Growth of Dogs on Purified Diet Plus Aureomycin and/or Vit. B_{12}. (19637)

LOTTE ARNRICH, EUNICE M. LEWIS, AND AGNES FAY MORGAN.
From the Department of Home Economics, University of California, Berkeley.

The basal diets used in nearly all the published studies on the effect of antibiotics on growth were practical mixtures of grains, soybeans and other crude feeds. Only one or two reports(1) have indicated the effectiveness of the antibiotics in chicks when purified adequate diets were given. The question of the value of addition of antibiotics to complete rations, adequate for normal growth of young animals maintained under sanitary conditions and disease-free, has been raised by Speer *et al.*(2). A study of the mechanism of any growth stimulation obtained under these conditions might throw further light on the problem. The reasons usually suggested for the beneficial effects observed are the production of favorable, or inhibition of unfavorable intestinal microflora under the influence of the antibiotics. This may result in the prevention of loss of dietary vitamins present in marginal amounts or in the production of additional vitamins favorable to the host.

Since in the dog intestinal bacterial activity is apparently less intense and varied than in some other non-ruminants(3) a study of the effect of antibiotics on dog growth might be illuminating. Good growth can be obtained in dogs with rather simple purified diets and the performance of the strain of cocker spaniels maintained in this laboratory for several generations on these diets has been recorded (4). The experiment was set up therefore to determine whether an antibiotic, aureomycin, when added in crystalline form to such a purified complete diet could increase the rate of growth. Vitamin B_{12} was also given to a group of animals both with and without the aureomycin, although there was no indication that these dogs required such an addition to this diet. An attempt was also made to determine the composition of the growth.

Experimental procedure. Two litters of weanling cocker spaniel puppies from the laboratory colony, weighing 1.5 kg were maintained on the experimental diet for approximately 20 weeks. Each dog was caged individually. The dogs were weighed 3 times per week and the amount of food given daily was based on the body weight, 50 g per kg at first, and about 25 g at the end of the period. The composition of the diet, given in a previous publication(4), insured generous provision of protein, 20% vitamin-free casein, all B vitamins including 60 mg per kg per day of choline and 30 of inositol, but no vit. B_{12}, and all necessary fat-soluble vitamins. Four groups were formed and fed as follows: I. The basal diet only, II. The basal diet containing 5 μg crystalline vit. B_{12}* per 100 g, III. The basal diet containing 5 μg crystalline vit. B_{12} and 10 mg crystalline aureomycin† hydrochloride per 100 g, IV. The basal diet containing 10 mg crystalline aureomycin hydrochloride per 100 g. Blood was taken from the femoral vein or artery at 4, 7, 10, 13, and 16 week intervals for determination by the usual methods(4) of the hemoglobin, non-protein nitrogen and serum proteins content. Three nitrogen balance periods of 4 days each were done. Periodic measurements of skin fold thickness were made by shaving an area in the thoracic region of the back and measuring the skin fold with a mm micrometer. On sacrifice, a lethal dose of sodium nembutal was administered intravenously, and as soon as the animal was relaxed, the jugular vein was severed for complete bleeding. The viscera were removed and each organ weighed separately. All visible fatty tissue in the abdominal and thoracic cavities was removed, pooled and weighed. The fatty tissue was dried in a vacuum oven at 60°C to constant weight and the fat content determined by continuous Soxhlet extraction for 72 hours with petroleum ether (boiling point 30-60°). Specific gravity determinations on the eviscerated animal were

* Crystalline vit. B_{12}. Lederle Laboratories Division, Cyanamid Co., Pearl River.

† Aureomycin HCl crystalline. Lederle Laboratories Division, American Cyanamid Co., Pearl River.

made by the water displacement method of Rathbun and Pace(5), and the per cent body fat calculated by their formula proposed for the conversion of human body specific gravity to the corresponding fat percentage. These authors proposed 2 formulas, one applicable to guinea pigs that was developed on the basis of actual determinations, and one for humans derived from the guinea pig formula. The difference between the two lies in the value for the specific gravity of body fat characteristic of the species. Since the average value for the specific gravity of dog body fat based on determinations from 5 animals used in this study was identical with that found for humans, we felt justified in using the following formula of Rathbun and Pace for calculating the per cent body fat from specific gravity determinations: $\%\ \text{fat} = 100\left(\frac{5.548}{\text{Sp. gr.}} - 5.044\right)$. The specific gravity of the fat of the 5 dogs was found to be 0.9164, 0.9150, 0.9210, 0.9206, 0.9120, mean 0.9170.

Results. No differences in hemoglobin, nonprotein nitrogen or serum protein levels were found among the 4 groups. As has often been noted the hemoglobin and serum protein levels increased steadily with age in all cases, with some tendency toward greater increases in the globulin than in the albumin fraction. All of these values were normal. The hemoglobin increased from 13% at 4 weeks on the diet to 15.5 at 16 weeks, and the serum protein from 4.4% at 4 weeks on the diet to 5.0 at 16 weeks. All of the dogs were in positive nitrogen balance, retaining on the average 651 mg per kg per day at 9 weeks on the diet, 447 at 13 weeks, and 348 at 16 weeks. No differences in intakes or retention were found among the diet groups and little variation in the individual animals.

Growth and body composition. There was a greater gain in weight shown by 2 of the 3 dogs fed vit. B_{12}, by 3 of the 4 given both vit. B_{12} and aureomycin, and by one of the 2 given aureomycin alone, than by the 2 control dogs. Unfortunately the sexes were not evenly divided among the groups but the normal performance of these young dogs has often been recorded. One animal in each of the 3 experimental groups lagged behind the others, although not behind the control group. The utilization of food for growth was slightly better in all the vit. B_{12} and aureomycin-fed dogs than in the controls, again excepting the same 3, which merely equalled the control performance (Table I). The eviscerated weights followed the same pattern, as did the carcass specific gravities. Three of the animals, 2 in the aureomycin plus vit. B_{12} group and one in the aureomycin group, were not sacrificed and these latter data are therefore not available for them. The 2 controls and the similar dogs given vit. B_{12} or aureomycin and vit. B_{12} had specific gravities ranging from 1.048 to 1.041, the other 4 overweight animals 1.031 to 1.013 (Table I).

When the carcass fat was calculated according to the formula of Rathbun and Pace(5) all of the experimental carcasses yielded higher value than did the 2 controls. Even dogs 5♂ and 8♀ which grew no better than the controls had slightly higher fat contents. The fat-free weights, moreover, of all but one of the dogs in the 3 experimental groups were no different from those of the controls. The one exception, 3♂, which had significantly greater fat-free weight than did the controls was the largest of the 11 dogs and the proportion of fat calculated for the live weight was the largest of the 8 animals sacrificed. The adipose tissue of the overgrown dogs was likewise richer in fat than in the controls and again this was true even though 2 of the former had no greater live weights than the latter.

The thickness of skin folds as suggested by Keys *et al.*(6) may be considered an indication of amount of subcutaneous fat. Only 3 of the dogs, 2 on vit. B_{12} and one on aureomycin, had obviously increased skin fold thickness over those of the controls. The heaviest dog, 3♂, showed normal fold thickness. For this group of dogs skin fold thickness did not correlate well with adiposity as measured by other means.

Discussion. Although the food intakes of these dogs were maintained equal in proportion to body weight and there was no difficulty of appetite in any case, the gain in weight varied from 18 to 25 g per 100 g food intake. Two of 3 dogs on the vit. B_{12} dose, 3 of 4 on

TABLE I. Growth and Body Composition of Young Dogs Fed a Purified Diet for 20 Weeks With and Without Vit. B_{12} and/or Aureomycin.

Diet, dog No., sex	Final live wt, kg	Total gain, kg	Gain/100 g food, g	Eviscerated wt, kg	Specific gravity	Carcass fat		Fat free wt, kg	Adipose tissue		Skin fold thickness, mm
						%	Total, kg		Wet wt, g	Fat, %	
Control											
1 ♀	6.3	4.3	18	4.9	1.048	25	1.57	4.7	145	76.5	4.3
9 ♀	6.7	4.5	19.2	5.3	1.042	28	1.88	4.8	162	59.7	5.5
Vit. B_{12}											
4 ♀	7.4	5.3	21.2	5.9	1.021	39	2.89	4.5	216	79.6	7
5 ♂	6.9	4.6	19.2	5.4	1.038	30.1	2.08	4.8	172	83.5	4.1
10 ♂	8.3	6.3	24.7	6.4	1.018	40.6	3.37	4.9	327	82.3	9.3
Vit. B_{12} + aureomycin											
2 ♀	8.3	6.5	24.1								
8 ♀	6.6	4.8	20.4	5.3	1.041	28.5	1.88	4.5	165	85.2	4.8
3 ♂	9.3	7.3	23.2	7.4	1.013	43.3	4.03	5.3	447	89.3	5.7
6 ♂	9	7.3	25								
Aureomycin											
11 ♀	7.6	5.5	20.5	6.1	1.031	33.7	2.56	5	294	85.2	8.5
7 ♀	6	4.2	18.1								

vit. B_{12} plus aureomycin, and one of 2 on aureomycin exceeded the control animals in total weight gain and in empty carcass weight. The specific gravity of the carcasses of these dogs was lower, the per cent carcass fat was higher, the adipose tissue richer in fat and in 3 cases the skin folds thicker than in the 2 controls and the 2 other dogs which resembled the controls in performance.

With such a small number of animals it is obvious that no certainty exists as to the reasons for these differences. In some cases vit. B_{12} and/or aureomycin caused better than usual feed utilization with production of extra body fat accounting for the weight additions. No corresponding increases in nitrogen retention were found nor was there any change in the composition of the nitrogenous blood constituents. If the intestinal microflora were involved in the extra fat deposition seen in these dogs it must have been through the stimulation by aureomycin of production of vit. B_{12} since one dog, 11♀, which received aureomycin but no vit. B_{12} accumulated extra fat. The other 5 overgrown dogs received vit. B_{12}. If this is true this is the first indication that young dogs fed a casein-rich diet may need dietary vit. B_{12}.

Determination of the composition of the increased growth produced by feeding antibiotics in chicks, turkeys and swine, not so far reported, is of obvious interest.

Summary. Eleven young cocker spaniels from 2 litters were fed from weaning for 20 weeks a purified complete diet which had been shown previously to produce optimum growth. Vit. B_{12} at the level of 5 μg %, aureomycin hydrochloride at the level of 10 mg % or both these substances were added to the diet of some of the animals. No differences resulted in nitrogen retention or in the nitrogenous constituents of the blood of any of the dogs. Two of the 3 dogs which received vit. B_{12}, 3 of the 4 which received both vit. B_{12} and aureomycin and one of the 2 which received only aureomycin gained more weight per 100 g food than did the 2 controls and the 3 others. These overgrown dogs had greater total gains, greater empty carcass weights, lower body specific gravity, more carcass fat, and more fat-rich adipose tissue than the 5 others. However, the fat-free carcasses were not significantly heavier. It is concluded that better food utilization may result in dogs in some cases from the addition of vit. B_{12} and/or aureomycin to a complete purified diet and that this growth is due solely to increased fat deposition.

1. Moore, P. R., Evenson, A., Luckey, T. D., Mc-

Coy, E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1946, v165, 437.

2. Speer, V. C., Vohs, R. L., Catron, D. V., Maddock, H. M., and Culbertson, C. C., *Arch. Biochem.*, 1950, v29, 452.

3. Axelrod, H. E., Gullberg, M. G., and Morgan, A. F., *Am. J. Physiol.*, 1951, v165, 604.

4. Mabee, D. M., and Morgan, A. F., *J. Nutrition*, 1951, v43, 261.

5. Rathbun, E. N., and Pace, N., *J. Biol. Chem.*, 1945, v158, 667.

6. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., Taylor, H. L., The Biology of Human Starvation, vol. I, 162, 1950, University of Minnesota Press, Minneapolis.

Received April 15, 1952. P.S.E.B.M., 1952, v80.

Complement-Fixing Murine Typhus Antibodies in Vitamin Deficiency States.* III. Riboflavin and Folic Acid Deficiencies. (19638)

KENNETH WERTMAN, FRANCIS D. CRISLEY, AND JOSEPH L. SARANDRIA.
(Introduced by F. S. Cheever.)

From the Division of Bacteriology, Department of Biological Sciences, University of Pittsburgh.

The purpose of this investigation, and others in progress, is an attempt to find an explanation for the increased susceptibility and mortality that occurs in malnourished animal and human populations exposed to typhus(1) and other rickettsial and bacterial infections. Wertman and Sarandria(2) reported that pantothenic acid and thiamine deficiencies influenced the antibody response when a relatively small amount of immunizing material was injected. However, the influence was not as apparent when relatively large amounts of the antigenic material was injected. In addition, they also reported(3) a severe impairment of antibody response in pyridoxine deficient rats when a rickettsial antigen (*Rickettsia typhi*) containing 0.0073 mg N was injected. However, rats deficient in nicotinic acid did respond and produce complement-fixing antibodies when injected with the same amount of antigen. Stoerk, Eisen, and John(4) reported normal serum antibody titers in thiamin, riboflavin and pantothenic acid deficiencies. However, Axelrod *et al.*(5) noted a greatly decreased titer in pantothenic acid, pyridoxine deficiencies and variable but lower titers in riboflavin deficiencies. Ludovici and Axelrod(6) reported severe impairment of antibody response in the pteroylglutamic acid-deficient rats and a moderate impairment in vit. A and niacin-tryptophan deficiencies. These investigators employed a 10% suspension of washed Group O, Rh positive human erythrocytes in physiological saline for antibody stimulation and measured the hemagglutinin production. The present paper deals with the effects of riboflavin, and folic acid deficiencies upon complement-fixing antibody production under similar experimental conditions(2,3).

Experimental. Male weanling albino rats of the Sprague-Dawley strain, distributed into groups as indicated in Table I, were housed individually in wide-meshed, screen-bottom cages and weighed at regular intervals. The ingredients used in preparing the different diets were obtained from General Biochemicals, Inc., and Nutritional Biochemicals Co. All ingredients were of the highest purity obtainable and each basal diet was formulated so that only a minimum amount of the B complex vitamins to be studied were present. The basal diet for the riboflavin groups was as follows: Sucrose, 58.75%; casein (vitamin-free), 25.00%; vegetable oil (hydrogenated), 10.00%; salt mixture No. 2 (U.S.P.), 4.00%; corn oil (Mazola), 2.00%; choline chloride, 0.20%; i-inositol, 0.03%; paraminobenzoic acid, 0.01%; dl-alpha-tocopherol acetate, 0.01%; and 2-methyl-1,4-naphthoquinone, 0.001%. The basal diet for the folic acid groups was as follows: Sucrose, 57.77%;

* This investigation was partially supported by a research grant from the Upjohn Co., Kalamazoo, Mich.