SOME EFFECTS OF DIETARY PENICILLIN ON THE GERMAN COCKROACH, *BLATTELLA GERMANICA* (L.)[1]

(ORTHOPTERA: BLATTIDAE)

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Although considerable information has appeared in the literature relative to the effects of the addition of small amounts of antibiotics to the diets of vertebrates, especially domesticated animals, comparatively little has been reported concerning the effects of these antibiotics on insects.

Working with aureomycin, Brooks and Richards (1955) obtained bacteroid free nymphs of the German cockroach by feeding their parents a dog food diet containing 1,000 ppm. of the antibiotic all of their lives. The bacteroids were not eliminated from the parents, but were absent from their offspring. The aposymbiotic (without intracellular bacteroids) nymphs thus produced were practically incapable of growth on a natural diet which was adequate for nymphs containing bacteroids. Higher concentrations of aureomycin resulted in excessive mortality.

De (1956) reported that streptomycin administered in the diet of stored grain beetles at 200 to 1,000 ppm. had no apparent effect on the insects. If anything, it extended the length of life. Steinhaus and Bell (1953), in attempting to obtain intracellular bacteroid free stored grain beetles by adding antibiotics to the grain, found chloromycetin, penicillin G, polymixin B, streptomycin, and terramycin to be highly toxic. At levels of 20,000 to 36,000 ppm., 100 percent of the beetles were dead in 30 to 60 days. Mengle and Fisk (1956) fed adult female German cockroaches high concentrations of 11 antibiotics, five of which produced little or

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no mortality even at 20 percent of the weight of the diet. The other six produced significant mortality when fed at the same level.

The work reported herein concerns some effects of the oral administration of fifteen different dietary concentrations (6 to 100,000 ppm.) of crystalline sodium penicillin G (Sharpe and Dohme) to the German cockroach. Starting with six parts per million, the penicillin concentration of each succeeding dietary level increased geometrically by a factor of two.

MATERIALS AND METHODS

The cockroaches used were from a normal (non-insecticide-resistant) strain which had been reared for several years on pulverized Purina dog checkers in the insect rearing room of The Ohio State University.

To obtain first instar nymphs for the dietary studies, approximately 30 capsulated female cockroaches were placed in each of two five gallon glass jars. The top two inches of each jar were coated on the inner surface with a mixture of petroleum jelly and mineral oil to prevent escape of the females and their offspring. In addition, the jars were securely covered with two layers of cheese cloth to prevent escape or contamination with other cockroaches. All other rearing chambers used in these experiments were similarly greased and covered.

The capsulated females in the five gallon jars received distilled water from a large vial plugged with absorbent cotton and a diet consisting of equal parts of vitamin free casein, dextrose, and powdered non-nutritive cellulose. The first instar nymphs received no other nutrients until placed on the test diets.

The following procedure was used for handling the first instar nymphs: 1) In three or four days, when most of the eggs from the capsulated females had hatched, the jars were placed in a deep freeze until the cockroaches were inactive. 2) The young were then removed by use of a wet camel's hair brush and groups of approximately 35 were placed in individual pyrex crystallizing dishes, five and three-fourths inches in diameter and three inches deep. 3) A few hours later, the cockroaches were again chilled and each group was weighed and reduced to 30 individuals in order to provide groups of cockroaches uniform as to initial weight. 4) Distilled water in a vial three inches by one inch in diameter, plugged with absorbent cotton and one of the prepared diets in a vial two inches by one and and one-half inches in diameter were added to each dish. 5) The dishes were placed in a constant temperature-humidity box set at $27 \pm 1°C$ and approximately 73 percent relative humidity. The humidity was controlled by placing two three quart capacity jars filled with saturated sodium chloride solution into the cabinet. Water was added to the jars as necessary. The insects were kept in the dark except when subsequent weekly weighings were made.

All weighings were performed with a Roller-Smith Torsion Balance graduated from 1 to 1,500 milligrams. The cockroaches were picked up individually by the antennae with a fine forceps and placed on the weighing pan. Weighings were made at weekly intervals from the first instar to the adult stage. For all but the initial weighing, the insects were anesthesized with carbon dioxide. New food and water were added weekly.

Cockroaches, even from the same egg capsule and reared under apparently identical conditions, reach the adult stage in different lengths of time. Because recordings of the weights of replicates containing both nymphs and adults would be of little value in determining the growth rate during the developmental period, it was necessary to designate a particular point as the "adult stage." Therefore, when 10 percent of the individuals in a test had actually reached the adult stage, or when the average weight per roach exceeded 42 mg., the whole group was considered to have reached the "adult stage." In following this procedure, it was found that approximately 40 to 50 percent of the insects would actually reach the adult stage in the following week.

The basic diet used throughout the study was micromixed pulverized "Purina Dog Chow Checkers," manufactured by the Ralston Purina Company of St. Louis, Missouri.

Sixteen diets were prepared, fifteen of which contained a different concentration of penicillin. The preparation of the diets was as follows: 1) Approximately 500 grams of the dog food were placed in a large beaker and mixed thoroughly; 2) For each diet a definite quantity of the dog food was weighed and placed into a small, wide mouth bottle to which was also added a small but definite quantity of Alphacel (powdered, non-nutritive cellulose) purchased from the Nutritional Biochemical Corporation, Cleveland, Ohio; 3) The appropriate quantity of sodium penicillin G was dissolved in five milliliters of water and added to each diet. Dilutions of a stock solution were used in the small concentrations for greater accuracy and convenience; 4) After the addition of penicillin, each diet was mixed thoroughly and dried in a vacuum oven at room temperature for 24 hours (table 1); 5) After drying, each of the various diets was again throughly mixed by grinding in a mortar and pestle. The prepared diets were stored in a deep freeze until needed.

TABLE 1

Composition of the diets employed in the rearing experiments

Diet No.	Grams Dog Food	Grams Alphacel	Grams Penicillin*	Ppm. Pen./Diet	Grams Total
1 (ck)	18.0	2.000	0.000000	0	20.000
2	18.0	2.000	0.000122	6	20.000
3	18.0	2.000	0.000244	12	20.000
4	18.0	2.000	0.000488	25	20.000
5	18.0	2.000	0.000976	50	20.001
6	18.0	1.990	0.001953	100	19.991
7	18.0	1.990	0.003906	195	19.994
8	18.0	1.990	0.007812	390	19.998
9	18.0	1.980	0.015625	781	19.995
10	18.0	1.968	0.031250	1562	19.999
11	18.0	1.938	0.062500	3125	20.000
12	18.0	1.875	0.125000	6250	20.000
13	18.0	1.750	0.250000	12500	20.000
14	18.0	1.500	0.500000	25000	20.000
15	18.0	1.000	1.000000	50000	20.000
16	18.0	0.000	2.000000	100000	20.000

*Dissolved in 5 ml. of H_2O by appropriate dilution of stock solution.

RESULTS AND DISCUSSION OF FIRST GENERATION STUDIES

As outlined above, for the first generation studies, fifteen diets containing different quantities of penicillin plus a check containing no penicillin were prepared. Two replicates of 30 German cockroaches each were set up on each test diet. The insects were weighed each week from the initial weighing to the adult stage. The results of this experiment, in average weight in milligrams per cockroach per week, are recorded in table 2.

Figure 1 indicates that between the fourth and fifth weeks of rearing there was a beginning of inhibition of growth in the cockroaches fed diets containing the higher levels of penicillin. Figure 1 also represents the differences in weight at nine weeks. At this time, the cockroaches reared on the check diet reached the adult stage.

An analysis of variance was used to estimate whether the rates of gain produced by all the treatments were equal or unequal (table 3). The results of the test indicated that the growth rates were not equal.

The differences between the individual treatments and the check were then tested for significance by the method of Least Significant Difference (L.S.D.). Difference was not encountered with diets 2 to 8, but was encountered with diets 9 to 16.

Therefore, growth rates of cockroaches reared on the test diets appeared to be equivalent to the check diet if the diets did not contain more than 400 ppm. of penicillin. However, this inhibitory trend did not become noticeable until the fifth week, even in the highest dietary levels. This seems important because the growth rate was more rapid (logarithmic) during the first four week period. The cockroaches almost doubled their weights from the initial weighing to the first week and also from the first to the second week. Since no inhibition was apparent

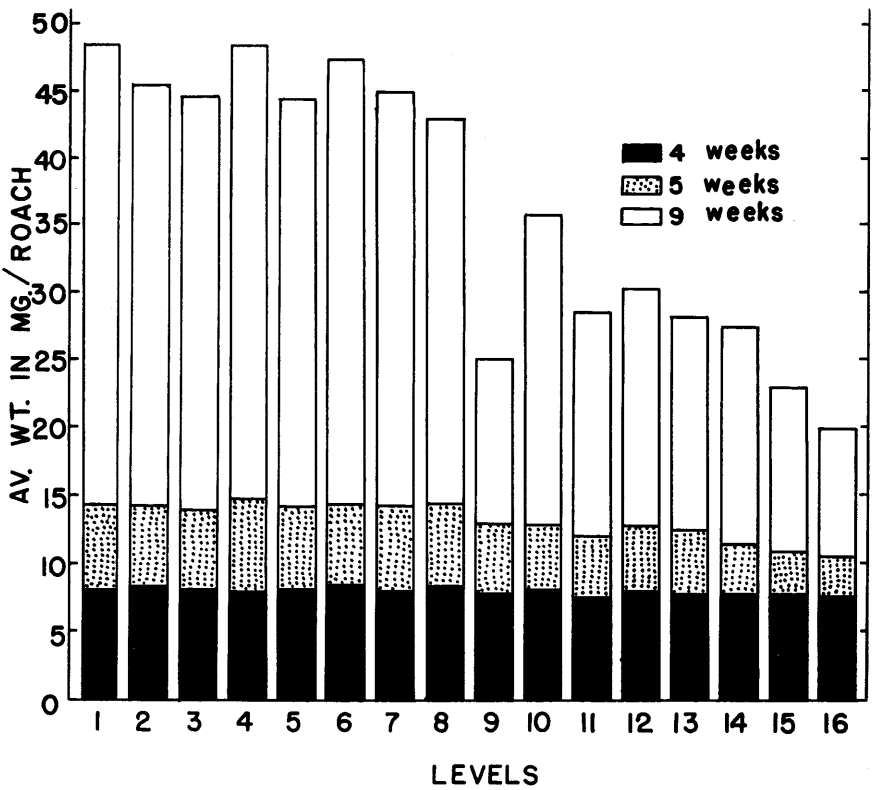

FIGURE 1. Growth of the German cockroach at four, five, and nine weeks, when reared on diets containing different concentrations of penicillin.

during these first weeks, the effect or effects of penicillin were latent or non existant at this time. Perhaps the penicillin inhibited the further accumulation or elaboration of some essential substance present in the nymphs at hatching. If this could occur, inhibition of growth would not be apparent until the supply of the unknown substance, present in the nymphs at hatching was exhausted. This supposition is somewhat substantiated by the fact that inhibition was evident earlier in the second generation where it was first observed between the second and fourth weeks.

It is possible that inhibition, particularly at higher levels, could have arisen through reduction of the intracellular bacteroids of the cockroaches. Brooks and

Richards (1955) found that 1,000 ppm. of aureomycin did not eliminate the bacteroids from the cockroaches. Higher levels eliminated the bacteroids, but also resulted in excessive mortality in three or four months time. However, since no such excessive mortality occurred in first generation cockroaches fed diets containing as much as 100,000 ppm. of penicillin, it is possible that this

TABLE 2

Weights of first generation roaches on test diets

Diet Number	Avg. Wt. in Mg./Roach Week									
	Initial	1	2	3	4	5	6	7	8	9
1 (check)	1.37*	2.59	4.78	7.19	8.01	14.27	20.68	26.47	41.47	48.52
2	1.33	2.56	4.78	6.88	8.24	14.20	20.24	25.94	39.17	45.63
3	1.32	2.47	4.67	6.80	8.01	13.83	19.07	25.86	37.81	44.07
4	1.34	2.46	4.72	7.53	7.88	14.56	19.50	27.01	43.27	48.23
5	1.35	2.62	4.60	7.07	7.96	13.78	18.92	27.92	31.52	43.53
6	1.34	2.61	4.75	7.07	8.32	14.33	20.01	26.09	39.62	46.77
7	1.33	2.43	4.47	6.70	7.75	13.90	18.57	26.34	38.67	45.03
8	1.33	2.43	4.56	7.18	8.35	14.31	19.18	26.29	38.70	42.71
9	1.37	2.62	4.44	6.82	7.62	13.15	17.52	23.77	20.45	25.03
10	1.32	2.44	4.70	6.52	7.89	13.26	15.82	22.78	30.78	35.70
11	1.33	2.38	4.25	6.44	7.20	11.86	13.82	18.34	24.35	28.01
12	1.33	2.47	4.55	6.64	7.68	12.80	14.56	19.74	26.60	30.18
13	1.33	2.66	4.24	7.00	7.79	12.40	14.49	19.92	25.46	28.61
14	1.34	2.45	4.53	6.31	7.60	11.61	13.59	19.16	22.62	26.92
15	1.37	2.44	4.42	6.20	7.54	11.03	12.64	18.28	19.71	23.01
16	1.39	2.64	4.17	6.20	7.14	10.36	11.36	14.92	17.00	19.58

*The two replicates of approximately 30 insects each agreed well as to total weight, and only the average of the two is recorded. Complete data are on file in the editor's office or may be obtained from the author upon request.

TABLE 2 continued

Weights of first generation roaches on test diets

Diet Number	Avg. Wt. in Mg./Roach Week							
	10	11	12	13	14	15	16	17
1 (check)	——§	——	——	——	——	——	——	——
2	——	——	——	——	——	——	——	——
3	——	——	——	——	——	——	——	——
4	——	——	——	——	——	——	——	——
5	——	——	——	——	——	——	——	——
6	——	——	——	——	——	——	——	——
7	——	——	——	——	——	——	——	——
8	——	——	——	——	——	——	——	——
9	37.08	52.00	——	——	——	——	——	——
10	41.62	46.43	——	——	——	——	——	——
11	33.72	38.75	——	——	——	——	——	——
12	35.70	40.26	——	——	——	——	——	——
13	32.80	36.76	——	——	——	——	——	——
14	31.21	33.00	38.76	44.05	——	——	——	——
15	27.38	30.82	34.70	42.50	——	——	——	——
16	20.60	23.30	26.28	30.00	33.36	35.56	36.00	37.50

§No records because the cockroaches had reached the adult stage.

antibiotic did not affect or completely eliminate the bacteroids. The possibility also exists that penicillin is much less toxic to cockroaches than is aureomycin, and the excessive mortality associated with high concentration of aureomycin was due to its direct toxic action on the insect.

First generation cockroaches receiving less than 780 ppm. of penicillin reached the adult stage at approximately the same time (fig. 2). This is well correlated with the estimate that the growth rates were statistically equivalent. In the levels containing 780 ppm. or more, however, there was an inverse relationship between time to adult stage and growth rate. The cockroaches on all diets in both generations molted to the true adult stage at approximately the same weight. Thus, smaller adults did not result from higher penicllin concentrations. It merely took them a longer time to achieve the normal size and weight for the molt to the adult stage.

Anorexia did not appear to be a problem in the various dietary levels with the possible exception of diet 16 (100,000 ppm. of penicillin). If the phenomenon

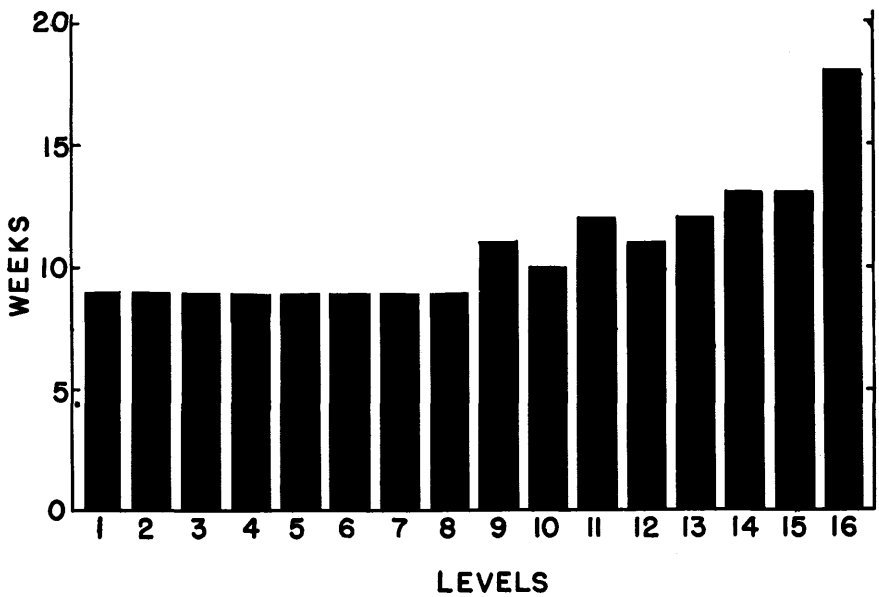

FIGURE 2. Time in which the German cockroach reached the adult stage, when reared on diets containing different concentrations of penicillin.

actually occurred, it was not evident until after the fourth week, because, even on diet 16, growth was almost as great as the check up to that point. The cockroaches did not appear to be repelled by the penicillin in any way.

The reviews of Jukes and Williams (1953), Stokstad (1954), and Jukes (1955) contain numerous references pertaining to the acceleration of growth in vertebrate animals, incited by the addition of small quantities of antibiotics in the food. In general, the antibiotics were added at about 10 ppm., and the extent to which it is absorbed by the animals at this low level is questionable. Most evidence to date indicates that the antibiotic exerts its action primarily on the intestinal biota.

No acceleration of growth was observed in this study. Had the cockroaches been reared under unsanitary conditions, or on a diet deficient in protein or vitamins, the results might have been different. In vertebrates reared in clean

quarters or under aseptic conditions, growth increases usually do not occur with antibiotic fortified feeds (Luckey *et al.*, 1956).

The mortality in the various levels in the first generation was generally low. There appeared to be little difference in mortality in the various treatments with the exception of diet 9. The high mortality with diet 9 (36 percent), however, cannot be satisfactorily explained. There is a good possibility that these insects became diseased early in the course of the test. Disregarding diet 9, the mortality varied between 3.7 and 11.9 percent, which is considered quite low in view of the fact that the insects were handled and anesthetized once each week from the time of hatching to the adult stage. However, Steinhaus and Bell (1953) found that 20,000 to 35,000 ppm. of penicillin added to grain killed one hundred percent of the two species of stored grain beetles tested, over a period of 30 to 60 days.

Reproduction by the first generation appeared to be decreased by all test diets containing 200 or more ppm. of penicillin (diets 7 to 16). This reduction was estimated to be about 40 to 50 percent at 200 ppm. (diet 7), and increased to about 99 percent at 50,000 ppm. (diet 15) and 100 percent at 100,000 ppm. (diet 16), where no egg capsules were produced. The egg capsules produced by the females at these levels were generally smaller than normal, and commonly misshapen. Many egg capsules were dropped prematurely, with none of the eggs hatching. In addition to being shriveled, some of the capsules were blackened and the embryos inside were dead. Figure 3 illustrates several of the deformed egg capsules compared with normal ones.

TABLE 3

Analysis of variance of the growth rates with the sixteen diets

Source	SS	DF	MS	F	F-5%	F-1%
Treatments	.3758	15	.02505	9.1221*	2.35	3.41
Error	.0209	16	.00131			
Total	.3967	31				

*Significant at 1% level.

Growth time equals the entire growth period. Test based on two replications of 30 cockroaches each, per treatment.

Since very few young were produced at the higher levels of penicillin concentration, and no egg capsules were formed at the highest level (100,000 ppm.), there is a good possibility that the ovaries of the females were impared from functioning normally. Brooks and Richards (1955) found 5,000 ppm. of aureomycin produced this effect with the German cockroach in 90 days. Glaser (1946) reported the same results with calcium and sodium penicillin on the American cockroach.

MATERIALS AND METHODS IN SECOND GENERATION STUDIES

In preparing tests for the second generation studies, three principal difficulties were encountered: 1) the first generation female cockroaches reared on any particular test diet matured at different times and therefore produced egg capsules at different times; 2) the egg capsules produced by these females contained varying numbers of viable embryos; 3) as a result of the foregoing, the numbers of second generation first instar nymphs of the same age available at any given time in any particular diet group was limited. This influenced the number and replications of some (diet levels 8 to 15) second generation tests.

The offspring from each first generation diet, where available, were reared on both the control diet and the diet of their respective parents. It was desired to have two replicates reared on these two diets, but in some cases. it was not possible

to obtain enough suitable insects for two replicates per diet. In such cases, only one was employed.

First instar nymphal cockroaches for the second generation studies were obtained as follows. The cockroaches which reached the adult stage on the test diets were allowed to mate and produce egg capsules, if any. Then first generation female cockroaches with darkly pigmented egg capsules were removed as they became available in each test group. The dark pigmentation indicated that the eggs would soon hatch. Two to four such females from each diet were placed in each of four clean crystallizing dishes, two containing the check diet and two containing the respective parental diet on which the young were to be reared.

When the eggs hatched, the adult females were removed, and the number of offspring in any one dish was reduced to thirty. The initial weights of the young were then recorded. In order to have enough nymphs for a test, it was necessary to use nymphs which hatched at slightly different times. Because of this variation in time of hatching, the first recorded weighings are for approximately one week old insects.

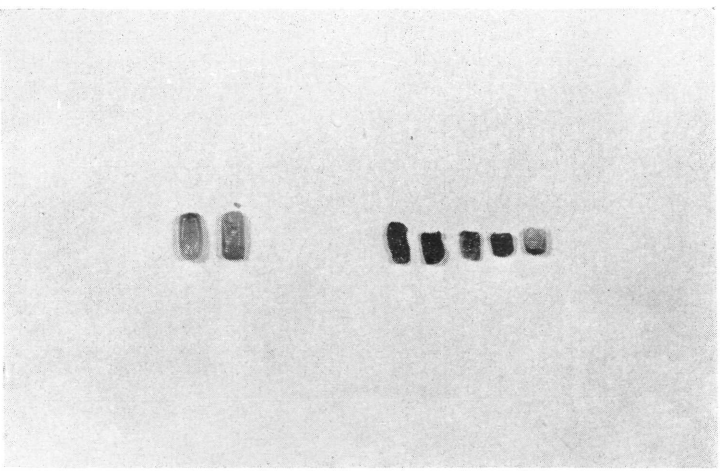

FIGURE 3. Normal egg capsules on the left; deformed egg capsules on the right.

The remainder of the second generation rearing procedures were the same as those used for the first generation studies. However, fresh diets, identical to those employed for the first generation were prepared for the second generation work.

RESULTS AND DISCUSSION OF SECOND GENERATION STUDIES

The results of the second generation experiments in average weight in milligrams per cockroach per week are given in table 4. These data are presented graphically for the second week, fourth week, and eighth week in figure 4. They indicate that inhibition possibly began between the second and fourth weeks of rearing. In the eighth week, the second generation cockroaches which were reared on the check diet reached the adult stage. Here, it appears that second generation cockroaches whose parents received 50 or more ppm. of penicillin (diets 5 to 8) were inhibited in growth.

An analysis of variance of the second generation data indicated that the growth rates were not the same for the various diets (table 5). Therefore, a test for a significant difference between the check mean and the means of the other individual treatments was performed by the method of Least Significant Difference. When no difference between the check mean and the treatment mean

was found, there was considered to be no inhibition of growth. When a difference between these means occurred, growth was considered to be inhibited.

From this, it can be seen that although inhibition of growth in the first generation was not evident until 780 ppm. of penicillin were present in the diet, the offspring of cockroaches which received as little as 50 ppm. exhibited reduced growth rates. This inhibition appeared whether the young were reared on the

TABLE 4

Weights of second generation roaches on test diets

Diet Number	Avg. Wt. in Mg./Roach Week								
	Initial	1	2	3	4	5	6	7	8
1 (check)	——	2.43*	3.63	6.80	10.50	16.23	24.70	33.76	44.86
2	——	2.20	3.46	7.96	12.06	17.13	24.12	33.03	42.75
2'	——	2.40	3.32	8.00	10.37	15.38	23.44	32.25	40.90
3	——	2.58	3.55	6.41	9.86	14.06	22.30	29.38	37.74
3'	——	2.61	3.46	6.45	9.77	16.32	21.61	30.55	35.62
4	——	2.60	3.08	4.30	11.08	16.11	20.69	33.44	47.80
4'	——	2.03	2.68	7.42	10.36	15.47	23.31	20.57	41.50
5	——	1.86	2.70	4.57	7.63	9.14	13.14	17.71	21.96
5'	——	2.10	3.06	4.56	7.84	8.08	12.20	14.79	21.20
6	1.14	2.48	4.19	6.21	8.04	12.28	17.08	17.31	25.86
6'	——	1.87	2.98	4.66	7.04	7.93	11.85	12.73	17.10
7	——	2.02	3.26	4.42	6.50	7.92	10.39	11.92	14.89
7'	——	1.69	2.56	6.35	7.27	9.23	10.31	10.81	15.73
8	——	2.00	2.81	4.01	5.03	7.05	8.27	10.59	13.07
8'	——	2.00	3.54	5.00	6.34	8.46	10.00	12.46	13.50

*The two replicates of approximately 30 insects each agreed well as to toal weight, and only the average of the two is recorded. Complete data are on file in the editor's office or may be obtained from the author upon request.

TABLE 4 continued

Weights of second generation roaches on test diets

Diet Number	Avg. Wt. in Mg./Roach Week						
	9	10	11	12	13	14	15
1 (check)	——§	——	——	——	——	——	——
2	——	——	——	——	——	——	——
2'	——	——	——	——	——	——	——
3	46.01	——	——	——	——	——	——
3'	50.65	——	——	——	——	——	——
4	——	——	——	——	——	——	——
4'	——	——	——	——	——	——	——
5	31.50	37.27	44.15	——	——	——	——
5'	29.88	36.86	44.53	——	——	——	——
6	34.55	46.88	——	——	——	——	——
6'	19.12	24.08	30.26	38.24	44.38	——	——
7	18.08	22.22	26.94	29.80	37.48	44.60	——
7'	18.99	26.08	32.61	38.57	44.04	——	——
8	10.02	18.74	24.41	28.78	34.11	33.27	44.07
8'	20.34	21.32	27.40	32.55	36.26	43.40	44.67

'Indicates use of parental diet. The check diet was used for the others.
§No records because the cockroaches had reached the adult stage.

control diet or on the parental diet. Therefore, it is concluded that in the first generation reared on diets containing 50 to 400 ppm. of penicillin, some irreparable damage occurred which was not evident until the second generation.

Figure 5 represents, graphically, the time in weeks in which the cockroaches reached the adult stage when reared on the various diets. Increase in time occurred chiefly if the parents of the young received 50 or more ppm. of penicillin, regardless of whether the young were reared on the check diet or on the parental diet.

In general, second generation cockroaches exhibited higher mortality than the first generation cockroaches, even if their parents received as little as 100 to 200 ppm. of penicillin.

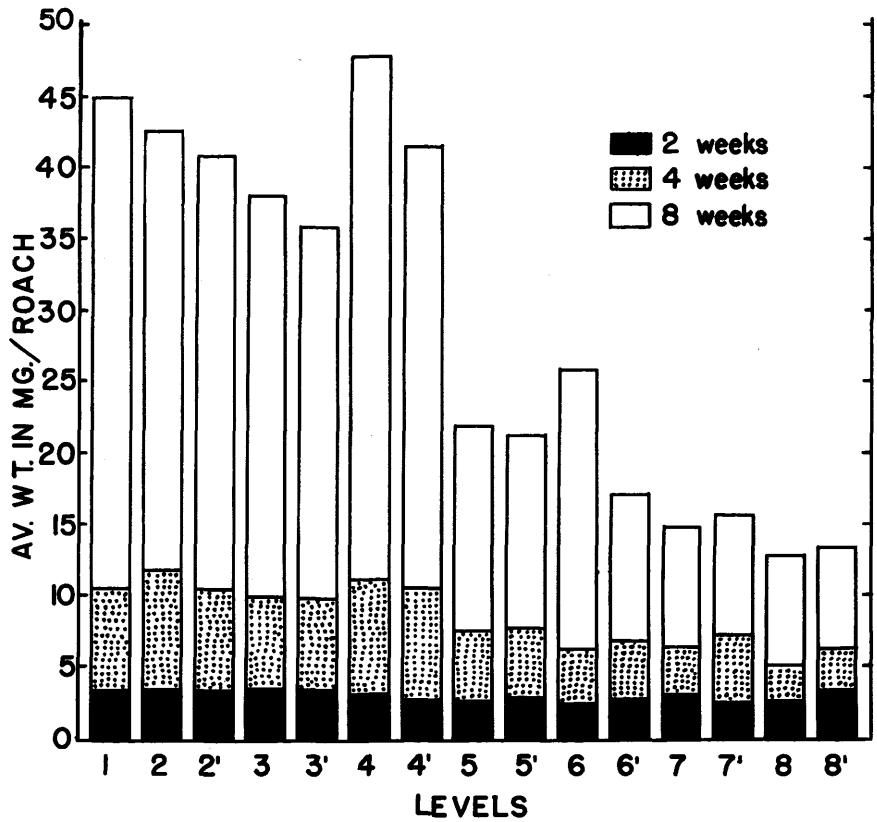

FIGURE 4. Growth of the second generation cockroaches at two, four, and eight weeks, when reared on the control and parental diets. (Primed numbers represent cockroaches reared on the diets of their respective parents; unprimed numbers represent cockroaches reared on the check diet.)

Second generation cockroaches, reared on either control or parental diets, appeared to reproduce normally if their parents received less than 50 ppm. of penicillin. However, third generation young produced by roaches whose parents received 50 to 100 ppm. of penicillin, hatched later than normal. Also, fewer nymphs per capsule were produced.

Cockroaches whose parents received 100 or more ppm. of antibiotic produced fewer egg capsules and these were often deformed. Again, this resulted regardless

of whether the second generation young were reared on the control or on the parental diets.

Experiments in rearing the young from adults receiving more than 400 ppm. of penicillin were rather limited and therefore inconclusive. In general, only enough insects were available to set up one replication. However, sometimes, not even the necessary 30 were available. On the average, these cockroaches reached the adult stage in about 13 to 16 weeks, regardless of whether they were reared on the control or parental diet. This was the case with 50 young from adults reared on diet 10 (1,560 ppm. of penicillin). The 50 young used for the test, however, were the total from fifteen egg capsules. Since a female cockroach normally produces 30 to 45 young per egg capsule, this sample was not considered adequate to reach any conclusions concerning the young which were produced.

Although fewer young were produced by adults receiving high concentrations of penicillin, those which did appear did not manifest all the characteristics shown by the aposymbiotic cockroaches which resulted from the work of Brooks and Richards (1955) with aureomycin at a level of 1000 ppm. They reported these nymphs possessed the following characteristics: 1) the nymphs were slightly smaller than normal and light gray in color rather than dark blackish brown; 2)

TABLE 5

Analysis of variance of the growth rates with second generation diets

Source	SS	DF	MS	F	F-5%	F-1%
Treatments	.4151	14	.02965	6.3545*	2.43	3.56
Error	.0700	15	.0046			
Total	.4851	29				

*Significant at 1% level.

Growth time equals the entire growth period. Test based on two replications of approximately 30 insects each, per treatment.

the embryonic cuticle, which is normally shed at the time of hatching, was not completely cast, but remained attached to the anal cerci; 3) the nymphs were weak and feeble; 4) some nymphs died immediately, while others lay on their backs for several days waving their antennae; and 5) the nymphs were almost completely unable to grow on the stock dog food diet. The controls molted approximately every 10 days on the dog food diet, and reached the adult stage at about 60 days. The young aposymbiotic nymphs had not molted once by the end of 30 days.

In this study, the offspring from adults receiving as much as 50,000 ppm. of penicillin did not show all of the above listed characteristics. The color appeared normal in about half of the insects, and the embryonic cuticles were completely detached. In addition, those that lived were able to reach the adult molt in 13 to 14 weeks on the normal dog food diet. This was not even twice the normal growth period. Antibiotics differ considerably in structure and mode of action. Therefore, it is quite likely that with dietary penicillin, the bacteroids were not eliminated or only partially so, and that the effects of the penicillin were directly on the cockroaches.

However, in all respects, the second generation cockroaches were much more adversely affected than the first.

SUMMARY

1. Some effects of the oral administration of penicillin on the German cockroach were studied. The antibiotic was added to a dog food diet at fifteen different

levels of concentration ranging from six to 100,000 ppm. Starting with six ppm., each succeeding dietary level increased geometrically by a factor of two. The insects were fed the various diets all their lives and through two successive generations.

2. The effects studied were: the growth rate; time to the adult stage; mortality; reproductive capacity; and these same effects in the second generation.

3. Test growth rates in the first generation appeared to be equivalent to the check if the particular diet did not contain more than 400 ppm. of penicillin. At 780 ppm. or more, inhibition of growth was significant and became increasingly more severe up to 100,000 ppm. In the second generation, the offspring of the cockroaches which received as little as 50 ppm. exhibited reduced growth rates, regardless of whether they were reared on the control or parental diet.

4. First generation cockroaches receiving diets containing less than 780 ppm. of penicillin reached the adult stage in approximately the same length of time. In levels containing 780 or more ppm., however, there was an inverse relationship between time to adult stage and growth rate. Offspring from first generation

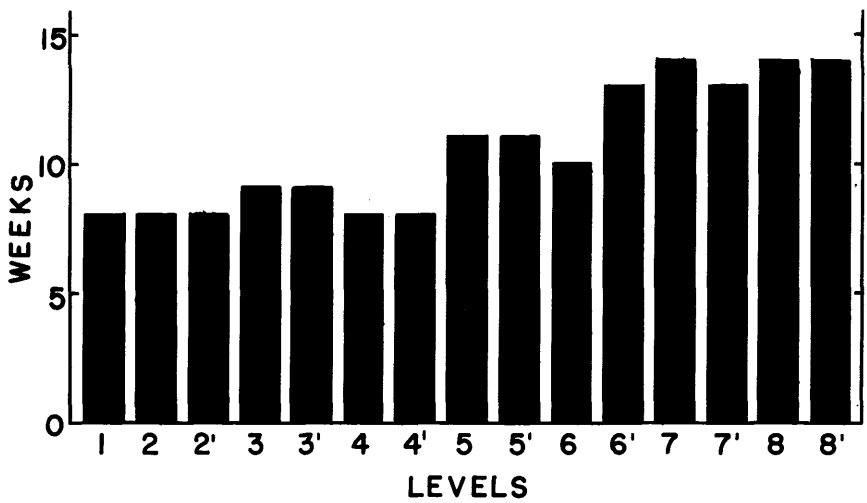

FIGURE 5. Time in which the second generation cockroaches reached the adult stage, when reared on diets containing different concentrations of penicillin. (Primed numbers represent cockroaches reared on the diets of their respective parents; unprimed numbers represent cockroaches reared on the check diet.)

adults receiving 50 or more ppm. of penicillin required a longer time to reach the adult stage than did the checks.

5. The first generation test cockroaches exhibited no appreciable mortality over the checks, even at the highest dietary levels of penicillin concentration. Second generation cockroaches exhibited higher mortality than the checks if their parents received as little as 100 to 200 ppm. of penicillin.

6. Reproductive capacity was apparently reduced in all dietary levels containing 200 or more ppm. of penicillin. No eggs were formed by the cockroaches receiving the diet containing 100,000 ppm. of penicillin. Offspring of adults receiving diets containing 50 or more ppm. of penicillin exhibited reduced reproductive capacity.

7. In all respects, the second generation cockroaches were much more adversely affected than the first.

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