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Effect of Orally Administered Penicillin-Resistant Microorganisms on Growth of Chicks.*† (20251)

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(Introduced by G. M. Briggs.)

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The exact mechanism of antibiotic stimulation of chick growth is still not clearly understood. Most theories concerning this mechanism explain it through changes in the intestinal microflora. These have been reviewed by Briggs(1). In experiments by Romoser *et al.*(2), definite changes occurred in the number and types of bacteria found in the ceca of four-week-old chicks following the oral administration of procaine penicillin G. The numbers of *Escherichia coli* and *Aerobacter aerogenes* present in the ceca were increased when penicillin G was fed at a level of 150 ppm of the diet. The growth rate was also improved. The addition of 5-15% lactose, in combination with the antibiotic, further increased chick growth and the numbers of these organisms. Anderson *et al.*(3) observed an increase in the coliform bacteria as a result of feeding penicillin to chicks. Similar increases, though less marked, were also secured by March and Biely(4) who used aureomycin. These workers also noted a marked decrease in the lactobacilli. Preliminary reports indicate that oral administration of *A. aerogenes*(5) or *E. coli*(6) increase the growth rate of chicks fed diets containing penicillin. From this it would appear that the growth-promoting action of antibiotics may result from: 1) the elimination of fastidious organisms which compete with the host for essential known or unidentified nutrients and/or, 2) the stimulation of other organisms which affect the host beneficially through the *in vivo* synthesis of one or more growth factors.

In this study the intestinal microflora was modified by feeding viable penicillin-resistant organisms to chicks, in order to investigate further the effect on chick growth.

Materials and methods. In all experiments, 15-20 day-old New Hampshire chicks of both sexes were used per experimental group. In Exp. 1, the chicks used were the progeny of dams housed on raised wire floors and fed a ration which was complete in all known nutrients but contained no animal protein supplements. Based on other studies, these chicks were considered to have a suboptimal "carry-over" of unidentified chick growth factors. In

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TABLE I. Composition of Basal Rations* Used.

Ingredients	%
Corn-soybean meal type ration (A)	
Ground yellow corn	60.85
Soybean oil meal, solvent, 44% protein	34.
Limestone	1.
Bone meal	3.25
Sodium chloride, iodized	.3
Cod liver oil (2250 IU vit. A, 300 ICU vit. D/g)	.25
Manganese sulfate	.025
DL-methionine	.05
	mg/lb
Riboflavin	1.6
Niacin	8.
Calcium pantothenate	2.
Choline chloride	200.
2-methyl-1, 4-naphthoquinone	.2
Vit. B_{12}	.01
Oat groat-fish meal type ration (B)	
	%
Oat groats	83.
Fish meal	15.
Calcium carbonate	1.25
Sodium chloride, iodized	.3
Dry vit. A and D supplement (4000 IU vit. A, 750 ICU vit. D/g)	.2
Manganese sulfate	.02
Copper sulfate	.001
Cobalt sulfate	.0001
DL-methionine	.08
Glycine	.3
	mg/lb
Riboflavin	1.6
Niacin	16.
Calcium pantothenate	5.
Choline chloride	200.
2-methyl-1, 4-naphthoquinone	.2
Alphatocopherol acetate	3.
Pyridoxine HCl	1.6
Biotin	.025
Folacin	.2
p-aminobenzoic acid	10.
Vit. B_{12}	.002

* These rations are considered adequate in all known nutrients required by the chick. However, ration A is considered to supply suboptimal amounts of 2 different unidentified growth factors, one of which is supplied by fish meal(7). Ration B was designed to supply ample quantities of the unidentified growth factor present in fish meal but contained no soybean oil meal, which also has been reported to supply unidentified growth factor activity(8,9).

all other experiments, the chicks were progeny of dams maintained on litter and fed a complete breeder ration. Feed and water were allowed to be consumed *ad libitum*. In no instance was excessive mortality encountered. The day-old chicks were individually wing-banded, placed in electrically heated battery brooders with raised wire floors, then weighed at weekly intervals during a 4-week experimental period. All experiments were conducted in rooms previously used for rearing chicks. Bacteriological methods employed have been described previously(2). The composition of the two basal rations used is presented in Table I. Ration A was fed in Exp. 1 through 6 and ration B was used in Exp. 7 and 8. Various bacterial preparations, made as described below, were fed as supplements. In Exp. 1 the mash used was prepared by fermenting a chick growing mash with *A. aerogenes* isolated from the ceca of a four-week-old chick which had been fed procaine penicillin G. The fermentation was effected by incubating the inoculated substrate at 35°C for 3 days under anaerobic conditions. The solid and liquid portions of the substrate were then dried separately and recombined. The organism supplement used in this experiment was prepared by incubating a skimmilk culture of *A. aerogenes* for 24 hours at 35°C. Three hundred ml of the resulting ferment were then added to 200 g of sterile ground corn and air dried. In the remaining experiments, *A. aerogenes* was grown on the surface of Eugon agar (Baltimore Biological Laboratory) in Kolle flasks. After 24 hours at 37°C, the surface growth was removed from each flask with 12 ml of a 10% solution of sterile Difco skimmilk. The cell suspension was dried by lyophilization. Thus higher concentrations of viable cells were obtained. In Exp. 3, *E. coli* was grown in the same manner. In addition, *A. aerogenes* was also cultured in skimmilk for 24 hours at 37°C under aerobic conditions, after which the complete culture was lyophilized. In all experiments involving the mixtures of dried organisms and skimmilk solids, a level of 0.022% was added to the diet. This level supplied approximately 0.003% dried bacterial cells. The cultures were added to the feed at the beginning of each experiment and stored at 10°C in containers with tight fitting lids during the 4-week experimental period.

Results. The results obtained in Exp. 1 are presented in Fig. 1. The average weight of the basal group was 219 g. Growth responses were obtained when the fermented

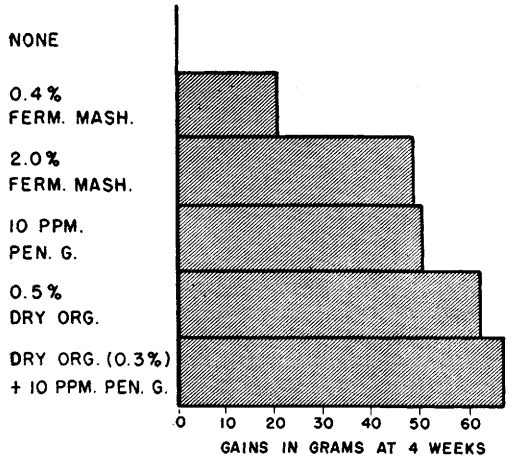

FIG. 1. Effect of *Aerobacter aerogenes* fermentation product and air dried bacterial cells on chick growth to 4 wk.

mash containing viable *A. aerogenes* was added to the diet. When the 2.0% level was used, the growth response was comparable to the addition of 10 ppm procaine penicillin G. When the preparation of dried organisms was added to the diet, alone or in combination with penicillin, slightly greater gains were observed. The relatively large gains noted here, as compared with those in the subsequent experiments, is believed to be due to the fact that the chicks used in this experiment had a lower "carry-over" of unidentified growth factors from their dams.

Exp. 2 was designed to determine the effectiveness of oral administration of lyophilized cultures of *A. aerogenes* and *E. coli*, alone and in combination with lactose and penicillin. The results of this experiment and number of viable organisms per gram of feed after 4 weeks storage are presented in Fig. 2. When lyophilized cultures of *A. aerogenes* or *A. aerogenes* plus *E. coli* were added to the basal diet without penicillin and lactose, the growth was only slightly improved. This improvement was equal to that obtained with procaine penicillin G when the culture of *E. coli* was fed. The chick growth response obtained when 3% lactose and 10 ppm procaine penicillin G were added to the basal diet is shown by the line bisecting the chart. When the cultures were fed in combination with penicillin and lactose chick growth was further stimulated, and in all cases exceeded that obtained with penicillin and lactose supplements alone. The results obtained by feeding lyophilized *A. aerogenes* and *E. coli* indicate that these viable cultures exerted a growth promoting effect particularly in the presence of the antibiotic.

In addition to the experiments described above, ration A was used in three other trials. Two experiments also have been performed in which ration B and *E. coli* cultures were employed. The results of all experiments are

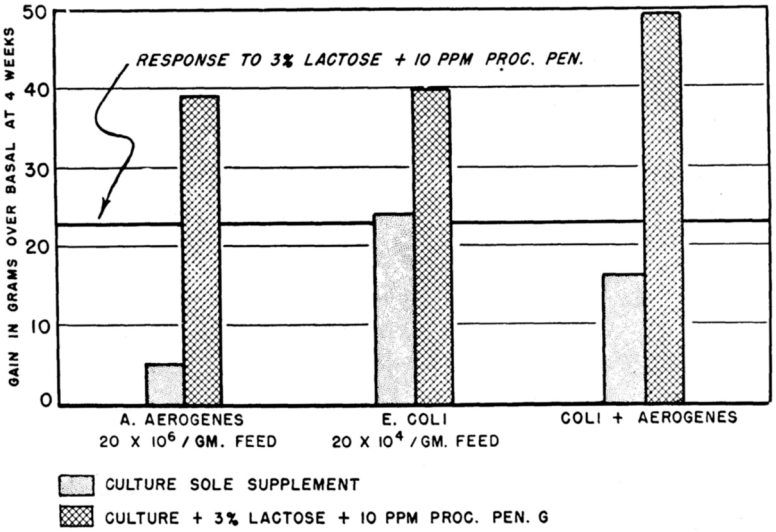

FIG. 2. Effect of lyophilized *Aerobacter aerogenes, Escherichia coli*, and their combination, with and without lactose and penicillin on chick growth to 4 wk.

summarized in Table II. Although the supplements containing viable organisms produced little growth response and in several instances seemed to cause a slight depression in growth when no antibiotic was fed, these cultures did improve the growth rate in every case when 10 ppm of procaine penicillin G were also fed. The average growth increase obtained with the addition of 10 ppm of procaine penicillin alone to rations A and B was 30 and 42 g, respectively. Further addition of viable organisms in the presence of the antibiotic resulted in average growth increases of 54 and 69 g over that obtained with basal rations A and B. Consequently, the growth promoting effect of the antibiotic was increased an average of 80 and 64% when viable cultures of *A. aerogenes* and/or *E. coli* were added to rations A and B. The average increase in chick weight in all 6 experiments, which resulted from the addition of cultures of viable organisms to ration A with added penicillin is statistically significant to the 1% level as measured by analysis of variance. These results reveal, therefore, that continuous feeding of certain viable penicillin-resistant bacterial cells was effective in promoting chick growth when procaine penicillin G was present in the ration.

TABLE II. Summary of Experiments Showing Effects of Viable Penicillin Resistant Microorganisms on Chick Growth to 4 Weeks with and without Antibiotic, 8 Exp.

Exp. No.	Without antibiotic; no added organisms	Organisms added	10 ppm procaine penicillin G: No added organisms	10 ppm procaine penicillin G: Organisms added
		Ration A		
1	219 (15)	282 (14)	270 (17)	285 (13)
2	312 (20)	328 (16)*	336 (19)	361 (16)
3	309 (27)	320 (14)†	349 (15)	384 (13)
		321 (14)‡		384 (14)
		351 (16)§		353 (15)
4	304 (18)	284 (18)	318 (20)	355 (17)
5	310 (18)	319 (20)	353 (20)	358 (20)
6	345 (18)	329 (18)	350 (18)	361 (14)
Avg	299	318	329	353
		Ration B		
7	261 (17)	267 (17)‖	307 (15)	333 (17)
8	268 (18)	258 (19)‖	307 (19)	335 (18)
Avg	265	263	307	334

* 0.022% each of lyophilized *E. coli* and *A. aerogenes* obtained from Kolle flask surfaces was added to diet.

† A lyophilized *A. aerogenes* culture obtained from Kolle flask surfaces was added to diet.

‡ A lyophilized skimmilk culture of *A. aerogenes* which contained 100 u of procaine penicillin G prior to fermentation added to diet.

§ A lyophilized skimmilk culture of *A. aerogenes* which contained no antibiotic prior to fermentation added to diet.

‖ Diet supplemented with a lyophilized culture of *E. coli* obtained from Kolle flask surfaces.

Figures in parentheses refer to No. of surviving chicks.

Discussion. Anderson, Slinger, and Pepper (6) have also obtained preliminary results similar to some of the observations reported previously(5) and in this paper. These investigators fed nutrient broth cultures of a mutant strain of *E. coli* to chicks and poults, adding them daily to the feed. The *E. coli* cultures promoted significant growth increases when fed in the presence of penicillin.

Continuous feeding of certain viable bacterial cultures to chicks accentuates the growth-stimulatory action of orally administered procaine penicillin G. This strongly indicates that the bacterial population in the intestinal tract exerts a marked effect on antibiotic function. The finding that antibiotics have no growth promoting properties when fed to chicks reared in germ-free environment(10) or in environments not used previously for rearing chicks(11,12) also support this view.

Although both *A. aerogenes* and *E. coli* are known to synthesize certain nutrients, one cannot conclude from these results that intestinal synthesis of unidentified nutrients is necessarily involved. Since significant stimulation in growth was obtained with cultures supplying such small amounts of dry matter, it is felt that the results obtained were due to effects of the viable organisms in the intestine rather than to the presence of unidentified nutrients in the cultures fed. Jones and Combs(13) reported that either penicillin or aureomycin were effective in sparing the dietary requirement of chicks for unidentified growth factors. Scott and Jensen(14) have reported recently that aureomycin appeared to spare the requirement of poults for an unidentified growth factor not present in fish meal to a greater extent than for the unidentified factor supplied by fish meal. It is also

possible that the continuous feeding of *A. aerogenes* and *E. coli* materially aided the antibiotic in reducing the numbers of less desirable organisms in the intestinal tract, thereby permitting more rapid chick growth.

Summary. Pure cultures of *E. coli* and *A. aerogenes* were grown, lyophilized, and fed to chicks as dietary supplements both in the presence and in the absence of procaine penicillin G. Little or no chick growth response was obtained when either of these organisms were added to the ration in the absence of the antibiotic. Greater gains were obtained when 10 ppm procaine penicillin G were fed. When viable cultures of *A. aerogenes* and *E. coli* were fed in combination with penicillin, growth was further increased significantly. The effectiveness of the antibiotic in promoting chick growth was increased 64 and 80% when these organisms were added to the feed. The results obtained illustrate the influence of bacterial environment on the antibiotic growth effect and in nutritional studies.

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Skim Milk Powders and Experimental Rat Caries. (20252)

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In a previous publication(1) it was reported that a diet containing 4 heat-processed cereal foods and 18% cerelose produced dental caries in white rats. The results were important because of the type of caries which developed on the smooth surfaces of the teeth, and because neither an excessive quantity of sugar nor coarse food particles were implicated as cariogenic factors. Moreover, the results suggest that serious attention should be given to the possibility of an effect of nutritive deterioration which may accompany the heat-processing of foods, on the etiology of experimental dental caries in white rats.

Perhaps no constituent of the diet has been more widely studied for the effects of heat-processing, than have the skim milk powders. Such studies are of obvious importance when it is realzed that in 1951 approximately 700,000,000 lb of skim milk powder, and 130,000,000

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