

SOME EFFECTS OF FOWL ASCARID PARASITISM UPON  
HOST RESISTANCE TO A BACTERIAL TOXIN

by

JOHN RICHARD EGERTON

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## INTRODUCTION AND REVIEW OF LITERATURE

Within the past two decades much experimentation has been undertaken to gain a better understanding of the factors involved in host resistance to parasitism. Ackert (1942) presented a summarization of the work to that date dealing with the factors affecting parasitism and natural host resistance.

That dietary constituents may influence the natural resistance of animals to helminthic infections was reported by Zimmerman, Vincent and Ackert (1926). Ackert et al. (1927, 1931) presented evidence that vitamin A deficiency lowered the resistance of fowls to Ascaridia galli (Syn. A. perspicillum, A. lineata). Further evidence of vitamin B (complex) as a factor in host resistance to parasitism was presented by Ackert and Nolf (1931).

Evidence that the age of a fowl is a factor in the resistance of a host to parasitism was presented by Ackert, Porter and Beach (1935), Ackert and Edgar (1938), and Ackert, Edgar and Frick (1939). These workers have shown that the resistance of a fowl to the large intestinal roundworm increased with the age of the chicken and reached a maximum at about 17 weeks of age.

That the genetic make up of the host animal may be an important factor in host resistance to parasitic infections was shown by Ackert, Eisenbrandt, Glading and Wilmoth (1933); Ackert and Wilmoth (1934); Ackert, Eisenbrandt, Wilmoth, Glading and Pratt (1935); and Ackert, Pratt and Freeman (1936).

As to the resistance of a parasitized host to a secondary

pathogenic entity very few experiments have been reported. Ackert and Folse (1946), working with a bacterial toxin, presented experimental evidence that a moderate infection of A. galli will predispose chickens to the effects of type A botulism toxin. Injection of botulinus toxin into both parasitized and nonparasitized chickens resulted in higher morbidity and mortality in the parasitized groups than in the nonparasitized groups, indicating a predisposition to the botulinus toxin in the parasitized chickens. Riedel (1950) working with Ascaridia galli and Eimeria tenella showed that chickens infected with A. galli were not predisposed to infection with E. tenella to any significant degree. Chicks infected with both the nematode and the protozoan showed a higher morbidity and a more retarded growth rate than did chicks infected only with E. tenella. However, the mortality and the number of caecal lesions were about the same in both groups of chicks, thus indicating no predisposition of chickens infected with A. galli to infection with E. tenella.

Since chickens reared under usual barnyard or range conditions usually are, or have been parasitized, it is desirable to know the factors influencing the resistance of a parasitized host to a secondary pathogen. This study was undertaken to determine relationships between fowl parasitism and host resistance to a bacterial toxin.

## MATERIALS AND METHODS

The chickens used in this study were Single Comb White Leghorns purchased from approved commercial hatcheries. The chicks were obtained in three lots, one lot for each experiment, placed in electrically heated brooders and fed standard rations. Within a week's time all chickens were banded, and when sufficiently matured, the chickens were placed in larger growing batteries. At four weeks of age the chickens were weighed and separated into four groups by selecting four birds of approximately equal weights and placing one into each of Groups I, II, III and IV until the desired number of chicks were included.

Egg cultures of Ascaridia galli were prepared from gravid females collected from the small intestines of chickens that were being dressed at a local packing plant. The anterior end of each worm was excised and the body contents squeezed out into a Petri dish. The uteri were then isolated and placed into another dish and covered with a few millimeters of tap water. The uteri were broken by mixing the contents of the dish with sharp strokes of a teasing needle. The entire dish was covered and placed in a constant temperature incubator at 28°C. and the ova allowed to mature. The ova used to infect the experimental chickens were allowed to incubate from 18-60 days.

At the age of four weeks and immediately following weighing and grouping, all chickens in Groups III and IV were fed 200<sup>±</sup>10 embryonated eggs of A. galli utilizing a modification

of the method of Riedel (1947). Ova were removed from the culturing dishes and placed in a small, cork stoppered shell vial containing a small amount of fine, clean sand. Water was added and the contents of the vial thoroughly mixed by agitation to enable the sand particles to break apart any clumps of ova, thus insuring an even distribution of ova throughout the liquid. A calibrated pipette was then quickly inserted into the suspension and the proper amount of suspension was drawn up into the pipette. This suspension was then delivered onto a glass microslide and the number of embryonated ova present were tabulated. The suspension was altered by the addition of water, or ova from the culture, until the proper number of ova were delivered from the pipette consistently. When the dosage had been standardized at  $200 \pm 10$  embryonated ova per each delivery of the pipette, all chickens of Groups III and IV were exposed to the ova by holding the beak open and delivering the contents of the pipette well back into the oral cavity. The vial was thoroughly agitated immediately preceding the extraction of each dose. In order to insure more uniform exposure of all individuals, five chickens from one group and then five from the other were exposed, alternately, until all animals had been dosed. The chickens were then replaced in the batteries for two weeks and left there except for obtaining the weekly weight records.

The toxin used in this study was dried botulinus toxin (Type A) which was supplied by the National Institutes of Health Laboratories, Bethesda, Maryland. In preparing the

toxin solutions, sterile physiological saline was used. As a preliminary experiment ten healthy chickens six weeks of age were injected with varying amounts of toxin in order to determine the amount of toxin which would produce clinical symptoms up to and including total prostration. For this experiment five groups of two animals each were injected with 0.015, 0.020, 0.025, 0.030 and 0.035 mg of toxin per kilogram of fowl body weight. For one week subsequent to these injections, observations were made periodically upon the clinical symptoms exhibited by the experimental chickens. The chickens which had been injected with 0.015 mg of toxin per kilogram of body weight showed total prostration but no fatalities, while chickens which had been injected with greater amounts succumbed to the effects of the toxin in most cases, death occurring sooner as the toxin dosage increased. Therefore, 0.015 mg/Kg of body weight was chosen as the experimental dosage to be used in the present study.

The toxin solution was prepared as follows: an excess of the dried toxin was placed upon the pan of an analytical balance, 10 mg was removed from the pan, and the toxin which had been removed was placed in a sterile, cotton plugged test tube. Using sterile technique, 10 ml of sterile physiological saline was then added to the tube containing the toxin. This solution now contained 1 mg of toxin per cubic milliliter of saline. Next, 1.5 ml of the above solution was added to 98.5 ml of sterile saline and thoroughly mixed. The resulting solution, containing 0.015 mg of toxin per cubic milliliter, was placed

into sterile rubber stoppered vaccine bottles for ease of handling during the injection of the fowl. When the chickens to be injected were weighed and their weights recorded in grams, it was necessary only to transpose grams to kilograms in order to determine the amount of toxin solution to be injected so that each individual received 0.015 mg/Kg of body weight. For example, a 650 gm chicken would weigh 0.650 Kg, and at the rate of 0.015 mg/Kg of body weight would require  $0.015 \text{ mg/Kg} \times 0.650 \text{ Kg}$  or 0.00975 mg of toxin to be injected. Since the toxin was in the concentration of 0.015 mg/ml, using the equation  $\frac{0.00975 \text{ mg}}{0.015 \text{ mg/ml}} = 0.65 \text{ ml}$  to obtain the milliliters of solution to be injected, the calculated answer will be equivalent to the weight of the chicken as expressed in kilograms. This method of deriving the volume of toxin solution appropriate for each individual eliminated the necessity of an individual calculation for each injection, thus reducing the possibility of experimental error. All injections were made utilizing a 1 cc tuberculin syringe graduated in 1/100 cc. The weight in Kg of the chicken to be injected was reduced to two significant figures which gave the volume of toxin to be injected.

At six weeks of age, all chickens were weighed, their weights recorded, and chickens from Groups II and III were injected with the chosen amount of a freshly prepared solution of the toxin. Each chicken to be injected was held ventral side up with wings and legs held securely. The area just posterior to the sternum was swabbed with disinfectant, and the toxin injection was made directly into the peritoneal cavity



at a point just posterior to the sternum.

In order to obtain the maximum effects from the toxin injection, it was decided to give the injections 14 days following exposure of the chickens to 200<sup>+</sup>10 embryonated ova of A. galli. At this time the greater number of larvae are in the tissue phase (Tugwell and Ackert, 1950), and this is the time at which the host suffers the greatest damage (Ackert and Herrick, 1928).

Quin (1946) reported that the heart rates of experimental animals injected with botulinus toxin increased, at times nearly doubled, their normal rates. Consequently, it was decided to record the heart rate of each experimental chicken to be used as a possible criterion for judging the effect of the toxin on the injected chickens. Just prior to the injection of the toxin the heart rate of each chicken was taken with the aid of a stethoscope, and for a period of one week until the termination of the experiment the heart rate of each chicken was taken twice daily.

Other criteria used for judging the effects of the toxin upon the experimental chickens were general weakness, inability to rise, and death. At the time when heart rates were taken, observations were made upon the chickens and data on their clinical appearance were recorded. The weight gain or loss of each group was also chosen as a possible added criterion for judging the effects produced by the toxin, so weekly weight records of all chickens were made from the time of exposure of Groups III and IV to embryonated ova of A. galli until the

termination of the experiment.

Three weeks following the exposure of Groups III and IV to the ova of A. galli, and one week following the injection of Groups II and III, the experiments were terminated. The parasitized chickens from Groups III and IV were killed, and the small intestine from the gizzard to the yolk sac diverticulum was excised and flushed under hydraulic pressure into glass containers according to the method of Ackert and Nolf (1929). The intestine was then opened with an enterotome and the intestinal mucosa scraped free and added to the flushings. The wing band of each chicken was removed and placed in the appropriate containers with the intestinal contents and scrapings. After allowing a time lapse of about eight hours for the worms to relax and straighten out, concentrated formaldehyde was added to each container in sufficient quantity to kill and preserve all nematodes. Collection of the nematodes was accomplished by pouring the contents of each container into a shallow, wide bottom glass dish and picking each nematode out with a small curved teasing needle. The nematodes and the wing band of the chicken from which they were collected were placed in small glass vials containing 10 per cent formalin.

Measurement of the worms was done by projecting the image of each worm, magnified six times to reduce error, upon a ground glass and making pencil tracings of the outline of the worm upon paper. The pencil tracings were then traversed with a milled wheel calibrated in millimeters. The results were then divided by a factor of six to obtain the true length of each specimen.

The sex of the worms being measured was determined with the aid of a compound dissecting microscope and a notation of the sex of each worm was placed beside its appropriate pencil tracing.

Because of the results which had been obtained in experiments I and II, it was decided at the termination of experiment III to collect blood serum samples from representative chickens of all four groups for the purpose of conducting precipitin tests. Four chickens from each group were selected at random and blood was collected from them by severing a large vein in the neck and allowing the blood to run into large, clean test tubes. The blood was allowed to clot at room temperature, and then the clots were ringed and centrifuged to separate the blood serum. The serum was then drawn off with sterile pipettes, placed in sterile stoppered test tubes, and put into a deep freeze unit for preservation at  $-40^{\circ}\text{C}$ .

Antigen used in conducting precipitin tests in the present study was prepared from living specimens of A. galli and from dried botulinus toxin. The worms were collected from the intestines of freshly drawn chickens, placed in physiological saline, and washed in five successive changes of saline to remove any clinging foreign matter. The worms were then passed briefly through a distilled water rinse bath and placed into a small vial. A one hole rubber stopper with a short length of glass tubing protruding was then firmly inserted into the vial and the vial partially immersed in a bath containing 95 per cent ethyl alcohol and solidified carbon dioxide. This treatment caused instantaneous freezing of the worms and kept

any denaturization of the protein which might have occurred to a minimum. The vial containing the worms was then attached to a vacuum pump and a partial vacuum was created. A condensation chamber consisting of an outer insulated chamber and an inner metallic chamber was in position between the pump and the vial containing the frozen worms. The inner container, which served to catch and hold the moisture which was drawn off from the specimens being lyophilized, was immersed in a bath of ethylene glycol monomethyl ether and solidified carbon dioxide contained within the outer insulated chamber. Desiccation was continuous for a period of 24 hours and continued at slightly under 200 microns for that period. At the termination of lyophilization the vial was sealed under a partial vacuum by fusing the glass tubing under a torch flame. The tube containing the desiccated worms was then weighed on an analytical balance, the worms removed to a sterile mortar, and the tube and stopper weighed again. The difference between the two weighings was equivalent to the dry weight of the worm protein. The worms were next macerated in the mortar and then returned to the vial. The mortar and pestle were rinsed with two washes of sterile saline using 5 cc for each rinse. The rinse was added to the contents of the vial, and an additional 12 cc of sterile saline was added to the macerated worms making a total of 22 cc. The resulting mixture was thoroughly agitated and then centrifuged at 30,000 R.P.M. for 20 minutes. Following centrifugation, a sterile pipette was used to remove 20 cc of the supernatant fluid. This antigen, containing 2.6 mg of protein per cc, was

placed into rubber stoppered tubes and placed in a deep freeze unit until used in the tests.

The botulism toxin used as antigenic material in conducting the precipitin test was mixed in the same manner as the toxin used in the injection of the fowl. The antigen was made in the concentration of 1 mg of toxin in 1 cc of saline, placed in rubber stoppered tubes and then in a deep freeze unit until used.

The tests were set up by cutting lengths of capillary tubing about one inch long and fusing one end in a bunsen burner flame. The tubes were set into small wooden blocks in an upright position and a sample of the serum being tested was placed in each by means of a long finely drawn pipette. The serum was then overlaid with appropriate antigens in varying dilutions.

The blocks containing the tubes were next placed in an incubator at 37°C. for one-half hour, removed, and placed in a refrigerator over night. The tubes were then examined for the typical "ring" formation and the results recorded.

## EXPERIMENTAL RESULTS

### Experiment I

Examination of the clinical symptoms of botulism exhibited by Groups II and III (Table 2) shows the relative effects the toxin produced. The unparasitized chickens (Group II) had one chicken weak at 19 hours following the toxin injection, two



Table 2. Record of weekly weights, worm lengths, worm numbers, and botulism symptoms of chickens in Experiment I.

Chicken number	Group I				Group II				Group III				Group IV																						
	Weight (gm)				Weight (gm)				Weight (gm)				Weight (gm)																						
	11/9/50	11/16	11/24	12/1/50	11/9/50	11/16	11/24	12/1/50	11/9/50	11/16	11/24	12/1/50	11/9/50	11/16	11/24	12/1/50	11/9/50	11/16	11/24	12/1/50															
A4090	356	462	652	810	A4092	262	353	460	594	A4101	266	374	468	552	1	1	15.8	2.2	15.8	2.2	15.8	2.2	A4088	312	426	525	641	9	9	4.0	14.5	1.5	1.8	2.9	3.7
A4093	276	372	454	550	A4094	264	354	427	527	A4102	306	420	532	630	1	3	4.0	14.3	4.0	3.5	4.0	7.6	A4089	314	402	529	643	1	0	3.5	-	3.5	-	3.5	-
A4095	290	364	510	644	A4097	324	426	575	637	A4105	252	340	440	528	6	6	4.2	4.2	1.7	3.3	2.9	3.7	A4091	306	405	528	633	0	1	-	3.7	-	3.7	-	3.7
A4098	342	448	573	759	A4103	286	400	522	622	A4116	324	420	558	688	7	3	5.0	2.2	1.7	1.7	2.9	2.0	A4096	256	350	476	592	7	8	8.0	26.8	1.3	5.0	4.3	21.1
A4099	322	445	592	792	A4106	278	368	486	600	A4119	332	444	574	712	4	4	13.3	11.7	2.5	1.7	7.8	5.0	A4104	302	362	454	576	3	1	3.0	2.3	2.3	2.3	2.6	2.3
A4100	244	356	483	617	A4107	256	458	496	573	A4121	358	464	573	702	11	11	7.2	3.3	0.8	1.3	2.5	2.4	A4111	280	382	482	591	2	2	4.7	17.5	3.3	6.0	4.0	11.7
A4109	264	354	486	582	A4115	308	387	494	567	A4123	264	364	443	554	5	7	16.3	18.3	3.0	3.3	5.8	13.6	A4126	294	391	508	586	9	4	4.5	4.5	1.5	1.8	3.1	2.9
A4110	232	310	391	492	A4128	248	334	426	362	A4124	318	393	509	636	1	2	4.2	3.7	4.2	1.7	4.2	1.8	A4127	358	482	626	756	2	1	2.5	2.3	1.3	2.3	1.9	2.3
A4112	328	428	547	695	A4129	360	484	638	642	A4131	344	485	635	770	3	0	3.0	-	2.5	-	2.7	-	A4144	348	476	609	749	12	3	5.7	3.7	2.7	3.2	3.9	3.4
A4114	268	358	500	594	A4135	338	444	556	611	A4134	296	397	503	579	4	4	20.8	20.5	3.3	1.3	8.3	10.3	A4149	338	434	565	720	6	6	6.0	4.8	3.5	2.7	4.5	3.8
A4118	284	394	558	692	A4150	280	372	494	562	A4136	302	400	496	607	13	4	3.7	3.3	1.8	3.0	2.8	3.2	A4153	324	451	546	640	2	5	4.3	4.8	3.2	2.5	3.7	2.6
A4120	360	497	665	820	A4151	286	352	479	572	A4137	262	357	422	553	1	1	2.0	1.7	2.0	1.7	2.0	1.7	A4156	264	374	469	606	4	1	4.2	1.8	1.5	1.8	3.3	1.8
A4145	304	391	471	600	A4155	310	432	517	604	A4138	352	438	542	667	3	4	2.5	9.2	1.7	1.7	1.9	4.5	A4161	264	359	450	588	1	1	3.5	3.0	3.5	3.0	3.5	3.0
A4148	286	360	478	594	A4159	284	387	511	506	A4139	284	379	476	582	0	1	-	2.5	-	2.5	-	2.5	A4164	230	298	356	444	6	4	4.2	4.7	1.5	1.7	2.7	2.8
A4152	262	335	422	511	A4160	302	366	535	660	A4140	336	449	564	722	1	3	17.2	2.3	17.2	1.7	17.2	1.8	A4169	282	386	527	673	1	0	2.8	-	2.8	-	2.8	-
A4154	302	381	526	661	A4168	270	347	452	516	A4141	310	358	445	555	8	9	3.0	18.3	0.8	1.7	2.0	5.9	A4173	356	468	582	708	0	1	-	23.2	-	23.2	-	23.2
A4157	320	417	539	691	A4172	350	467	625	770	A4146	272	371	442	528	1	1	14.7	15.8	14.7	15.8	14.7	15.8	A4177	284	357	445	545	0	0	-	-	-	-	-	-
A4167	338	436	581	702	A4175	316	376	540	662	A4147	246	344	406	470	4	19	4.7	4.7	2.0	2.0	3.5	3.3	A4180	320	424	537	750	0	1	-	3.2	-	3.2	-	3.2
A4174	256	330	434	530	A4183	256	340	482	575	A4162	280	364	456	569	2	3	4.2	2.5	1.7	2.2	2.9	2.4	A4181	266	348	441	554	6	1	3.3	4.0	2.0	4.0	2.7	4.0
A4178	314	404	520	639	A4237	318	410	529	550	A4170	286	358	451	560	2	2	4.0	4.5	3.8	3.3	3.9	3.9	A4182	282	383	448	586	1	1	3.8	4.8	3.8	4.8	3.8	4.8
A4186	282	374	453	555						A4184	234	282	320	410	1	2	2.3	4.0	2.3	1.7	2.3	2.8	A4239	242	320	403	513	6	4	4.7	5.3	3.3	2.0	4.1	3.8
Average	296	391	516	645		296	389	513	597		296	391	488	598	3.8	4.3					4.0	4.8		296	394	500	623	3.7	2.6				3.4	6.7	

\* The figures in this column refer to hours following toxin injection.

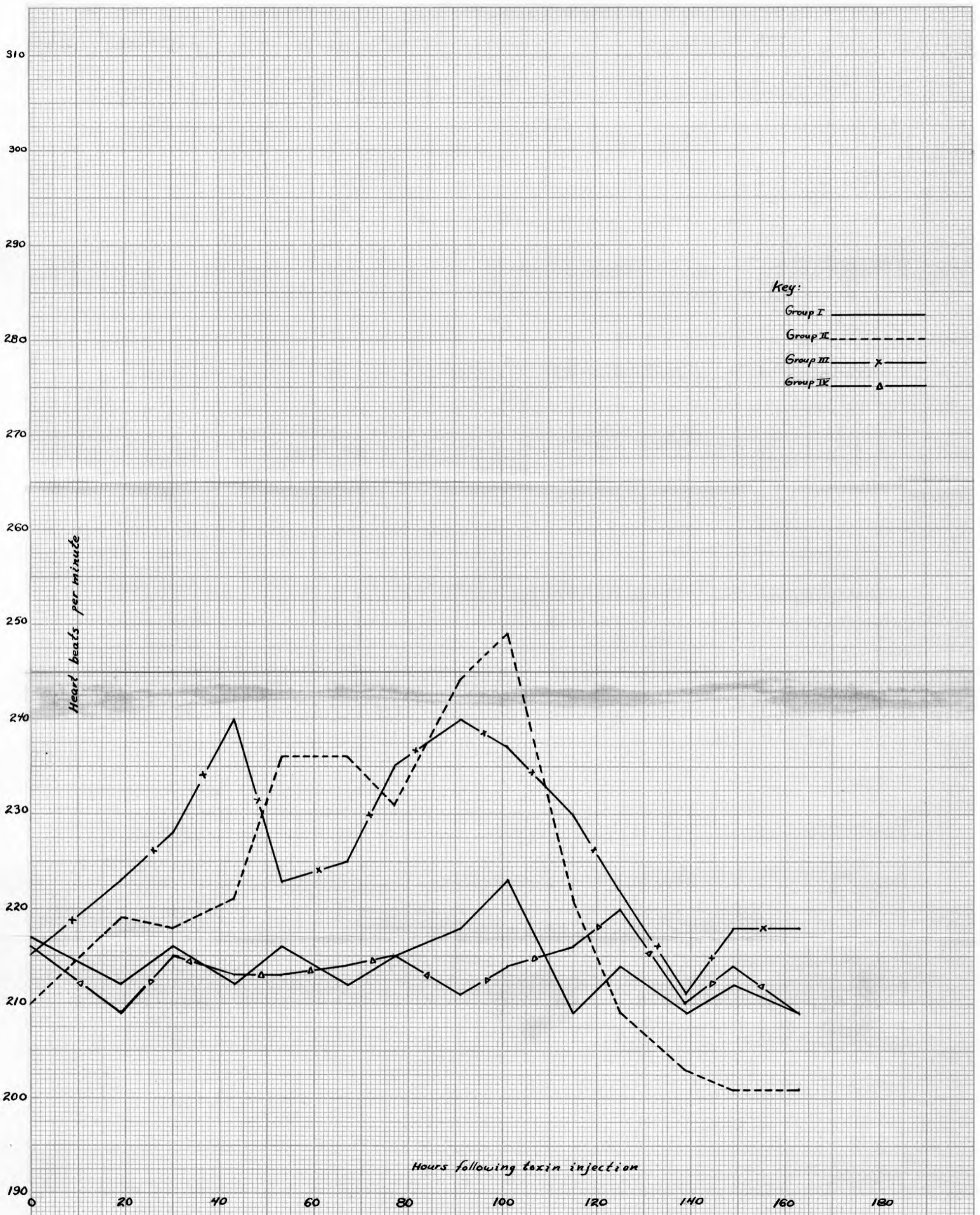


Fig. 1. Average heart rates of chickens in Experiment I.



more at 30 hours, another at 43 hours, and a total of 5 by the fifty-third hour. At that time two chickens were unable to rise. At 67 hours following injection the third and last chicken was prostrate, but four additional fowls became weakened by the ninety-first hour. In contrast to this the parasitized chickens (Group III) exhibited no symptoms of botulism.

Figure 1 and Table 1 show that the average heart rate of the parasitized fowls (Group III) began increasing sooner than that of the unparasitized ones (Group II). But the average heart rate of Group II reached a peak exceeding that of Group III and returned to normal sooner than did that of Group III.

Examination of the weekly weight records reveals that the average weight gain of the chickens in Group II was exceeded by that of Group III following the toxin injections.

As to worm infections, Group III yielded a total of 169 worms (79 males and 90 females) and Group IV (parasitized but uninjected) harbored a total of 132 worms (78 males and 54 females). The average number of worms per chicken in Group III was 8.1 as compared to an average of 6.2 in Group IV. The average lengths of the worms recovered from Group III were 3.96 mm for the males and 4.79 mm for the females as compared to 3.44 mm for the males and 6.71 for the females in Group IV. The female A. galli from Group IV averaged slightly larger than those in Group III while the males varied only to a small degree.

The results from injecting Group II (unparasitized) and Group III (parasitized) with botulinus toxin (Type A) at the rate of 0.015 mg/Kg of fowl body weight showed that the Group

III (parasitized) chickens were affected first by the toxin as indicated by the heart rate increasing at an earlier time than did that of the unparasitized group. However, when clinical symptoms are considered, the parasitized chickens (Group III) were more resistant to the toxin than the unparasitized chickens (Group II) since they lacked the morbidity shown by the latter in which a total of nine fowls exhibited clinical symptoms of botulism.

#### Experiment II

The results of the observations made upon the injected chickens in this experiment are recorded in Table 4. The unparasitized chickens (Group II) manifested the first observable clinical symptoms at 19 hours following the injection of the toxin, at which time four individuals exhibited a weakened condition. Twenty-eight hours following injection three chickens from this group were prostrate, and by the forty-third hour two more, making a total of five, were unable to rise. The last animal to be affected was prostrate at 52 hours, but no deaths had occurred as yet. The first fatality occurred by the sixty-seventh hour, and the last deaths of a total of six were observed 72 hours later at 139 hours subsequent to the initial toxin injection.

In comparison to this, only four individuals of the parasitized fowls in Group III exhibited any symptoms of botulism which were observable, and the toxin produced no

effects beyond a weakened condition in any one of the four. The first observable symptom appeared at 43 hours, 24 hours following the first symptom exhibited by Group II.

As in the preceding experiment, the average heart rate of the parasitized group increased sooner than that of the unparasitized group (Fig. 2 and Table 3). Contrary to Experiment I, however, was the peak reached by the two groups. Group III reached a peak in the average heart rate which exceeded that of Group II. The return to normal was similar in both groups.

The weekly weight records following the toxin injection show that the parasitized Group III, on the average, weighed less than the nonparasitized Group II (Table 4).

Data from the same table show that the chickens (Group III) injected with the botulinus toxin harbored a total of 297 worms (166 males and 131 females) and the uninjected chickens (Group IV) harbored a total of 176 worms (82 males and 94 females). The average number of worms per chicken recovered from Group III was 16.5 as compared to an average of 8.8 worms per chicken in Group IV. The average length of the male worms recovered from Group III was 19.3 mm as compared to an average length of 19.6 mm for the males of Group IV; while the average length of the females in Group III was 19.9 mm and that of Group IV was 25.5 mm. In this experiment the male worms from both groups were similar with regard to average lengths, but the average length of the females from Group IV was greater than that of the females from Group III.

The results of this experiment show that effects of the



Table 4. Record of weekly weights, worm lengths, worm numbers, and botulism symptoms of chickens in Experiment II.

Group I				Group II				Group III				Group IV																																
Weight (gm)				Weight (gm)				* Clinical symptoms				Worms																																
Chicken number	2/22/51	3/1/51	3/8/51	3/15/51	Chicken number	2/22/51	3/1/51	3/8/51	3/15/51	Weakness	Inability to rise	Death	Chicken number	2/22/51	3/1/51	3/8/51	3/15/51	Weakness	Inability to rise	Death	Total	Maximum length (mm)	Minimum length (mm)	Average length (mm)	Chicken number	2/22/51	3/1/51	3/8/51	3/15/51	Total	Maximum length (mm)	Minimum length (mm)	Average length (mm)											
A4365	194	308	438	600	A4359	228	320	414	-				A4363	196	316	442	534							26	9	31.4	28.2	3.5	8.7	19.3	21.5	A4360	186	408	542	620	5	4	24.7	31.7	4.0	27.0	16.6	29.0
A4366	238	338	468	584	A4375	192	266	358	530	28	43	139	A4371	154	242	317	400				2	0	24.7	-	20.5	-	22.6	-	A4362	208	308	401	508	1	1	23.8	26.4	23.8	26.4	23.8	26.4			
A4376	145	206	310	434	A4389	187	266	328	424				A4372	228	310	399	500				30	31	36.0	39.4	14.7	16.5	28.2	32.6	A4369	258	362	446	562	0	0	-	-	-	-	-	-			
A4401	184	271	370	503	A4407	194	270	380	-	19	28	91	A4378	180	252	338	450				6	4	27.5	30.0	8.2	8.7	18.9	21.4	A4370	194	288	370	480	5	3	28.4	33.0	4.3	30.0	16.6	31.5			
A4416	178	198	260	362	A4409	158	275	400	-	19	28	76	A4399	210	252	328	339	43			7	6	21.7	27.6	5.8	8.0	16.5	18.5	A4377	210	256	348	466	1	0	5.0	-	5.0	-	5.0	-			
A4427	234	296	382	534	A4410	242	331	406	534				A4386	182	194	252	358				10	7	28.4	27.5	6.7	8.0	18.2	19.8	A4380	148	199	260	316	3	6	23.7	26.7	10.0	3.2	18.4	12.8			
A4433	196	295	406	518	A4414	278	423	566	713				A4393	174	271	396	528				2	0	4.3	-	3.5	-	3.9	-	A4385	154	240	318	410	1	4	27.0	30.2	27.0	13.2	27.0	23.8			
A4434	282	405	536	700	A4417	244	350	478	628				A4398	194	205	276	367				1	1	9.7	4.2	9.7	4.2	9.7	4.2	A4387	252	350	438	594	0	0	-	-	-	-	-	-			
A4441	255	338	424	536	A4422	166	278	244	350				A4404	228	292	372	458				11	13	30.0	35.4	4.2	9.2	22.0	26.6	A4392	236	320	408	530	6	11	29.0	33.2	15.3	18.7	23.5	27.6			
A4442	186	276	382	498	A4424	230	344	440	616				A4406	186	292	410	404	91			28	19	30.0	32.2	5.2	6.3	19.4	24.5	A4394	169	270	358	456	2	3	19.4	25.4	14.0	18.8	16.7	21.1			
A4446	277	358	472	626	A4425	251	368	486	602				A4418	291	338	444	552				6	6	27.4	32.0	16.7	5.0	22.2	20.9	A4397	274	409	520	560	14	16	31.9	34.8	3.7	16.0	24.1	29.7			
A4451	163	214	276	380	A4430	204	231	304	368	43			A4419	252	332	430	528				0	1	-	13.2	-	13.2	-	13.2	A4402	182	276	366	462	10	9	26.8	35.6	3.8	12.2	13.7	23.4			
A4454	229	278	382	514	A4432	220	276	352	-	43	52	139	A4421	244	326	412	504				2	1	20.2	3.5	16.5	3.5	18.3	3.5	A4403	174	202	210	292	13	10	29.8	32.8	11.8	8.2	21.1	22.9			
A4457	216	236	240	324	A4436	192	230	328	440	76			A4429	232	306	368	521				5	3	26.7	26.9	12.8	22.8	20.5	25.0	A4412	196	282	350	446	2	4	18.7	28.5	3.5	18.3	11.2	21.8			
A4459	266	352	460	651	A4438	195	258	334	-	19	43	91	A4444	222	250	296	314	52			7	3	18.8	15.2	2.5	3.2	6.5	7.2	A4415	226	270	336	442	2	1	15.2	3.5	3.3	3.5	9.2	3.5			
A4460	271	391	520	648	A4440	227	326	426	-	19	28	67	A4445	278	330	450	560				3	1	21.6	25.8	8.7	25.8	17.3	25.8	A4420	228	289	410	532	7	15	28.7	34.5	9.0	16.3	22.7	29.8			
A4461	203	246	322	429	A4449	177	252	318	452				A4447	212	290	365	422	91			10	13	4.7	6.2	2.5	3.3	3.7	4.1	A4423	222	304	396	524	1	0	8.8	-	8.8	-	8.8	-			
					A4456	277	312	406	550				A4455	260	274	340	436				10	13	20.0	24.8	4.8	10.3	14.4	18.4	A4428	193	250	324	426	1	1	11.8	12.7	11.8	12.7	11.8	12.7			
					A4661	268	322	410	538																				A4435	234	395	513	620	8	6	25.4	28.6	21.7	22.7	23.6	26.4			
																													A4443	235	266	367	510	0	0	-	-	-	-	-	-			
Average	221	298	391	519		217	299	388	518					218	282	368	429				9.2	7.3					19.3	19.9		211	297	381	488	4.1	4.7					19.6	25.5			

\* The figures in this column refer to hours following toxin injection.

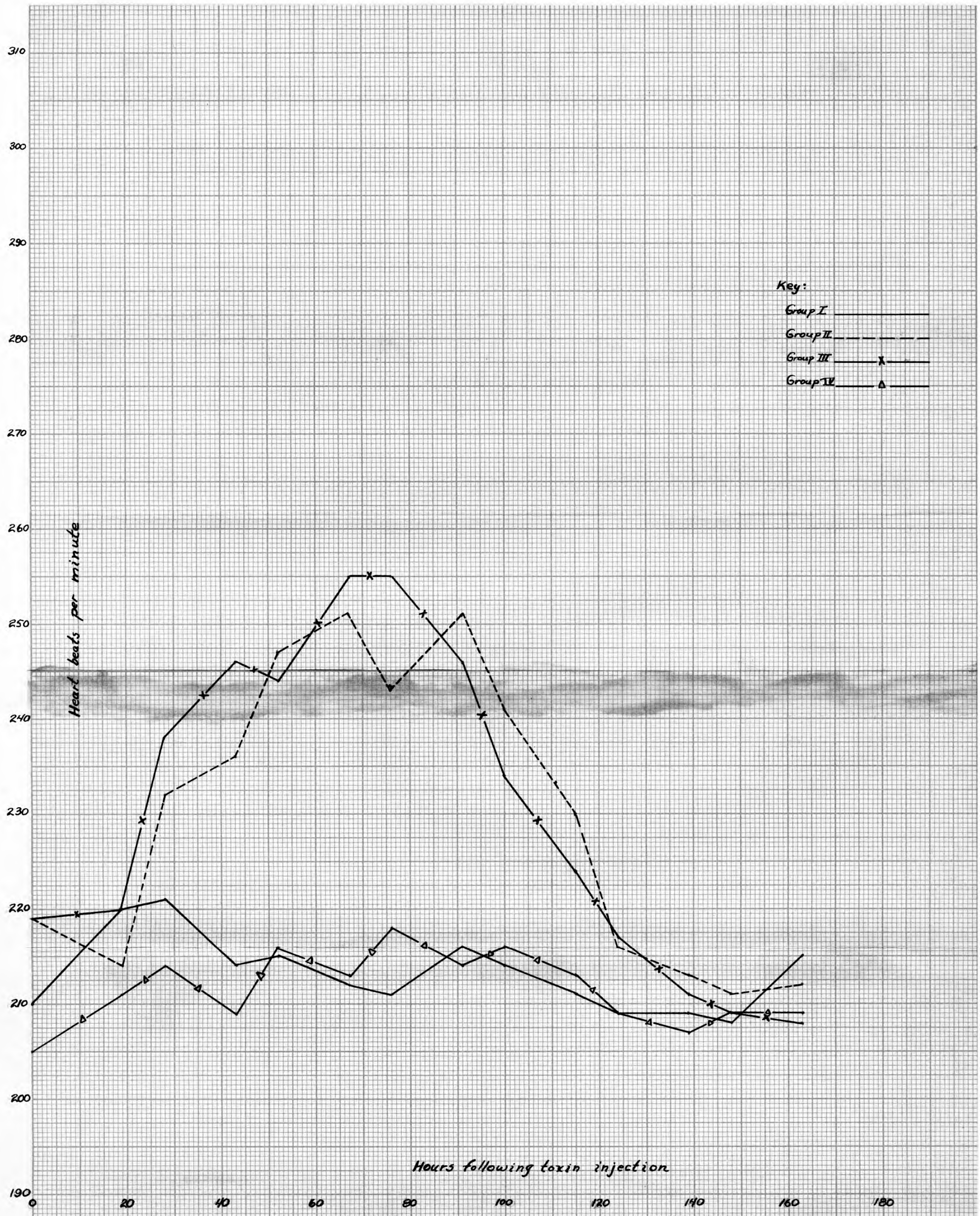


Fig. 2. Average heart rates of chickens in Experiment II.

botulinus toxin are manifested first in the group of chickens harboring the fowl nematode Ascaridia galli as indicated by the rise in heart rate occurring at an earlier hour following injection of the toxin, but that the most severe effects of the toxin occur in the unparasitized group as indicated by the higher morbidity shown in this group than in the parasitized group.

### Experiment III

This experiment was similar in all respects to the two preceding experiments with the exception of the collection of blood samples from some chickens from all four groups at the termination of the experiment.

Examination of the clinical symptoms as recorded in Table 6 reveals that the groups injected with the botulinus toxin exhibited the severest symptoms of botulism of all the experiments. Seventeen chickens of a total of 21 in Group II suffered from the toxin to such an extent that the clinical picture was observable as a weakened condition, 10 of these chickens were prostrate at some time subsequent to the toxin injection, and eight of these cases ended fatally. Weakness of the chickens was first observed 19 hours following the injection of the toxin and appeared periodically up to and including 52 hours. The first chicken exhibiting an inability to rise was observed at 28 hours following the injection.

Six individuals were prostrate by the forty-third hour, an additional two by the fifty-second hour, and the last of the total of ten was prostrate by the ninety-first hour. The first fatality occurred by the ninety-first hour following the toxin injection, with a total of eight chickens succumbing from that time including the final one by the hour at which the experiment was terminated.

Of a total of 21 chickens in Group III, 16 exhibited recognizable symptoms of weakness, nine of these 16 were so affected as to be unable to rise, and 5 of these 9 chickens succumbed to the effects of the toxin. Weakness was first observed periodically throughout the course of the experiment up to the one hundredth hour, at which time the last chicken exhibiting weakness was observed. The initial appearance of an inability to rise was at the forty-third hour following the injection at which time five chickens were affected. Four additional chickens were unable to rise through the period up to 148 hours. The first fatality occurred at this time. By the termination of the experiment four additional chickens had succumbed.

The average heart rates of Groups II and III, as shown in Figure 3 and Table 5, began to increase at about the same time with that of Group II slightly ahead of Group III. The peaks in the average rate of each group were for all practical purposes identical, but Group III began the descent toward normal slightly ahead of Group II.

The average weight of the chickens in Group II remained





Table 6. Record of weekly weights, worm lengths, worm numbers, and botulism symptoms of chickens in Experiment III.

Group I				Group II				Group III												Group IV																					
Weight (gm)				Weight (gm)				* Clinical symptoms				Weight (gm)				* Clinical symptoms				Worms				Weight (gm)				Worms													
Chicken number	4/17/51	4/24/51	5/1/51	5/8/51	Chicken number	4/17/51	4/24/51	5/1/51	5/8/51	Weakness	to	Death	Chicken number	4/17/51	4/24/51	5/1/51	5/8/51	Weakness	to	Death	Total	Maximum length (mm)	Minimum length (mm)	Average length (mm)	Chicken number	4/17/51	4/24/51	5/1/51	5/8/51	Total	Maximum length (mm)	Minimum length (mm)	Average length (mm)								
										rise								rise			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀							
A4928	260	350	479	516	A4931	186	253	329	-	19	43	115	A4943	224	279	346	328				6	6	6.7	8.7	3.3	1.8	3.9	4.6	A4965	262	351	472	518	16	15	27.7	35.0	11.8	25.2	24.2	30.8
A4929	190	287	389	420	A4934	200	274	351	-	19	91	115	A4944	222	298	372	463				16	14	16.7	20.0	3.5	3.5	7.6	11.6	A4969	229	329	426	470	7	7	10.0	31.8	3.3	4.8	4.9	11.5
A4930	233	304	396	460	A4935	206	286	372	-	28	43	139	A4948	206	375	361	280	100			7	5	16.7	16.8	3.2	2.3	8.9	10.1	A4970	224	280	344	360	14	2	6.0	4.2	3.3	3.8	4.5	4.0
A4932	165	236	349	334	A4940	224	313	413	312	91			A4954	258	347	452	-	28	43	115	2	0	3.8	-	3.5	-	3.7	-	A4972	207	265	310	316	4	3	4.0	4.8	2.2	3.2	3.2	3.9
A4933	198	284	369	410	A4942	221	317	416	-	19	43	124	A4956	236	316	413	-	28	43	163	2	1	4.5	3.3	3.5	3.3	4.2	3.3	A4976	236	291	372	405	1	3	11.7	26.9	11.7	18.5	11.7	22.0
A4936	224	296	394	453	A4947	266	370	486	416	91			A4962	262	358	460	382	52			11	13	11.3	12.3	5.2	4.3	8.9	10.1	A4981	219	299	380	408	4	2	15.0	18.7	3.5	5.3	6.6	12.0
A4937	242	310	409	454	A4950	245	339	438	296	91			A4963	208	315	414	352	28	43		3	1	5.0	4.2	4.0	4.2	2.1	4.2	A4984	234	326	414	436	4	2	4.3	4.7	2.2	3.2	3.7	3.9
A4938	246	336	414	430	A4952	256	352	442	398	43			A4968	278	371	488	398	28			13	6	8.5	10.0	2.5	3.2	4.9	7.1	A4985	270	372	496	552	5	1	16.7	21.7	4.2	21.7	12.4	21.7
A4939	204	300	437	470	A4953	238	309	382	372	52			A4975	243	330	429	357	43			10	22	14.8	20.8	6.7	9.5	11.0	14.0	A4993	212	279	351	392	3	1	21.6	3.3	3.3	3.3	12.1	3.3
A4941	218	312	427	472	A4955	204	282	479	-	19	28	91	A4979	234	294	390	225	43	148		0	0	-	-	-	-	-	-	A4995	264	376	483	496	1	1	9.0	3.2	9.0	3.2	9.0	3.2
A4945	236	328	432	482	A4960	240	306	404	472				A4983	235	320	419	386	124			5	2	24.2	23.4	3.8	22.5	13.5	22.9	A4996	200	266	352	274	2	1	28.4	4.0	4.0	4.0	16.1	4.0
A4946	252	334	440	521	A4964	270	360	472	362	28	52		A4986	169	234	332	262	19	43		1	1	5.0	4.0	5.0	4.0	5.0	4.0	A4999	238	319	386	410	11	5	8.0	11.5	1.8	2.7	4.5	5.8
A4949	278	366	489	530	A4967	228	316	422	-	43	52	163	A4988	245	357	478	-	19	43	115	1	1	5.0	3.5	5.0	3.5	5.0	3.5	A5000	248	334	441	480	17	10	18.4	23.5	1.8	3.3	5.1	12.6
A4957	213	292	394	409	A4973	212	288	370	423				A4989	200	282	368	350	43			1	2	4.5	21.6	4.5	17.3	4.5	19.5	A5008	204	291	402	406	1	1	3.3	4.7	3.3	4.7	3.3	4.7
A4959	229	306	402	430	A4974	168	221	285	-	19	43	124	A4992	246	328	436	304	43			16	13	13.5	14.8	3.5	7.5	10.0	11.1	A5009	256	362	476	514	5	3	19.7	30.8	4.2	4.7	11.7	21.5
A4961	222	312	426	492	A4977	234	326	420	455	28	43		A4997	187	264	316	388	43			13	10	24.3	16.7	3.2	8.2	10.5	13.4	A5010	222	308	397	426	1	2	4.8	18.4	4.8	4.0	4.8	11.2
A4966	268	355	464	498	A4980	248	339	442	537				A4998	230	325	426	-	28	91	115	8	7	4.7	4.5	3.2	2.0	3.8	3.4	A5011	188	273	372	430	6	3	5.2	5.0	2.8	3.3	3.6	4.2
A4971	238	308	420	473	A4982	202	288	382	310	52			A5001	148	211	310	196				2	7	16.2	16.5	7.2	12.3	11.7	14.3	A5013	169	240	326	353	2	2	16.3	10.2	14.6	5.0	15.5	7.6
A4973	202	300	406	472	A4987	211	295	376	295	91			A5002	240	332	458	334	91	115		6	3	5.2	5.2	1.7	3.5	3.8	4.2	A5014	202	278	358	394	2	4	13.2	26.8	8.2	23.4	10.8	25.2
A5003	216	288	330	372	A4994	192	273	352	-	19	43	115	A5004	202	279	374	464				6	2	26.8	26.8	4.2	6.2	20.6	16.5	A5015	200	263	362	379	5	4	26.6	31.8	23.5	5.0	25.1	22.6
A5007	208	282	385	409	A5012	262	365	488	620				A5005	210	322	400	-	43	100	148	10	9	4.8	5.2	3.3	3.5	4.1	4.9	A5185	242	340	434	450	1	0	14.3	-	14.3	-	14.3	-
Average	225	308	412	453		225	308	406	406					223	311	404	342				6.6	5.9				8.1	10.5		225	306	398	427	5.3	3.4					9.9	16.1	

\* The figures in this column refer to hours following toxin injection.

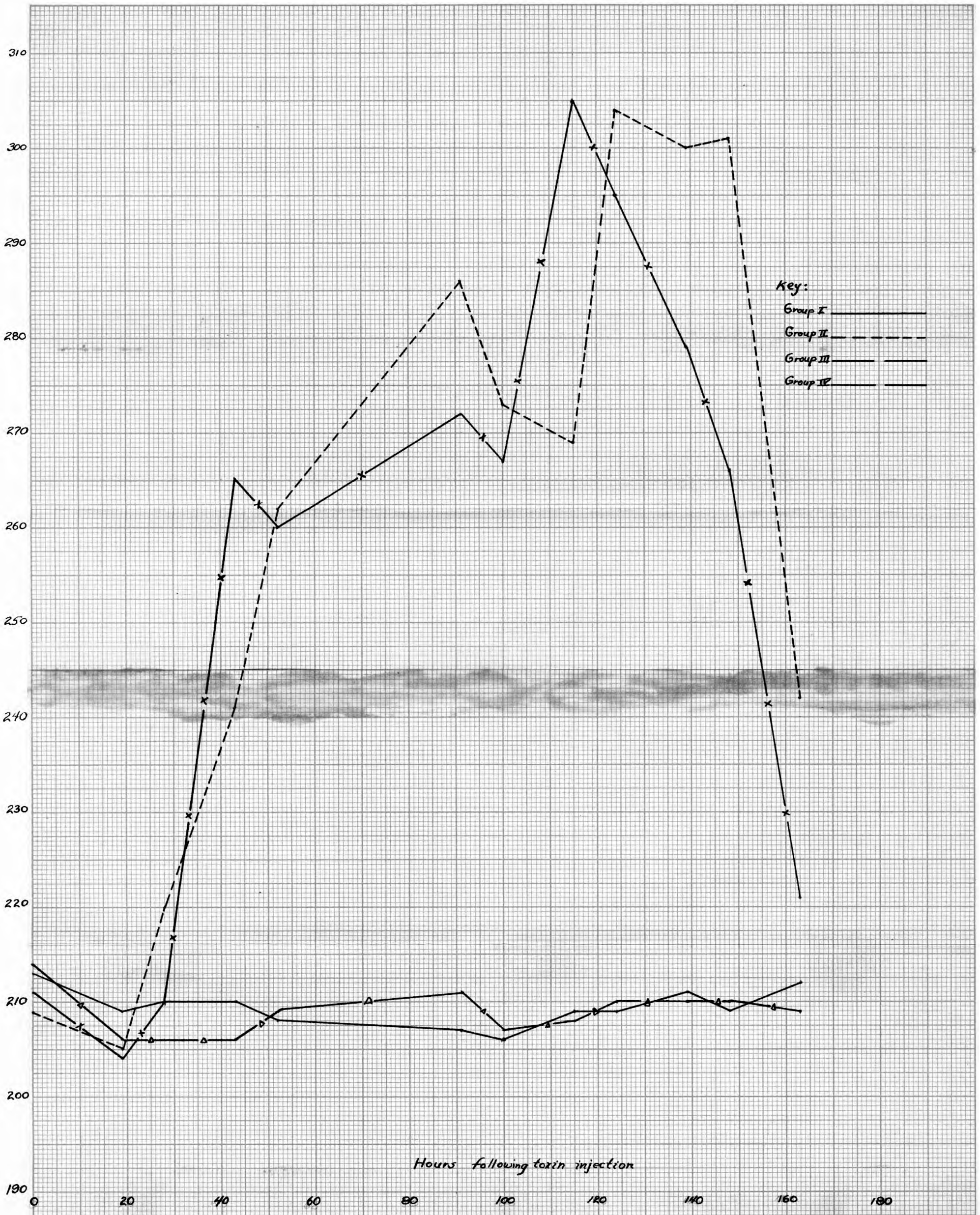


Fig. 3. Average heart rates of chickens in Experiment III.

the same as it had been before the toxin injection while the chickens in Group III suffered a weight loss on the average, as shown in Table 6.

The total number of worms harbored by Group III was 264 (139 males and 125 females) with an average of 12.6 worms per chicken. Group IV yielded a total of 184 worms (112 males and 72 females) with an average of 8.8 worms per chicken. The average lengths of the males in Group III was 8.1 mm as compared to an average length of 9.9 mm for the males of Group IV. The females of Group III averaged 10.5 mm, but the females of Group IV were larger in that they measured 16.1 mm on the average.

Precipitin tests were run utilizing a saline extract of lyophilized Ascaridia galli and a saline solution of botulinus toxin Type A as antigen. The tests were set up with a seven tube series for each antigen. Dilutions of each antigen were made from 1:10 to 1:320 with an additional test utilizing the undiluted antigen. The results of the precipitin tests were negative in all of the serum samples in both the worm extract antigen and the botulinus toxin antigen.

The results of this experiment indicate that the effects of the botulinus toxin were manifested in the nonparasitized group and the parasitized group at about the same time as indicated by the average heart rate of both groups increasing at approximately the same time. The effects of the toxin were most severe in the group which was free from any nematode parasitism as evidenced by the greater morbidity and mortality

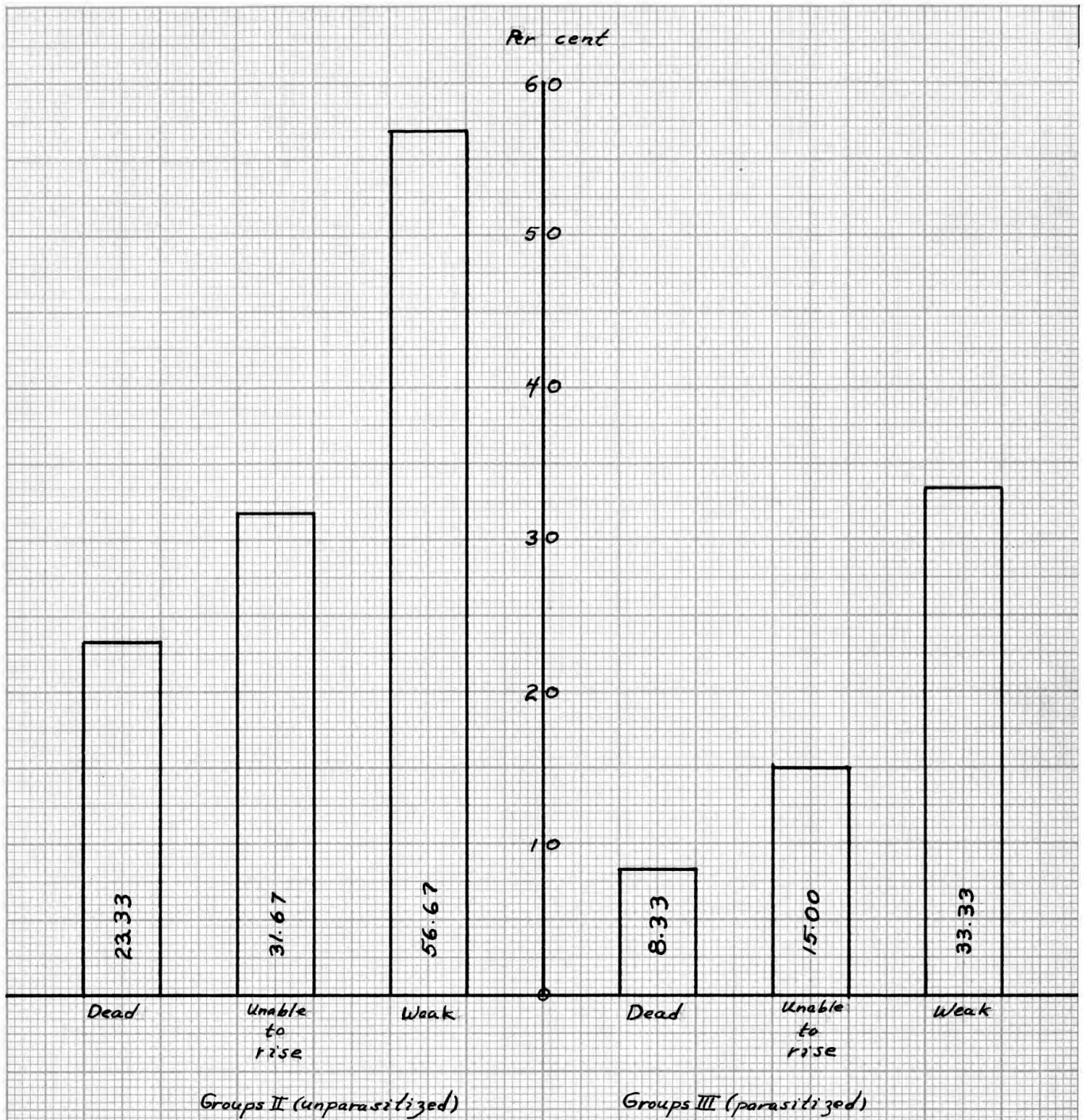


Fig. 4. Comparative morbidity and mortality of chickens injected with botulinus toxin.

occurring in Group II as compared with that of Group III.

#### DISCUSSION

During the course of this study it was observed that chickens which had been injected with botulinus toxin and were affected by the toxin did not consume feed and water at the same rate as did the chickens which had not been injected with the toxin. The amount of feed and water consumed was about inversely proportional to the effect produced by the toxin upon the fowl; i.e., the chickens most severely affected consumed the least food and water, while the chickens least affected consumed the larger amount.

Parasitized chickens manifested symptoms of botulism prior to, or at about the same time, as did the unparasitized chickens, as evidenced by the comparative heart rates of the chickens in the present study. With regard to the severity of the toxic effects, however, the situation was different. In all three of the experiments performed, the most severe effects of the toxin were exhibited by the group of chickens which harbored no parasites as evidenced by the morbidity and mortality of the parasitized group in comparison with those of the unparasitized group. The results obtained from these experiments indicated the possibility that the presence of a moderate infection of the fowl nematode A. galli, rather than decreasing the resistance of a chicken to 0.015 mg/Kg of botulism toxin, actually enhanced

the chance of survival of the animal. It was for this reason that blood serum samples were collected from the chickens used in Experiment III. If the presence of the nematode increased the host resistance to the botulism toxin injected at the experimental level, it was probable that the basis for such resistance was an antigen-antibody relationship. The precipitin tests which were run on the blood sera of the experimental chickens were to search for an antibody-antigen reaction. The results were negative in all tests.

Even though no antibodies could be demonstrated by the precipitin test used in the present study, one cannot conclude that such antibodies did not exist. Sadun (1949) reported that the antibody basis for immunity in chickens to A. galli was dependent upon antigenic stimulation by a metabolic product of the nematode. He could not demonstrate any antibody reaction when a whole worm extract was used as the antigen. The present study complements his results in this respect. He did, however, demonstrate an antibody reaction in an in vitro test which utilized living larvae of the nematode and serum from infected chickens.

The fact that the unparasitized chickens were more susceptible to the experimental dosage of botulinus toxin than were the parasitized chickens might be explained on an antigen-antibody basis. The presence of the nematodes and the production of a metabolic product from these nematodes stimulates the immunological system, generally regarded to be the reticulo-

endothelial system, to the production of antibodies against the antigenic metabolic product, or products, of the worms. These antibodies are normally utilized in aiding the fowl to resist the invasion and subsequent development of the parasite. If a similarity between the antigenic fraction, or haptophor, of the botulinus toxin and the antigenic fraction of the metabolic product of the nematode is hypothesized, a similarity between the antibodies which are produced in response to the two antigens can be assumed. Therefore, when the botulinus toxin was injected into the parasitized chickens which already possessed antibodies against A. galli, these antibodies could have combined to some extent with the haptophor fraction of the toxin, thus neutralizing a portion of the botulinus toxin. The lower mortality and morbidity among the chickens in the parasitized groups as compared to the nonparasitized groups (Fig. 4) suggests that the toxin was, to some extent, neutralized in the parasitized chickens. Furthermore, at the time of injection of the toxin the reticulo-endothelial system, once having produced antibodies against the nematode antigen, would be stimulated quickly to produce more of the same type of antibodies by the anamnestic reaction. This phenomenon would increase the rate of antibody production well above that which would be found in a chicken previously unexposed to the parasite. While the toxin was affecting the host which had not been previously exposed to the parasitic infection and subsequent antibody production, the antibody present in the blood plasma of the parasitized host was neutralizing the toxic effects normally manifested in the injected animal.



Table 7. Statistical data on worm lengths and worm numbers of combined experiments.

Subject	Sum squared deviation		Degrees freedom		Variance		Variance ratio, F	95 per cent value F	99 per cent value F
	Between groups	Within groups	Between groups	Within groups	Between groups	Within groups			
Worm lengths:									
males	208.63	58,720.31	1	654	208.63	89.79	2.32	3.86	6.70
females	1,945.10	69,835.11	1	564	1,945.10	123.82	15.70**	3.86	6.70
combined	469.74	134,103.06	1	1,220	469.74	109.92	4.27*	3.84	6.64
Worm numbers:									
	471.82	12,090.78	1	120	471.82	100.76	4.89*	3.94	6.90

\* Significant, five per cent level.

\*\* Highly significant, one per cent level.

That the nematodes themselves were affected to some extent by the toxin can be reasoned from the evidence dealing with the comparative worm lengths and worm numbers from the two parasitized groups of the three experiments. The total number of worms harbored by the parasitized groups which were injected with the botulinus toxin (Groups III) exceeded that of the uninjected ones (Group IV). Analysis of variance of the data dealing with total numbers of worms gave an F value which was significant at the 5 per cent level (Table 7). This indicates that the normal resistance of the host to the invading parasite was altered in some manner by the presence of the botulinus toxin. Reverting to the hypothesis of a similarity of antigen-antibody structure between the toxin and a metabolic product of the nematode, one can suggest that antibodies which normally would be used in combatting the parasites are diverted to the neutralization of the toxin, thereby allowing a significantly greater than normal number of the nematodes to develop.

A comparison of the data dealing with the lengths of the nematodes recovered from Groups III and IV shows another interesting feature regarding the effect of the toxin upon the nematodes. Taken by sexes, the males from Groups III were slightly longer, on the average, than were those from Groups IV. In contrast, the females recovered from Groups IV were much longer on the average than the females from Groups III (Tables 2, 4, 6). Analysis of variance of these data (Table 7) showed that the difference between the males of the two groups yielded an F value that was not statistically

significant while the difference between the females of both groups yielded an F value that was highly significant; i.e., beyond the 1 per cent level. Analysis of the data of the two groups, obtained by combining the lengths of the males and females in each group, yielded an F value that was significant at the 5 per cent level. This indicates that the presence of the botulism toxin had a detrimental effect upon the growth of the female nematode. Possibly the fact that the chickens which were injected with botulism toxin did not maintain the normal intake of feed and water would explain the shorter lengths of the female nematodes in Group III than in Group IV (Ackert, Whitlock and Freeman, 1940). Inasmuch as the female worm is normally longer than the male, any metabolic interference which would inhibit the normal growth would be more obvious first in the female, since the length of the female must increase at a more rapid rate than that of the male, assuming that maturation of males and females occurs at approximately the same time.

In view of the results of the statistical analyses conducted upon the lengths of the worms, by sexes, it may not be advisable to combine the lengths of both sexes when using worm lengths as a criterion for determining the relative resistance of a host animal to a parasite.

#### SUMMARY

Three experiments involving 242 chickens were conducted

to determine the effect of a moderate fowl ascarid infection upon host resistance to a bacterial toxin. The chickens in each experiment were divided into four groups: Group I, controls; Group II, injected with 0.015 mg of botulinus toxin per kilogram of fowl body weight at six weeks of age; Group III, fed 200 $\pm$ 10 embryonated ova of A. galli per chicken at four weeks of age and injected with 0.015 mg of botulinus toxin per kilogram of fowl body weight at six weeks of age; and Group IV, fed 200 $\pm$ 10 embryonated ova of A. galli per chicken at four weeks of age. Heart beat records of each chicken were made following the toxin injections until the termination of the experiment.

A total of 1,222 nematodes was collected from the intestines of the parasitized groups of chickens. The nematodes were measured and the sex of each was determined. Blood serum for precipitin tests was collected from all groups at the termination of Experiment III. Statistical treatment; i.e., analysis of variance, was made on the data on worm lengths and worm numbers. The results of the experiments were as follows:

1. The comparative heart rates of Groups II and III showed that chickens infected with A. galli and then injected with botulinus toxin 14 days following exposure to the embryonated ova showed the effects of the toxin somewhat sooner than did chickens which had no parasitic infection and were injected with botulinus toxin.

2. Chickens harboring A. galli manifested less severe symptoms of botulism than did chickens without a parasitic

infection, as shown by the greater morbidity and mortality exhibited by the groups harboring no parasites.

3. Precipitin tests run to determine whether or not the increased resistance shown by the parasitized chickens to the effects of botulinus toxin was an antigen-antibody reaction yielded negative results.

4. Chickens harboring A. galli and injected with botulinus toxin (Group III) had more worms than did the parasitized chickens unexposed to botulinus toxin (Group IV). The difference which was statistically significant indicates an interference with host resistance to the nematodes in the injected groups.

5. Statistical analysis of the lengths of the male worms recovered from these groups which harbored A. galli showed that the difference between the lengths of the male nematodes collected from Groups III and Groups IV was not significant.

6. Statistical analysis of the lengths of the female nematodes collected from Groups III and IV yielded an F value which was significant beyond the one per cent level. The female worms from the botulinus injected Groups III were shorter on the average than the females from Groups IV, indicating a metabolic disturbance which affected the normal growth rate of the females recovered from the botulinus injected chickens.

7. Statistical analysis of the lengths of the combined males and females from Groups III and IV showed that the A. galli from Groups IV were longer than those from Groups III. The difference in length was significant at the 5 per cent level.

8. An hypothesis is presented which suggests the possibility of an antigenic similarity between a metabolic product of the nematode A. galli and botulinus toxin (Type A).

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**SOME EFFECTS OF FOWL ASCARID PARASITISM UPON  
HOST RESISTANCE TO A BACTERIAL TOXIN**

by

**JOHN RICHARD EGERTON**

**B. S., Colorado Agricultural and Mechanical College, 1951**

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**AN ABSTRACT OF A THESIS**

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**requirements for the degree**

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Three experiments involving 242 chickens were conducted to determine the effects of a moderate fowl ascarid infection upon host resistance to a bacterial toxin. The chickens in each experiment were divided into four groups: Group I, controls; Group II, injected with botulinus toxin; Group III, fed 200<sup>+</sup>-10 embryonated ova of Ascaridia galli and injected with botulinus toxin; and Group IV, fed 200<sup>+</sup>-10 embryonated ova of A. galli.

A preliminary experiment was conducted to determine the level at which the toxin was to be injected. The results of the experiment indicated that the desired experimental level was 0.015 mg of botulinus (Type A) toxin per kilogram of fowl body weight.

Ova of A. galli were obtained from live gravid females. The uteri containing the ova were removed and allowed to mature in Petri dishes half filled with water. At the time of experimental infection of Groups III and IV embryonated ova were placed in a vial containing sand and water, agitated, and delivered by calibrated pipette onto a glass microslide. The ova were counted, and adjustments made until the desired number of embryonated ova were delivered consistently.

At four weeks of age the chickens from Groups III and Groups IV were fed 200<sup>+</sup>-10 embryonated ova of A. galli per chicken by delivering the contents of the calibrated pipette into the oral cavity.

The toxin solutions prepared for use were freshly mixed from dried toxin prior to each experiment. The toxin was

weighed on an analytical balance and added to sufficient sterile saline to make a solution containing 0.015 mg of toxin per ml of solution. At this concentration it was necessary only to weigh the chickens to be injected, transpose the weights to kilograms, and use this value in cubic centimeters as the amount to be injected.

At six weeks of age the chickens from Groups II and Groups III were injected with 0.015 mg of toxin per kilogram of body weight. The injection was made just posterior to the sternum and directly into the peritoneal cavity.

A record of the heart beat of each chicken was made with the aid of a stethoscope immediately prior to the toxin injection and continued throughout each experiment. Observations upon the botulism symptoms exhibited by the chickens injected with the toxin were made throughout the experiments.

At the termination of the experiments the chickens were killed, the small intestine from the gizzard to the yolk sac diverticulum was removed, flushed with water under pressure and the mucosa was scraped free and added to the flushings. The worms were allowed to stretch out and die, at which time quantities of concentrated formalin were added as a preservative. The worms were then collected, counted and placed in vials containing 10 per cent formalin.

Measurement of each worm was done by projecting its image, magnified six times, upon a ground glass plate. The outline of the projected image was traced upon paper, and then the pencil drawing was traced with a calibrated wheel. The results

were then divided by a factor of six to obtain the true lengths of the nematodes.

At the termination of Experiment III blood serum samples were collected from all four experimental groups. The results of Experiments I and II showed an increased resistance of the parasitized hosts to the toxin, so precipitin tests were run upon the blood serum for the purpose of determining whether or not this resistance was an antigen-antibody reaction.

Statistical treatment; i.e., analysis of variance, was made upon the data relative to worm lengths and worm numbers. The results of the experiments are as follows:

1. The comparative heart rates of Groups II and III showed that chickens infected with A. galli and then injected with botulinus toxin 14 days following exposure to the embryonated ova showed the effects of the toxin somewhat sooner than did chickens injected with botulinus toxin but unparasitized.
2. Chickens harboring A. galli manifested less severe symptoms of botulism than did chickens without a parasitic infection, as shown by the greater morbidity and mortality exhibited by the groups harboring no parasites.
3. Precipitin tests run to determine whether or not the increased resistance shown by the parasitized chickens to the effects of botulinus toxin was an antigen-antibody reaction yielded negative results.
4. Chickens harboring A. galli and injected with botulinus toxin yielded a statistically significant greater number of worms

than did parasitized chickens unexposed to botulinus toxin, indicating an interference with host resistance to the nematodes in the injected groups.

5. Statistical analysis of the lengths of the male worms recovered from both groups which harbored A. galli showed that the difference between the lengths of the male nematodes collected from Groups III and IV was not significant.

6. Statistical analysis of the lengths of the female nematodes collected from Groups III and IV yielded an F value which was significant beyond the one per cent level. The female worms from the botulinus injected Groups III were shorter on the average than the females of Group IV, indicating a metabolic disturbance which affected the normal growth rate of the females recovered from the botulinus injected chickens.

7. Statistical analysis of the lengths of the combined males and females from both groups showed the difference in length to be significant at the five per cent level.

8. An hypothesis is presented which suggests the possibility of an antigenic similarity between a metabolic product of the nematode A. galli and botulinus (Type A) toxin.