posed to their normal antagonism in bacteriostasis. A direct antioxygenic reaction might be the cause for this synergism which hitherto has defied any plausible explanation. The enhancing effect of p-aminobenzoic acid on sulfonamides in the retardation of rancidity in fat has recently been experimentally demonstrated.[13]

More experimental evidence is needed before the link between antioxygenic activity of thiourea and related substances and their effect on metabolism and the thyroid gland can be regarded as established.[13]

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VITAMIN B_6 DEFICIENCY ANEMIA IN THE DOG[1]

HYPOCHROMIC microcytic anemia typical of vitamin B_6 deficiency in puppies and adult dogs has been produced in this laboratory consistently, thus confirming the work of others.[2,3,4,5,6] The synthetic basic diet used was high in protein and consisted of the following ingredients: casein 40 per cent., sucrose 36, cotton seed oil 18, cod liver oil 2, mineral salts[7] 4 per cent. This diet is essentially free from all members of the vitamin-B complex.

EXPERIMENTAL GROUPS

Group I—consisting of nine animals—received in addition to the basic diet seven synthetic members of the vitamin B complex as follows: thiamin 1.4 mg, riboflavin 0.7 mg, nicotinic acid 6 mg, inositol 6 mg, pantothenic acid 6 mg, para aminobenzoic acid 6 mg, and choline 30 mg per dog per day, but no vitamin B_6. All these dogs developed the hypochromic microcytic anemia observed in dogs lacking vitamin B_6. The initial hemoglobin of these dogs averaged 15.5 grams. This rose to a peak of 18.8 grams in approximately 7 weeks, then declined to values averaging 7.7 grams after 22 weeks on the diet. For other blood values see Table I.

Group II. As a control group five puppies were placed on the same régime except that vitamin B_6 was given as a supplement (6 mg per dog per day) from the beginning of the experiment along with the other seven synthetic vitamins. Much to our surprise under these conditions the blood values (determined every two weeks) followed a pattern quite similar to that of the vitamin B_6 deficient animals (Fig. 1). Their initial hemoglobin values averaged 14.5 grams. These rose to a high value of 18.6 grams in 4 weeks, then declined gradually to 9.4 grams over a period of 20 weeks.

Group III. As an additional control group, 4 animals (1 adult and 3 puppies) received the basic diet altered to contain brewers' yeast, as a source of all the B-complex vitamins, at a level of 10 per cent., replacing an equivalent amount of carbohydrate. The initial hemoglobin values in this group averaged 14.5 grams, rose to a peak of 19.8 grams after 17 weeks on the diet and then returned to a value around 18 grams, a level which was maintained throughout the rest of the experiment.

Group IV—composed of 4 puppies—received the same yeast control diet as group III, but in addition these animals received the eight synthetic vitamins given to group II and in the same daily amounts. Here the average initial hemoglobin was 13.5 grams with a peak averaging 20.5 grams after 21 weeks and a subsequent return to a value around 18 grams. Fig. I illustrates the contrast in behavior between the animals receiving only synthetic vitamins and their yeast controls.

One puppy of group I after being depleted of vitamin B_6 was subsequently treated with vitamin B_6 and later with brewers' yeast. The results substantiated the findings in groups II and III. At the end of the depletion period of 36 weeks this dog had a Hgb. of 6 grams, a red blood count of 4,570,000 and a hematocrit of 18.5 volumes per cent. Treatment with vitamin B_6 resulted in the usual prompt rise in hemoglobin, which increased 4 grams in 6 days with a corresponding rise in the other blood values. The hemoglobin then gradually rose to 15.5 grams in 10 weeks with a corresponding RBC of 6,720,000 and a hematocrit of 41.9. The hemoglobin could not be

[13] In preliminary experiments (in collaboration with Dr. R. M. Tomarelli) hydroquinone monobenzyl ether (0.5 per cent. in the diet) has no effect on the size of the thyroid in rats when administered for fourteen days. In this connection it should be noted that the effect of various antioxidants depends, to a large extent, on the substrate on which they are tested.

[1] Evidence that vitamin factors found in brewers' yeast in addition to vitamin B_6 are essential for maintaining hemoglobin in the dog.

[2] Only two adult dogs were used, one in Group I, the other in Group III.

[3] P. J. Fouts, O. M. Helmer, S. Lepkovsky, and T. H. Jukes, *Jour. Nutrition*, 16: 197, August, 1938.

[4] P. J. Fouts, O. M. Helmer, and S. Lepkovsky, *Am. Jour. Med. Sci.*, 199: 163, 1940.

[5] H. J. Borson and R. S. Mettier, *Proc. Soc. Expt. Biol. and Med.*, 43: 429, 1940.

[6] J. M. McKibbin, A. E. Schaefer, D. V. Frost and C. A. Elvehjem, *Jour. Biol. Chem.*, 142: 77, 1942.

[7] Mineral salt mixture: Bone meal (steamed), 57.8 per cent.; sodium chloride, 24.4 per cent.; lime stone (oyster shell flour), 12.2 per cent.; iron sulfate (U.S.P.), 3.7 per cent.; magnesium oxide (U.S.P.), 1.2 per cent.; copper sulfate (reagent), 0.3 per cent.; manganese sulfate (reagent), 0.1 per cent.; zinc oxide (reagent), 0.1 per cent.; cobalt carborate, 0.1 per cent.; potassium iodide, 0.1 per cent.

maintained at this level and fell gradually over a period of 6 weeks to 12 grams (RBC 5,580,000, hematocrit 37 vols. per cent.), after which it began to rise under the influence of the yeast treatment. After 13 weeks on this régime a hemoglobin value of 18.5 grams with a red count of 7,500,000 and hematocrit of 46.8 vols. per cent. was obtained. The peak values were reached after 19 weeks on the yeast diet and were maintained for 14 weeks, the hemoglobin not falling below 20 grams during this time. After that the hemoglobin fell slightly and was maintained at approximately 18 grams throughout the remainder of the treatment period of one year.

The vitamin B_6 therapy had no discernible effect on the general condition of this dog. The animal weighed 5 kilograms at the beginning of the treatment and at the end of 100 days, or approximately 14 weeks, the weight was exactly the same. There was no improvement in appetite, vitality, condition of the skin, which had a severe fungus infection, or the general alopecia. The effect of the yeast treatment on the general condition of the animal as well as the hematologic changes in the blood elements was almost miraculous. In 16 weeks the dog had doubled its weight, and almost doubled its hemoglobin and hematocrit, completely cleared up the fungus infection and grown a sleek

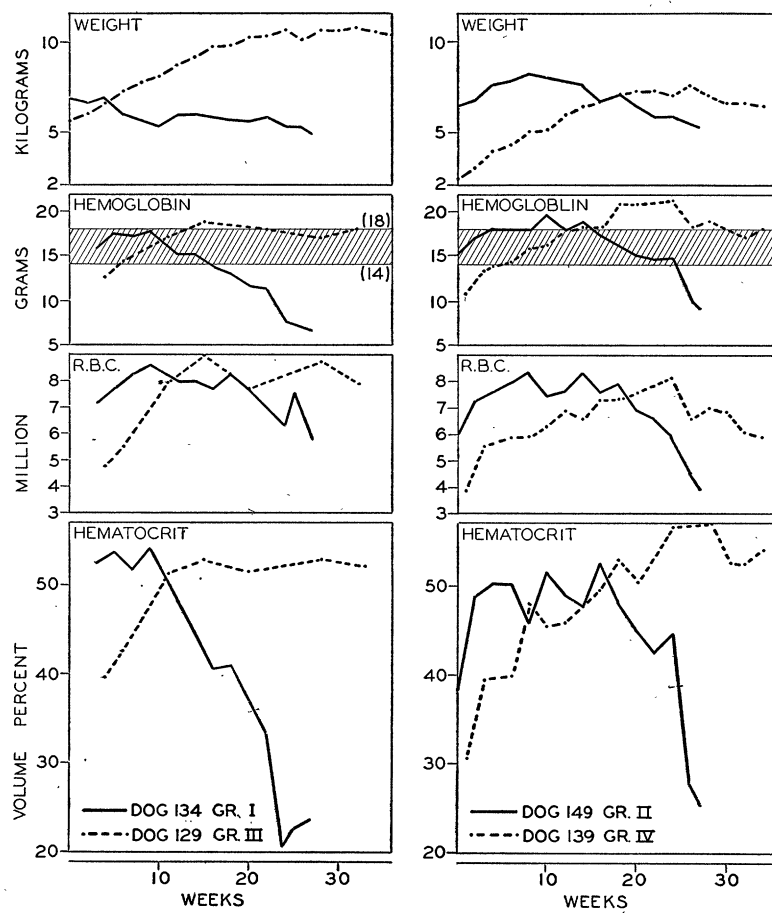

TABLE I

Gr	No dogs	Weeks to peak	Weeks on expt.	Diet	Weight			Hemoglobin			Red count		Hematocrit		
					I	H	F	I	H	F	I	H	F	H	F	I
					Kilo			grams			million		vol per cent.		
I	9	6	22	BD + (V–B_6)	5.6	5.8	4.6	15.2	18.8	7.7	6.36	8.36	5.12	47.2	26.5	52.7
II	5	4	27	BD + V	4.9	5.9	4.0	14.5	18.6	9.4	5.84	8.16	4.78	40.1	30.9	52.1
III	4	15	53	C. D.	5.2	10.6	9.6	14.5	19.7	18.0	5.98	8.92	7.83	39.8	51.6	55.8
IV	4	10	36	CD + V	4.5	11.5	11.1	13.5	20.0	19.2	5.36	8.25	7.72	37.8	54.5	54.5

I = Initial H = High F = Final

healthy coat over areas that appeared miserably "moth eaten" at the time the treatment started. The changes in this animal served as a dramatic illustration of the importance of administering the entire B complex which is to be found only in natural sources.

Although vitamin B_6 therapy in the case of a dog with vitamin B_6 deficiency anemia produces a dramatic and apparently specific effect, yet it has been observed that B_6 alone will not bring the hemoglobin back to normal. Borson and Mettier[5] found that some of the filtrate factors were needed for more complete blood regeneration in their B_6 deficient dogs, and McKibbin and his associates[6] found that certain factors present in liver were needed before normal hemoglobin values ("13–14" grams) could be reached in their deficient animals. This was also borne out in Wintrobe's study[8] with vitamin B_6 deficient swine. In the present study the giving of all the other synthetic B-complex factors in adequate amounts was sufficient to enable the addition of vitamin B_6 alone to bring the hemoglobin back to 15.5 grams but not to maintain it at this level. Brewers' yeast at a level of 10 per cent. not only maintained the hemoglobin level but kept it at a constant value considerably higher than the accepted normal of 14.0 grams.[9] These extremely high values in the yeast-treated dogs, *i.e.*, 18–20 grams, as compared with 14 grams were viewed with some scepticism at first, but they have been repeatedly confirmed when checked in other laboratories of this hospital. The observance of hemoglobin values from 4–5 grams higher than those usually found in dogs probably reflects the fact that dogs under natural conditions of living never receive an optimum supply of the vitamin-B complex. It is not urged that this is essential, but it is probably of value in situations not fully understood at the present moment.

From these data it is concluded that even though a diet lacking vitamin B_6 results in a typical hypochromic anemia in dogs which responds specifically to vitamin B_6 treatment, vitamin B_6 alone or even combined with the known synthetic factors of the B complex is not sufficient to maintain the hemoglobin at optimum levels. There is at least one factor, possibly more, in brewers' yeast in addition to vitamin B_6 which serves to stimulate hemoglobin production in the dog.

ACKNOWLEDGMENTS

We are indebted to Merck and Company, Rahway, N. J., for the Vitamin B_6 and calcium pantothenate used in these studies, to the Fleishmann Laboratories, Standard Brands, Inc., New York City, for the brewers' yeast and to Lederle Laboratories for the material used in immunizing the puppies against distemper.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

TECHNIQUE FOR STROBOSCOPIC STUDIES OF INSECT FLIGHT[1]

SINCE the application of the Edgerton stroboscope[2] to studies of insect flight,[3, 4] it has become clear that the frequency of wing-beat of standardized insects offers a rigorous technique in quantitative biology. There is reason to believe that the usefulness of such measurements will not be limited to studies of insect physiology, but may extend into other fields such as genetics and experimental pharmacology. It is therefore our purpose to describe the apparatus and technique that we have found most suitable for determining wing-beat frequency.

A survey of the flight of numerous insects has demonstrated that *Drosophila* is a most favorable experimental animal. Advantages offered by this genus include the year-round availability of numerous species and varieties and the generally consistent response under the experimental conditions, as well as the large body of information which already exists in regard to their structure and physiology. Furthermore, wing-beat frequency varies not only with the age and sex of the individual[5] but also with the temperature at which they are reared and flown,[6] so that these factors must be carefully controlled. The use of homogeneous, inbred strains is also highly desirable.[6] Procedures for regulating these details are better established and more easily applied for *Drosophila* than for most other insects. While the small size of *Drosophila* may be a hindrance in some types of work, as in analysis of chemical changes during flight, it simplifies the problem of mounting specimens for observation of wing movement. The relatively stiff

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[1] This study was aided by a grant from the Josiah Macy Junior Foundation.

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