Effect of Feeding Penicillin on the Life Cycle of the Chick.* (18726)

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Stokstad and Jukes(1) and Whitehill *et al.*(2) reported that aureomycin, streptomycin, penicillin, and sulfasuxidine promoted the growth of chicks adequately supplied with vit. B_{12}. Reed and Couch(3) failed to obtain an increase in the growth of chicks kept on sand litter by adding an APF concentrate, which contained only vit. B_{12}, to an all-vegetable-protein diet, but obtained a very significant increase in chick growth by adding an APF obtained from *Streptomyces aureofaciens* fermentation. The type of dietary carbohydrate has been reported to influence the microflora in fecal droppings from laying pullets(4). These workers found that dextrin and lactose stimulated the development of coliform bacteria, whereas, sucrose had a depressing effect on the fecal coliforms. Penicillin and terramycin have been reported to have an inhibitory effect on *Clostridium perfringens* in the intestinal tract of turkeys(5). These workers stated that it appeared possible that these antibiotics might promote growth through such inhibition.

With these reports in mind it appeared desirable to determine the effect of feeding penicillin on growth, egg production, hatchability, and on the fecal microflora of the birds fed this antibiotic.

Experimental. Crossbred chicks (N.H. × S.C.W.L.) used in these studies were obtained from hens which had been fed a practical all-mash diet(6). This diet has been used to produce B_{12}-depleted chicks and has been shown to produce eggs with yolks of low B_{12} content(7). The chicks were kept in batteries with raised screen floors with feed and water supplied *ad libitum.* The birds were weighed at weekly intervals during an experimental period of 32 weeks. Cockerels were removed at the end of the 10th week. Pullets were transferred to individual laying cages at 9 weeks of age. The management and experimental procedures with regard to the care and handling of the pullets, insemination, incubation, and embryonic mortality records were the same as those previously reported by Couch *et al.*(8), and Olcese, Couch, and Lyman(9). The basal diet (G-1) used in this study was the same as that reported earlier by Couch and Olcese(10). One hundred chicks were randomized into 4 groups of 25 chicks each. The first and second groups were fed the basal diet. Each chick in the second group received an injection of 1 μg of crystalline vit. B_{12} per chick per week into the pectoral muscle. Groups 3 and 4 were fed the basal diet supplemented with 33 mg of procaine penicillin (G) per kg of diet. Each chick in Group 4 was injected with 1 μg of crystalline vit. B_{12} weekly.

Six cockerels from each group were killed

* This work was supported by a grant-in-aid from the Williams-Waterman Fund for the combat of dietary disease, administered by the Research Corporation, New York. Crystalline vit. B_{12} and procaine penicillin (G) were supplied through the courtesy of Dr. D. F. Green, Veterinary Division, Merck and Co., Rahway, N. J.

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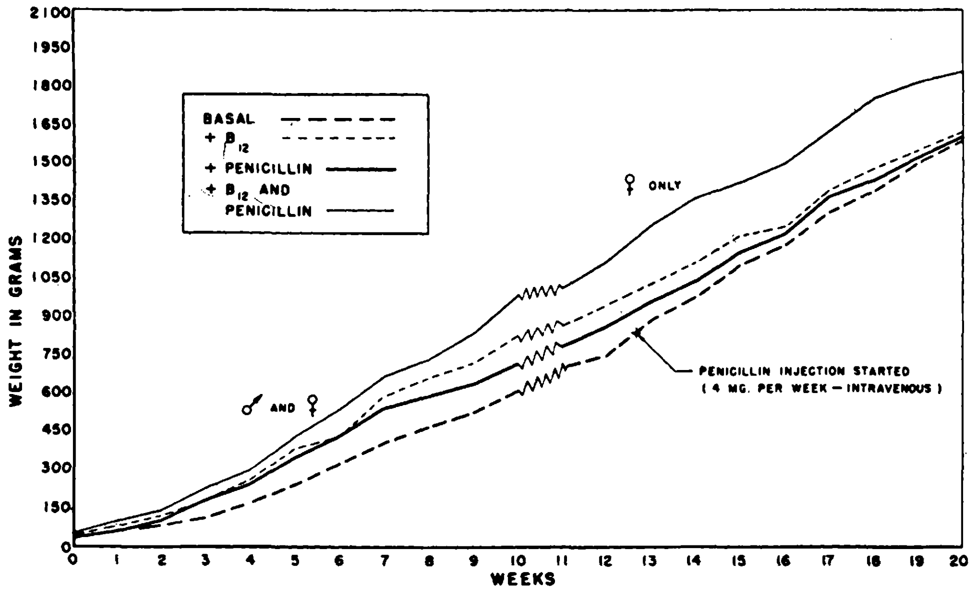

FIG. 1. Effect of feeding penicillin and injecting B_{12} on weights of crossbred chicks.

at the end of the 10th week and the livers and kidneys were removed for vit. B_{12} determination. Samples of liver and kidney tissue were prepared for assay according to the method of Couch and Olcese(10). Eggs were collected for B_{12} determination during the 26th and 27th weeks of the test. Egg yolks from each group were pooled and an aliquot of the yolks was prepared for vit. B_{12} assay according to the method of Halick and Couch(7). The vit. B_{12} content of the tissues and egg yolks was determined according to the method of Skeggs *et al.*(11). Fecal droppings for bacteriological analysis were collected on clean waxed paper between 5:00 and 6:00 a.m. at weekly intervals from the fifth through the 15th and from the 18th through the 30th weeks. Droppings from each group were thoroughly mixed with 50 ml of sterile 0.1% tryptone solution in a 100 ml dilution bottle until all visible clumps were dispensed. Serial decimal dilutions were made in sterile 0.1% tryptone solution up to 10^{-10} dilution. Inoculations from such dilutions were made into the sterile media described below. The thioglycollate medium (Baltimore Biological Laboratories) was used for making total dilution counts. The enterococci dilution counts were made by using the "SF" broth of Hajna and Perry(12). Difco eosin methylene blue agar ("EMB") was used for making coliform plate counts. Yeast counts were made by using the potato-dextrose agar acidified to pH of 3.5 with citric acid (13). Carrot-liver extract ("CL") agar shake tubes were used for counting lactic acid bacteria(14). Plate counts of gram-negative bacteria were made with crystal violet agar. Penicillin resistant bacteria were determined by plating in nutrient agar containing 100 μg of procaine penicillin (G) per ml. The thioglycollate medium, Difco eosin methylene blue agar, crystal violet agar, and penicillin medium were incubated at 37°C for 48 hours. "SF" broth was incubated at 45°C for 3 days. Potato-dextrose agar was incubated at room temperature for 5 days. Carrot-liver extract agar was incubated at 37°C for 72 hours. All media were plated and tubed in duplicate with

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TABLE I. Effect of Feeding Penicillin and Injecting Vit. B_{12} on Egg Production, Hatchability and B_{12} Content of the Liver and Kidney Tissues, and Egg Yolk.

Supplement to basal diet	Egg production, %	Hatchability, %	μg B_{12} per 100 g Liver	μg B_{12} per 100 g Kidney	$m\mu g$ B_{12}/g egg yolk
None*	29	0	11.7	11.9	1.7
1 μg B_{12} inj./bird/week	38	72	15.2	16.5	3.4
Penicillin (33 mg/kg)	22	39	13.2	11.6	2.1
Penicillin (33 mg/kg) + 1 μg B_{12} inj./bird/week	50	80	17.7	18.4	3.1

* Four mg penicillin was inj. intrav. from 14th through 32nd week.

the exception of thioglycollate and "SF" broth. The latter were run in a series of 5 tubes per dilution. For convenience in handling, each individual count was converted to its respective logarithm to the base 10 (the mantissa was carried out to the second place). An analysis of variance for counts obtained on each medium was made, and the F value (ratio of appropriate mean square values from an analysis of variance table) was determined. Least significant differences (LSD) were calculated where the F value was significant at the 1 or 5% level of probability.

Results and discussion. A summary of results up to the time the males were removed (10 weeks) shows that the addition of penicillin to the basal diet resulted in increased growth (Fig. 1). The injection of vit. B_{12} resulted in a greater increase in growth than did addition of penicillin, though this difference did not appear until the seventh week. When the birds received both vit. B_{12} and penicillin the increase in weight was greater than either given alone.

Due to the excessive mortality in the basal group it seemed advisable to modify the experiment at the end of the 14th week. Since stimulation has been shown by incorporating penicillin in the diet it seemed worthwhile to evaluate the influence of intravenous injection of this antibiotic. The basal birds were, therefore, given an intravenous injection of 4 mg of procaine penicillin (G) at weekly intervals for the duration of the experiment. This break in continuity necessitated treating the experimental data as two separate experiments. Growth of these birds was improved by such injection and the weight obtained at the end of the 20th week was almost identical with that of birds receiving penicillin and of those receiving B_{12} throughout the period. All birds started to lay at 19-23 weeks of age. Egg production and hatchability data were collected for a period of 12 weeks (Table I). Birds fed penicillin and injected with vit. B_{12} had the highest egg production during this period. Couch *et al.*(6) have shown that vit. B_{12} is necessary for egg production.

No live chicks were obtained from eggs laid by pullets from the basal group which received an intravenous injection of penicillin after the 14th week. Typical symptoms of vit. B_{12} deficiency, as described by Olcese *et al.*(15) were observed in these embryos. In contrast, 39% hatchability was observed in eggs from birds fed penicillin continuously. It is possible that the feeding of the antibiotic continuously led to the establishment of a more favorable microflora which may have synthesized vit. B_{12} at a sub-optimal level. The per cent hatchability of eggs from pullets injected with B_{12} and those injected with B_{12} and fed penicillin is considered to be within the normal range.

The vit. B_{12} content of the liver and kidney tissues from cockerels at 10 weeks of age was increased by injecting the vitamin into the birds, but apparently was not increased to any appreciable extent by feeding the antibiotic (Table I). This is in agreement with the work of Couch and Olcese(10).

The injection of vit. B_{12} into the hens increased the concentration of this vitamin in the egg yolk (Table I). There appears to be little difference in the B_{12} content of the eggs from the birds receiving the two types of penicillin treatment. It is possible that vit. B_{12} was not the limiting factor influencing the

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TABLE II. Summary of Statistical Analysis of Data from Fecal Droppings of Chickens up to 14 Weeks of Age.

Media and type of count	Avg Basal	Avg B_{12}	Avg Penicillin	Avg Penicillin + B_{12}	F-value
Thioglycollate (Total dilution count)	8.11*	7.71	8.76	8.91	5.72†
Carrot-liver (Lactics)	7.81	7.15	7.79	7.99	1.70
"SF" broth (Enterococci)	4.43	4.55	5.87	6.10	11.96†
Penicillin (Resistant penicillin)	2.96	4.07	6.16	5.96	8.44†
Eosin methylene blue (Coliform)	7.01	6.68	7.89	7.92	2.84
Crystal violet (Gram-negative)	7.36	6.39	7.50	7.40	1.92
Potato-dextrose (Yeast)	3.35	3.42	4.04	4.44	1.71

* Avg log of number of bacteria per g feces (wet wt).
† Significant at 1% level (probability of chance occurrence less than 1%).

TABLE III. Summary of Statistical Analysis of Data from Fecal Droppings of Chickens from 18 to 30 Weeks of Age.

Media and type of count	Avg Basal	Avg B_{12}	Avg Penicillin	Avg Penicillin + B_{12}	F-value
Thioglycollate (Total dilution count)	9.19*	9.03	9.67	9.72	6.65†
Carrot-liver (Lactics)	8.66	8.60	8.72	8.51	.40
"SF" broth (Enterococci)	5.25	4.88	5.11	5.54	.97
Penicillin (Resistant penicillin)	5.13	4.44	5.35	6.05	8.40†
Eosin methylene blue (Coliform)	6.81	6.68	6.98	7.18	1.19
Crystal violet (Gram-negative)	6.57	6.39	6.80	6.95	2.22
Potato-dextrose (Yeast)	3.15	2.15	4.27	4.92	69.27†

* Avg log of number of bacteria per g feces (wet wt).
† Significant at 1% level (probability of chance occurrence less than 1%).

hatchability in the latter groups. This observation is in agreement with Halick and Couch(7) who reported that eggs containing as little as 1.7 mμg of B_{12} per g gave normal hachability.

The feeding of penicillin produced an increase in the total dilution count (thioglycollate), enterococci ("SF" broth), and an increase in the penicillin resistant organisms (Table II), from the 5th through the 14th week of the test.

Apparently there was some adjustment in the intestinal microflora after the 14th week of test (Table III). The difference in the enterococci counts, observed as being traceable to feeding penicillin was not significant from the 18th through the 30th weeks of test. The injection of the antibiotic had a rather definite effect on the number of penicillin resistant organisms and on the number of yeasts in the intestinal tract.

It seems likewise evident that the injection of this antibiotic stimulated the growth of these birds through a shift in the microflora of the gastrointestinal tract.

Summary. 1. The feeding of penicillin stimulated the growth of birds fed an all-vegetable-protein diet. 2. Injection of the antibiotic into the birds fed the basal diet stopped the mortality and appeared to stimulate the growth. 3. Eggs were obtained from all groups. Pullets fed penicillin and injected

with vit. B_{12} had the highest egg production. Hatchability of eggs from birds injected with B_{12} was within the normal range. No live chicks were obtained from pullets injected with penicillin from the 14th week. A 39% hatch was obtained from the eggs of pullets fed penicillin continuously. The B_{12} content of the liver and kidney tissues and of the egg yolks was directly related to the injection of the vitamin into the birds. 4. Feeding of penicillin caused a significant increase in the total number of intestinal micro-organisms as indicated by thioglycollate counts. Penicillin likewise caused a significant increase in the number of enterococci of the intestine during the first 14 weeks of the experiment. The administration of penicillin either orally or intravenously resulted in a marked increase in the number of penicillin resistant microorganisms in the intestinal tract.

Received April 25, 1951. P.S.E.B.M., 1951, v77.

Inhibition of Growth of C3H Mice by Ethionine and its Alleviation by Methionine or Choline.* (18727)

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In a recent communication we reported(1) that either optical isomer of ethionine (S-ethyl-homocysteine) inhibited the growth of albino rats which were maintained on a complete casein diet. The inhibition could be alleviated by either isomer of methionine or by choline, but not by cystine, homocystine, or cystathionine. The observation that choline was effective suggested that ethionine was interfering with the economy of the labile methyl group. Further studies employing ethionine labeled with C-14 in the ethyl group revealed that the ethyl group carbon was incorporated into the molecule of choline and creatine of rat tissues. This suggested the possible "transethylation" of ethionine(2). Similar conclusions were made in experiments employing S^{35}-ethionine(2). It appeared of interest to extend these observations to other species, such as the mouse.

Experimental. Weanling mice of C3H strain, inbred by brother-sister method, born and raised in the laboratory, were used. At the age of 23 days the males and females were separated and placed in separate cages, 3 mice per cage. A total of 66 mice were used. The composition of the basal diet was as follows: Labco casein 30; corn starch 36; sucrose 15; Crisco 10; inorganic salts (USP XII No. 2) 4; cod liver oil 5%. Per kg this diet was fortified with the following vitamins: thiamin hydrochloride 20; riboflavin 20; calcium pantothenate 50; pyridoxine hydrochloride 20; nicotinamide 20; folic acid 5; inositol 1000; p-amino-benzoic acid 1000; biotin 1.0; alpha-tocopherol 300 mg; and vit. B_{12} 150 μg. No choline was added to the basal diet. The preparation and analytical purity of ethionine, methionine, cystine, and homocystine used in the present study were previously described (1). The food and water were fed *ad libitum,* the mice were weighed twice a week, and the food consumption was recorded. At the end of the experimental period the livers of mice in each group were pooled, and their lipid content was determined gravimetrically(1). The supplements fed with the basal diet are indicated in Table I.

Results. Since the incorporation of 0.5% of DL-ethionine into the diet proved fatal to the growing mice within 24 to 48 hr after its ingestion, the concentration was reduced to 0.25%. As will be observed from the data in Table I, this amount retarded or inhibited the growth. Supplementation of this ethionine-containing diet with 0.25% of methionine per-

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