Therefore, appearance of serum inhibitors cannot be considered by itself as evidence of infection with a member of the EMC group of viruses. As a matter of fact, the failure of human sera to neutralize the virus seems to prove that the inhibiting capacity is not due to the presence of specific antibodies and, thus, to exclude definitely the assumption of EMC virus as an etiologic factor in the cases in question. The inhibitor found might still be an antibody, although heterologous, or it might be a non-specific substance appearing in the blood in connection with disturbances of the central nervous system. This question will be the subject of future experiments.

Summary. 1. Hemagglutination of sheep red blood cells by the Col-MM strain of the EMC virus was inhibited by polyvalent anions, the inhibitor titers (HI-titers) of the different negative ions being roughly proportionate to their net electrical charge. 2. Whereas the HI-titer of specific antibody was inversely proportionate to the amounts of virus, indicating a stoichiometric combination of virus and antibody, the HI-titers of sodium hexametaphosphate (6-valent anions) were only slightly influenced by the quantity of virus added. The order of mixing of the reactants was of great importance for the serum inhibition but without effect on the activity of hexametaphosphate. 3. The strong hemagglutination-inhibiting effect of sodium hexametaphosphate was not paralleled by any virus neutralizing activity. 4. A standard serological HI-test, based on the results of a study of the Col-MM virus hemagglutination, is described. Applied on 569 human sera, this method gave a high incidence (12.5%) of strongly positive hemagglutination-inhibition titers among 384 sera obtained from patients showing distinct involvement of the central nervous system. Weakly positive reactions were encountered in 14.3%. A control group, consisting of 146 sera collected from healthy humans showed only 0.7% strong and 11% weak reactions, while 39 sera from patients with various ailments but apparently free from neurotropic infections were negative throughout. 5. Even strongly inhibiting human sera failed to neutralize Col-MM virus in animal experiments.

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Effect of Various Levels of Vitamin B_{12} Upon Growth Response Produced by Aureomycin in Chicks. (18391)

E. L. R. STOKSTAD AND T. H. JUKES.

From the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

It was reported by Stokstad and coworkers(1) that the growth of vit. B_{12}-deficient chicks was increased by the addition of a crude supplement prepared from fermentation with *Streptomyces aureofaciens*. The presence of vit. B_{12} and B_{12b} in this fermentation was subsequently demonstrated (2) and it was shown that the supplement also contained aureomycin which was found to increase the growth rate of chicks on diets either adequate(3,4) or deficient(5) in vitamin B_{12}. It thus became evident that either aureomycin or vit. B_{12} would produce a growth response in chicks on an all-vegetable diet and some evidence was obtained(5) which suggested that aureomycin had a spar-

1. Stokstad, E. L. R., Jukes, T. H., Pierce, J., Page, A. C., Jr., and Franklin, A. L., *J. Biol. Chem.*, 1949, v180, 647.

2. Pierce, J. V., Page, A. C., Jr., Stokstad, E. L. R., and Jukes, T. H., *J. Am. Chem. Soc.*, 1949, v71, 2952.

3. Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 523.

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5. Stokstad, E. L. R., and Jukes, T. H., Paper presented at 117th meeting of *Am. Chem. Soc.*, Philadelphia, Pa., April, 1950.

ing action on the requirements of the chick for vit. B_{12}. It seemed of interest to investigate more fully the effect of aureomycin on the vit. B_{12} requirement of the chick.

Methods. Chicks (Barred Rock x New Hampshire) were obtained from eggs laid by hens on a corn-soybean-alfalfa diet as described previously(1) and were fed the experimental diets immediately. The chicks were markedly deficient in vit. B_{12}, but the severity of the deficiency varied from month to month as indicated by variations in rate of growth of chicks on the basal diets. Such variations are probably reflections of differences in the extent of carry-over of vit. B_{12} from the hen through the egg to the chick. This carry-over may change although the diet fed to the hens is kept constant, for it has been shown that vit. B_{12}-like factors are produced by microorganisms which are present in the feces(6) and coprophagy by the hens may be presumed to occur. The production of such factors in the litter of the henhouse is apparently influenced by the environmental temperature(7). Basal diets 1 (30% soybean meal) and 2 (70% soybean meal) which were supplemented with minerals, 0.3 DL-methionine and vitamins including 0.2% choline chloride have been previously described(3). Aureomycin HCl was recrystallized either twice or thrice before use.* Its vit. B_{12} content was found to be less than 0.2 μg per g by microbiological assay with *Lactobacillus leichmannii*.† An assay of a sample with *Euglena gracilis* indicated that the content was 0.1 μg per g according to a communication from Dr. W. J. Robbins. Solutions of vit. B_{12} for addition to the diets were prepared from crystalline compound and were standardized spectrophotometrically at 261 mu. $E^{1\%}_{1cm} = 208$.

TABLE I. Effect of Aureomycin on Growth at Different Levels of Vitamin B_{12}.

Exp. No.	% increase in growth* due to 100 mg aureomycin per kg diet at various levels of vit. B_{12}						Evidence of B_{12}-sparing action
	0 μg B_{12}	3 μg B_{12}	5 μg B_{12}	10 μg B_{12}	20 μg B_{12}	50 μg B_{12}	
1	−1			18	13	23	No
2	2		37	17		9	Yes
3	34		18	11	11		Yes
4	6	−4	12	11	17	15	No
5	10				9		No

* Weighed at 25 days. Diet 2 (70% soybean meal) was used in Exp. 1, 2, 3, and 4, and diet 1 (30% soybean meal) in Exp. 5.

Results. Various experiments were made in which graded levels of vit. B_{12} were fed to chicks in the presence and absence of aureomycin. The results, summarized in Table I, show the growth increases produced by aureomycin at different levels of vit. B_{12}. A sparing action of aureomycin on vit. B_{12} was noted in some experiments, such as Exp. 2, but not in others. In Exp. 6, Table II, 10 μg of vit. B_{12} in the presence of aureomycin gave almost the maximum response obtained with the vitamin but in the absence of aureomycin, 50 μg of vit. B_{12} per kilogram of diet produced a markedly greater response than 10 μg.

Aureomycin often produced little or no growth response but markedly decreased mortality in chicks when on the basal vit. B_{12}-deficient diet or when inadequate levels of vit. B_{12} were added. The chicks at hatching were markedly deficient in vit. B_{12} due to the fact that they were obtained from eggs laid by hens which were on a diet deficient in this vitamin. A high mortality of such chicks has been noted when they are placed on diets deficient in vit. B_{12}(1,8). A summary of all experiments pertaining to the effect of aureomycin on the mortality of vit. B_{12}-deficient chicks is presented in Table III. It shows that in every case the mortality was lower when aureomycin was added. Two explanations are possible for this reduction of mortality. First, aureomycin may reduce the requirement of the chicks for vit. B_{12}. Sec-

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7. Bird, H. R., and Marvel, J. A., *Poultry Sci.*, 1943, v22, 403.

* Prepared by the Chemical Production Section, Lederle Laboratories.

† Carried out by Dr. H. P. Broquist.

8. Bird, F. H., *Poultry Sci.*, 1950, v29, 314.

TABLE II. Effect of Aureomycin on Growth of Chicks on Diet 2 at Various Dietary Levels of Vit. B_{12}.
Basal diet 1 (70% soybean meal) 16 chicks per group.

Exp. No.	Vit. B_{12} per kg diet, μg	Body wt and survivors ( ) at 25 days*		% increase in wt due to aureomycin†
		No aureomycin, g	Aureomycin added, g†	
6	0	120 (6) ± 11‡	132(10) ± 11	10
	2	120 (6) ± 9	190(14) ± 12	58
	4	201(13) ± 18	240(14) ± 15	19
	5	203(14) ± 20	260(15) ± 17	28
	10	227(13) ± 20	337(16) ± 16	44
	50	331(15) ± 10	357(16) ± 14	8
7	0	164(15) ± 14	185(16) ± 12	13
	2	220(16) ± 9	271(16) ± 11	23
	4	237(15) ± 14	284(16) ± 12	20
	10	266(15) ± 8	273(15) ± 12	3
	50	294(15) ± 15	300(16) ± 12	2
8	0	125 (2) ± 0	129(10) ± 11	3
	3	146 (7) ± 19	197(15) ± 17	35
	5	200(10) ± 23	234(15) ± 18	17
	10	253(15) ± 18	309(16) ± 16	22
	20	303(16) ± 14	347(16) ± 10	14

* Average of 2 groups of 8 chicks each.
† Exp. 6 and 7, 100 mg of aureomycin; Exp. 8, 50 mg of aureomycin added per kilo of diet.
‡ Standard error $= \sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" = deviation from mean and "n" = number of observations.

ond, the deficient chicks may have a higher susceptibility than normal chicks to the deleterious effect of certain microorganisms in the intestinal tract, and aureomycin may decrease the number of these organisms.

The effect of another bacteriostatic substance, sulfamethazine, on the requirement of chicks for vit. B_{12} was investigated and the results are shown in Table IV. No sparing effect of sulfamethazine on the requirement for vit. B_{12} was found nor was a growth-promoting effect noted for sulfamethazine.

TABLE III. Effect of Aureomycin on Mortality at Low Levels of Vit. B_{12}.

Exp. No.	Vit. B_{12} per kg diet, μg	Mortality at 21 days	
		No aureomycin, %	100 mg aureomycin per kg diet, %
1	0	25	8
2	0	58	8
	5	58	8
3	0	7	0
4	0	67	16
	5	41	8
5	0	50	31
	2	56	12
6*	0	87	25
	3	50	0
7	0	6	0
8	0	87	37
	3	56	6
	Avg	50	12

* 50 mg aureomycin added per kg diet in this experiment.

Discussion. It was shown by various investigators in studies with purified diets that the addition of bacteriostatic substances such as sulfaguanidine and succinylsulfathiazole produced a deficiency in the rat for certain water-soluble vitamins such as biotin and pteroylglutamic acid. This is usually interpreted as indicating that rats on purified diets are dependent upon their intestinal bacteria as a source of certain vitamins of the B complex. The present observations with chicks may indicate that under certain circumstances the use of an antibacterial substance may serve to increase rather than to decrease the supply of water-soluble vitamins. Moore *et al.*(9) reported that at marginal levels of pteroylglutamic acid the addition of succinylsulfathiazole produced a depression of

9. Moore, P. R., Evanson, A., Luckey, T. D., McCoy, E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1946, v165, 437.

TABLE IV. Effect of Sulfamethazine on the Response to Vitamin B_{12}. Basal diet 1 (70% soybean meal) 12 chicks per group.

	Body wt and survivors at 21 to 22 days				
	No sulfamethazine		0.5% sulfamethazine		
Vit. B_{12} per kg diet, μg	Individual groups, g	Avg, g	Individual groups, g	Avg, g	Response to sulfamethazine, %
0	116 (5) 91 (4)	103	117 (3) —	117	14
3	146 (8) 137 (5)	142	124 (4) 144 (9)	138	−4
5	159 (9) 194(11)	177	169(10) 148(10)	159	−10
10	188(12) 220(12)	204	221(11) 195(12)	208	2
20	230(12) 223(12)	227	225(10) 218(11)	222	−2
50	219(12) 245(11)	232	243(12) 227(11)	235	1

growth which may have been due to a decrease in the amount of pteroylglutamic acid which was synthesized by the intestinal bacteria. However, in the presence of an excess of dietary pteroylglutamic acid, the addition of succinylsulfathiazole produced an increase in the rate of growth, perhaps due to the suppression of certain deleterious intestinal bacteria. The effect of succinylsulfathiazole in depressing growth at marginal levels of pteroylglutamic acid may be contrasted with the effect of aureomycin in improving growth at marginal levels of vit. B_{12} in chicks in some of the present experiments. The effect of aureomycin in producing a hematological response in patients with pernicious anemia as noted by Lichtman and co-workers(10) may be compared with the effect of aureomycin in producing a growth response in chicks which were receiving an insufficient dietary supply of vitamin B_{12}. The oral administration of high levels of streptomycin to rats was reported by Smith and Robinson(11) to decrease the number of *E. coli* organisms in the digestive tract and to produce a simultaneous increase in *Bacillus megatherium*, the organism which was shown by Lewis and co-workers(12) to produce large quantities of vit. B_{12} by fermentation.

The presence or absence of a vit. B_{12}-sparing effect for aureomycin as shown by the variation in results in different experiments may be due to differences in the intestinal flora encountered. It would seem probable that variability may be expected when the growth response to a substance depends on the nature of the intestinal flora which are present in the test animal.

Summary. 1. The vit. B_{12} requirement for growth of chicks was studied on a deficient diet with and without the addition of aureomycin. In some experiments, a "sparing effect" of aureomycin upon vit. B_{12} requirement was noted but in others no effect was observed. A growth-promoting effect for aureomycin was noted both in the presence and absence of added vit. B_{12}. 2. The mortality of deficient chicks on the diet containing no added vit. B_{12} was markedly reduced by aureomycin. 3. The addition of sulfamethazine to the diet did not appear to effect the quantitative requirement of chicks for vit. B_{12}.

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