TABLE III. Electrophoretic Distribution of Plasma Proteins in Pulmonary Tuberculosis before and after ACTH Therapy. (Last patient, Table II.)

Date	ACTH admin., mg/day	Albumin, %	Fibrinogen, %	Gamma globulin, %	A/G ratio	Total protein, g %
8– 8–49	Control	34.9	13.2	16.2	.67	6.72
9–11	100					
12–15–18	75	41.3	8.4	16.0	.82	6.65
19–25	100	43.2	9.4	14.2	.91	5.86
9–12–15		47.2	8.0	12.6	1.05	6.62
16–22	75					
23	25					
24–26	0	35.4	14.6	13.6	.86	5.91

Summary. 1. Albumin level and A/G ratio of pulmonary tuberculosis patients are reduced from normal value. 2. Levels of gamma globulin, fibrinogen, and alpha 1 globulin are considerably higher than those of normal individuals. 3. ACTH depresses the gamma globulin and fibrinogen, and raises the albumin values and A/G ratio toward normal levels. Gamma globulin is less susceptible to immediate changes than the latter two components. 4. Cessation or reduction of ACTH therapy brought about a reversion of protein fractions to values similar to those noted in control samples. 5. Values for alpha 1, alpha 2, and beta globulins appeared to be variable under the influence of ACTH.

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Effect of Dietary *Clostridia* upon Growth-Promoting Responses of Penicillin.* (20980)

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Elam *et al.*(1) reported that penicillin greatly reduced the total number of *Clostridia* present in the intestinal tract of the chick and that small amounts of the antibiotic inhibited the growth of *Clostridia* isolates *in vitro*. Jacobs *et al.*(2) later reported that antibiotics (directly or indirectly) stimulated growth by reducing the total number of *Clostridia* in the intestinal tract of the chick and that the antibiotics may be ineffective in stimulating growth in a clean environment where the *Clostridia* population was low. Kiser *et al.* (3) reported that a number of *Clostridia* were

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destroyed, in *in vitro* studies, by the addition of aureomycin, penicillin or terramycin. The growth-promoting effect of penicillin and terramycin has been explained as in inhibition of *Clostridium perfringens* in the intestinal tract of turkeys by Sieburth *et al.*(4). Elam *et al.* (5) observed that when meat scraps were added to the basal diet of swine the total number of *Clostridia* was increased and this number was greatly lowered by the addition of chloromycetin which resulted in an increase in growth, whereas the addition of chloromycetin to the basal diet not containing meat scraps (where the total number of *Clostridia* was low) failed to stimulate growth. Coates *et al.* (6,7) reported that chicks reared in quarters not previously used for chick experiments failed to show a growth response to penicillin, whereas a positive response occurred in old quarters previously used for chick experiments. These authors concluded that the reason for the antibiotic response in the old quarters was the presence of an "infection" that made the chicks sensitive to the antibiotic. Similar studies have been reported by others (8-10).

The two experiments described in this paper were conducted to determine the effect of feeding fecal *Clostridia* to the chick in old and clean environment with and without an antibiotic.

Methods. Data from 2 separate experiments are reported in the present study. In each experiment parallel tests were carried out in old and clean quarters. The New Hampshire chicks used in these studies were obtained from hens that had been fed the diet described earlier by Elam *et al.*(11). For the test in the clean environment the birds were housed in a clean room which had been washed daily for a period of 2 weeks before the beginning of the experiments. The room was washed daily during the entire experimental period. In the test carried out in old quarters the birds were housed in quarters which had been used continuously for the past 3 years for chick experiments. The birds were maintained in batteries with raised screen floors. Feed and water were supplied *ad libitum* and the chicks were weighed at weekly intervals during an experimental period of 10 weeks. For the first experiment 350 day-old New Hampshire chicks were randomized into 7 groups of 50 chicks each, and in the second experiment 320 chicks were randomized into 8 groups of 40 chicks each. The *basal diet* used in this study was the all-vegetable protein diet described earlier by Jacobs *et al.*(2). Penicillin was administered at a level of 2 mg per lb of feed. Fresh *fecal material* containing approximately 15,000 *Clostridia* per gram of feces was put in a water solution and mixed directly into the feed every other day at a level of 8 ml (which contained 0.350 g of fecal material per ml) per pound of feed. The water solution of fecal *Clostridia* was prepared by suspending 0.350 g of fecal material per ml of water. The water suspension was then heat shocked for 30 minutes at 65-70°C to kill the aerobic microorganisms. The autoclaved fecal material was prepared by autoclaving the water suspension for 15 minutes at 15 lb of pressure at 121-125°C, and then fed at the same level as the fecal *Clostridia*. Fecal samples were collected once a week and were prepared as described by Elam, Gee and Couch(12). The total number of *Clostridia* per gram of feces was determined by the method described by Jacobs *et al.*(2). An analysis of variance for the *Clostridia* counts and chick weights was made, and statistical significance was determined from the F value (ratio of appropriate mean square values from an analysis of variance table).

Results. In the first experiment the addition of penicillin produced a significant increase in the growth of the chicks and decreased the total number of *Clostridia* per gram of feces when maintained in old quarters (Table I). This was not the case when penicillin was added to the diet of chicks maintained in clean quarters. Under the latter conditions the addition of penicillin was ineffective in increasing growth or reducing the total number of *Clostridia*. This is in agreement with the work reported earlier by Jacobs *et al.*(2). There is also a significant difference between the two control groups as the birds reared in the clean quarters grew more rapidly and had a much lower *Clostridia* count regardless of the antibiotic supplement.

The addition of fecal *Clostridia* to the basal

TABLE I. Effect of Feeding Penicillin and Fecal *Clostridia* upon Weights and Number of *Clostridia* in Old and Clean Quarters.

Group	Avg wt at 10 wk (g)	Total No. of Clostridium isolates (per g of feces)
First experiment		
Old quarters		
Control	969	15570
Penicillin	1085*	265*
Clean quarters		
Control	1167	1215
Penicillin	1210	615
Fecal *Clostridia*	1042*	10260*
" " + penicillin	1180	450
Autoclaved fecal *Clostridia*	1150	1110
Second experiment		
Old quarters		
Control	1134	12450
Penicillin	1262*	640*
Fecal *Clostridia*	1170	16710
" " + penicillin	1310*	740*
Clean quarters		
Control	1324	940
Penicillin	1343	650
Fecal *Clostridia*	1210*	9440*
" " + penicillin	1339	625

* Significant at the 1% level.

diet of birds reared under clean conditions significantly reduced the growth of the birds and increased the total number of *Clostridia* (Table I). When penicillin and fecal *Clostridia* were fed in combination, the growth of the birds was not retarded nor was the total number of *Clostridia* increased. The addition of autoclaved fecal *Clostridia* had no effect on the growth or the *Clostridia* count. The latter was administered to determine whether or not the solution of fecal material would inhibit growth in the absence of *Clostridia*.

In the second experiment the addition of penicillin increased growth and decreased the total number of *Clostridia* in old quarters but failed to have any effect on the growth of the birds or on the fecal *Clostridia* count under clean conditions where the *Clostridia* count was low (Table I). The addition of fecal *Clostridia* failed to have any effect on growth or *Clostridia* count in old quarters as the *Clostridia* population was already high enough to retard growth. As in the first experiment the feeding of fecal *Clostridia* under clean conditions decreased the growth of the birds and increased the *Clostridia* count. The incorporation of penicillin corrected this growth retardation and decreased the *Clostridia* count (Table I).

It should be pointed out that in comparing the old and clean quarters that there is a considerable difference between the *Clostridia* population in the control groups which explains in part the difference in weights between the two quarters. This also explains the response obtained from the antibiotics in contaminated but not in the clean quarters.

Summary. 1. The feeding of penicillin stimulated growth and decreased the *Clostridia* count per gram of feces in birds maintained in old quarters. 2. Penicillin failed to stimulate growth or decrease the *Clostridia* count in birds maintained in clean quarters where the *Clostridia* population was low. 3. The feeding of fecal *Clostridia* decreased the growth rate of birds reared in clean quarters. This decrease was overcome by addition of penicillin to the diet. The feeding of fecal *Clostridia* to birds reared in old quarters where the *Clostridia* population was high failed to depress growth. 5. Penicillin produced a growth stimulation only in cases where there was a decrease in the *Clostridia* count per gram of feces. This indicated that one explanation for antibiotics stimulating growth is through an action on the anaerobic (*Clostridia*) microflora.

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Role of Magnesium Ions in Donath-Landsteiner Hemolytic Reaction of Paroxysmal Cold Hemoglobinuria.* (20981)

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The effect of certain metallic ions on complement activity has attracted considerable attention in recent years(1-5). The activity of guinea pig complement against sensitized sheep erythrocytes has been shown to depend on the concentration of Mg^{++} used in the reagents(1,6,7), and it has been stated(5) that calcium as well as magnesium is essential for this immune hemolysis. Other investigators, however, have found that these ions enhance but are not essential for the activity of rabbit(8) or human complements(9). While studying the mechanism of hemolysis in paroxysmal nocturnal hemoglobinuria, Harris, Jordan, Pillemer and Desforges(10) found that Mg^{++} is essential for the activity of the serum factor, or factors, which hemolyse the abnormal erythrocytes. No immune mechanism, however, has been demonstrated in this unusual human hemolytic system(11,12). Accordingly, the role of magnesium was studied in another human hemolytic system, that of paroxysmal cold hemoglobinuria (PCH), in which hemolysin and complement are involved.

The PCH system is unique among the hemolytic systems in that it requires the presence of complement both for antibody fixation in the cold phase of the Donath-Landsteiner reaction as well as for hemolysis in the warm phase(13). The experiments described in this paper examine the part that Mg^{++} plays in these two phases. The sheep cell hemolytic system was used to demonstrate any change which might have occurred in complement activity when Mg^{++} was removed from guinea pig serum by various methods.

Materials and methods. PCH sera. Sera from three patients with paroxysmal cold hemoglobinuria were used. Case No. 1 and case No. 2 have been described previously (13). Case No. 3 was seen in February, 1952, at Crile Veterans Administration Hospital through the courtesy of Dr. Norman Shumway. This 30-year-old colored male had had episodes of cold hemoglobinuria since 1945. Despite a number of courses of penicillin, he was still having frequent episodes of hemoglobinuria. Kahn and Kolmer tests were positive. The Donath-Landsteiner hemolysin titer was 1:4; the PCH antibody titer measured by the indirect Coombs test was 1:16. *Complement titration.* Lyophilized guinea pig serum was used, and was titered with a modification of the method described by Kabat and Mayer(14), using the Coleman Jr. spectrophotometer. The 50% unit of complement was determined by plotting the hemolysis curve on logarithmic paper. All titrations were made in duplicate, and repeated titration showed that the results were reproducible within 5%. *Buffer.* Barbital buffer(1) con-

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