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Importance of Choline in Synthetic Rations for Dogs.*

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During studies on the newer members of the vitamin B complex with dogs^{1, 2} we observed that a highly purified ration supplemented with synthetic thiamin, riboflavin, nicotinic acid, pyridoxine, and pantothenic acid does not permit survival. Evidence was presented for the existence of two other factors present in liver extract essential for the dog. Since choline was found to be essential for the prevention of fatty livers in depancreatized dogs,³ we suspected very early that choline may have been one of these factors. The administration of choline, however, produced no effect in the deficient animals and it was concluded that these factors were distinct from choline. But in the light of more recent work with both rats and chicks we must conclude that the dose used was far below that found necessary for these other animals. We have, therefore, restudied the significance of choline in the nutrition of the dog using higher levels of choline.

Two litters of mongrel puppies were used in these experiments. The ration, as previously described, contained sucrose 66%, acid washed casein 19%, cottonseed oil 8%, cod liver oil 3%, and salt mixture 4%. This was supplemented with thiamin and riboflavin at a level of 100 γ per kg of body weight per day, nicotinic acid at a level of 2 mg, pyridoxine at 60 γ , and calcium pantothenate at 500 γ per kilo per day. These vitamins were administered orally in water solution twice weekly. Dogs 1, 2, 3, 4, and 5 were placed on this ration at the age of 8 weeks. Dogs 6, 7, and 8 of the other litter

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¹ McKibbin, J. M., Madden, R. J., Black, S., and Elvehjem, C. A., *Am. J. Physiol.*, 1939, **128**, 102.

² McKibbin, J. M., Black, S., and Elvehjem, C. A., *Am. J. Physiol.*, 1940, **130**, 365.

³ Best, C. H., and Ridout, J. H., *Ann. Rev. Biochem.*, 1939, p. 349.

were placed on the ration at 6 weeks of age. The growth curves of these dogs are shown on the graph. It can be seen that dogs 1, 2, 3, 4, 5, and 8 receiving the choline-low ration showed a quick growth plateau followed by anorexia and loss of weight. No other symptoms were observed. The administration of choline at a dosage of 100 mg per kg of body weight per day produced an immediate growth response in these dogs. Dogs 6 and 7 received 50 mg of choline per kg per day from the beginning of the experiment and serve as positive controls. These animals gained 1.85 and 2.1 kg, respectively, while the litter mate dog No. 8 had gained only 0.9 kg during the 25-day period. The addition of choline to the diet of dog No. 8 resulted in a weight increase of 1.75 kg in 12 days. The condition of the animals at the time of treatment was more critical than would appear from the growth curves. It has been our experience with puppies on this ration that prolongation of the anorexia is usually fatal. It is possible that difficulties of this kind encountered in our earlier studies were at least in part due to choline deficiency. We are now studying the choline content of the various liver fractions used to determine the relation of choline to the activity of these fractions. A high incidence of yellow livers observed in these dogs suggests a parallel to those found in choline-deficient depancreatized dogs.

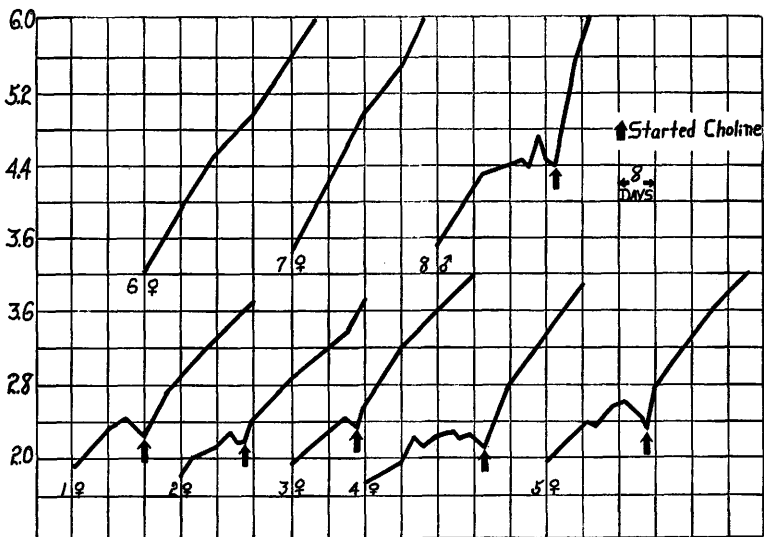


FIG. 1.

Growth curves of young dogs on the basal synthetic ration with and without choline.

The speed of onset of the deficiency is comparable to that of the other B vitamins. We have found in extensive studies on this ration involving some 128 dogs over a 3-year period that acute fatal deficiencies of thiamin, riboflavin, nicotinic acid, pyridoxine, and probably pantothenic acid may be expected within 2 to 6 weeks. This is in distinct opposition to the claim of Morgan⁴ that "Even in very young animals several months are required for the development of overt symptoms of deficiency in any of the B vitamins, with the possible exception of B₁."

The requirement of choline may be supposed to be largely dependent on the amounts of methionine and cystine in the diet.^{5, 6, 7} Increasing the level of casein, therefore, lowers the choline requirement of rats. Griffith⁶ found that the hemorrhagic degeneration of choline deficiency in rats was prevented by a low fat diet containing 47% casein even in the presence of 0.5% cystine. Most other laboratories have used higher levels of casein than we have used and this might entirely nullify the requirement for choline. Thus Fouts, *et al.*,⁸ used 41.4%, Borson and Mettier,⁹ 37% casein in their vitamin B₆ deficiency studies with dogs. In addition, the natural vitamin B complex sources such as liver extract and rice bran preparations contain considerable quantities of choline. The choline would have to be removed from these preparations in order to demonstrate this deficiency.

The growth rate of puppies maintained on this ration supplemented with thiamin, riboflavin, nicotinic acid, pyridoxine, and pantothenic acid is very suboptimal and erratic even with added choline. The ration is, therefore, still not entirely satisfactory for studying the uncomplicated deficiency of any of the 6 synthetic vitamins without the addition of a crude vitamin B complex preparation, although we have used a ration of this kind for nicotinic acid assay of foods over limited periods of time. Studies on other substances in liver extract necessary for the normal growth of the dog are now in progress.

Summary. In addition to thiamin, riboflavin, nicotinic acid, pyridoxine, and pantothenic acid, young puppies also require choline

⁴ Morgan, A., *Science*, 1941, **93**, 261.

⁵ du Vigneaud, V., Chandler, J. P., Moyer, A. W., and Keppel, D. M., *J. Biol. Chem.*, 1939, **131**, 57.

⁶ Griffith, W. H., *J. Biol. Chem.*, 1940, **132**, 639.

⁷ Griffith, W. H., and Wade, N. J., *J. Biol. Chem.*, 1940, **132**, 627.

⁸ Fouts, P. J., Helmer, O. M., and Lepkovsky, S., *J. Nutr.*, 1940, **19**, 393.

⁹ Borson, H. J., and Mettier, S. R., *Proc. Soc. Exp. Biol. and Med.*, 1940, **43**, 429.

and probably other factors of the vitamin B complex for normal growth under the conditions of these studies. Possible factors altering the requirement of choline for dogs on synthetic rations are discussed.

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Isolation and Pure Cultivation of the Smaller Mouth Spirochetes by an Improved Method.

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The characteristics and properties of the so-called commensal spirochetes of mucous membranes, and their individual or contributing rôles in fuso-spirochetal infections, remain inadequately understood largely because of a lack of effective means for their isolation and maintenance in pure culture. *Treponema vincenti*, the large delicate loosely wound spirochete of the mouth, seems never to have been obtained in pure culture; *T. buccale*, the large, thick, loosely wound form, has been reported in culture rarely,^{1, 2, 3} apparently under conditions which could not be duplicated. *T. microdentium*, the small mouth spirochete which resembles *T. pallidum* in morphology and is cultivable with the least difficulty,⁴⁻⁸ seems for that reason to be the only member of the group which can be accepted with little doubt as a distinct species. Studies of pure cultures of *T. microdentium*, moreover, have yielded interesting information on the pathogenesis of fuso-spirochetal infections.^{5, 6, 9, 2} On the other hand, none of the other varieties which have been described and named, such as Noguchi's *T. macrodentium*⁴ or the several species named by Seguin and Vinzent,¹⁰ is so clearly defined.

1 Smith, D. T., *Oral Spirochetes and Related Organisms in Fuso-Spirochetal Disease*, Baltimore, Williams and Wilkins Co., 1932, p. 11.

2 Proske, H. O., and Sayers, R. R., *U. S. Pub. Health Rep.*, 1934, **49**, 839, 1212.

3 Vinzent, R., and Daufresne, M., *Compt. rend. Soc. Biol.*, 1934, **116**, 490.

4 Noguchi, H., *J. Exp. Med.*, 1912, **15**, 81.

5 Kritehewski, B., and Seguin, P., *Rev. stomatol.*, 1920, **22**, 613.

6 Smith, D. T., *Am. Rev. Tuberculosis*, 1927, **16**, 584.

7 Smith, D. T., *loc. cit.*, 1932, p. 8.

8 Seguin, P., and Vinzent, R., *Ann. de l'Inst. Pasteur*, 1938, **61**, 255.

9 Smith, D. T., *loc. cit.*, 1932, p. 124.

10 Seguin, P., and Vinzent, R., *Compt. rend. Soc. Biol.*, 1936, **121**, 408.