue diet fed was that designated by Goldberger⁵ as No. 123. Its composition has been described previously.³ In an extensive series of experiments black-tongue has never resulted from the feeding of this diet in less than 5 weeks nor more than 10. The normal diet fed was a mixture of cooked beef, bread, and dog biscuit, which is known to be capable of maintaining dogs in good health over a period of several years. The amidopyrine employed was lot 1433, manufactured by the H. A. Metz Laboratories. A 5% solution in water was effected by gentle heating. The animals were bled twice weekly from the jugular veins. The blood was collected in bottles containing potassium oxalate. Hemoglobin values were estimated by the Sahli method, using calibrated tubes and standards.

The report includes observations of 17 dogs divided into 4 groups.

Experiment 1. Six dogs were fed the black-tongue diet and 0.5 gm. of amidopyrine was administered daily. All of these animals showed pronounced anemia after periods ranging from 8 to 35 days. When the amidopyrine was discontinued, although the black-tongue diet was still fed, the blood levels returned to normal.

Experiment 2. Three dogs were fed a normal diet and received 0.5 gm. of amidopyrine daily. This series served to control Experiment 1. In no instance was anemia observed.

Experiment 3. In this series 3 dogs were fed the black-tongue diet and 2.0 gm. of amidopyrine were administered daily. Severe anemia occurred in all 3; one animal died after a convulsion and in the remaining 2 the drug was discontinued and recovery ensued.

Experiment 4. This group, composed of 5 animals, tested the effect of the administration of 2.0 gm. of amidopyrine daily while a normal diet was fed. In every instance a well-defined decrease of

³ Miller, D. K., and Rhoads, C. P., *J. Exp. Med.*, 1933, **58**, 585.

⁴ Day, P. L., Langston, W. C., and Shukers, C. F., *J. Nutrition*, 1935, **9**, 637.

⁵ Goldberger, J., and Wheeler, G. A., *Bull. Hyg. Lab. U. S. P. N. S.*, No. 120, 1920, 7.

erythrocyte and hemoglobin levels occurred. Two animals died, one of a pneumonitis and one after a convulsion. The remaining animals recovered after the amidopyrine was discontinued.

Specimens of tibial bone marrow were removed at autopsy of the animals which died at the height of the anemia. The marrows were cellular but the number of adult forms of both red and white cells was distinctly less than normal, suggesting an inhibition of maturation. Necrosis was not observed.

Goldberger⁵ has reported that the oral administration of yeast is effective in preventing the symptoms of black-tongue and has suggested that the disease is due to a lack in the diet of a vitamin which is contained in yeast. This observation suggested that yeast might be preventive also of the anemia produced by amidopyrine administered coincidentally with the diet feeding.

Experiment 5. Two dogs were fed the black-tongue diet and at the same time 0.5 gm. of amidopyrine was administered daily by stomach tube. When a severe degree of anemia had resulted 10 gm. of Vegex, a commercial yeast autolysate, were administered daily. This was followed by a sharp rise in leukocytes, in erythrocytes, and in hemoglobin. The treatment with yeast was given daily for 20 days and then was discontinued, all other factors remaining unchanged.

Following the omission of yeast a slow progressive fall in blood levels occurred. Yeast was administered a second time and on this occasion the reticulocytes were counted. The institution of treatment was followed by an increase in the number of reticulocytes to 10 and 14% respectively and a rise once more in the levels of red and white cells and of hemoglobin.

To substantiate further the conclusion that the anemia resulting from amidopyrine administration was due to the lack of some constituent of yeast, 2 dogs were fed the diet plus 10 gm. of autolyzed yeast daily. At the same time 0.5 gm. of amidopyrine was administered daily by stomach tube. No anemia occurred during the 2-month period of observation, although a fall of blood levels occurred in every dog so treated without the supplement of yeast.

Summary and Conclusions. The daily administration of 0.5 gm. of amidopyrine causes anemia in dogs fed a diet which causes canine black-tongue. No anemia results when a normal diet is fed. The anemia is associated with a suppression of maturation of hemopoietic bone marrow cells and it may be prevented and cured by supplementing the diet with autolyzed yeast.