EFFECT OF HELMINTHIASIS ON RESISTANCE TO PARASITISM

by

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INTRODUCTION

It seems a general biological law that the human body, and for that matter any animal body, develops resistance or immunity to certain types of bacterial infection. A classic example is smallpox. It is almost universally recognized that a person who has recovered from smallpox is immune or at least very resistant to a second attack of the disease. Naturally then the question arises, does the body develop a similar resistance to the invasion of

animal parasites? And if there is such a resistance, are the laws governing it in any way comparable to those which govern various types of bacterial immunity?

Numerous investigators have taken up this question from various points of view, using various hosts and parasites with the result that there has been amassed a large amount of suggestive data but little conclusive evidence either for or against the development of immunity to metazoan parasites. As animals in general show great individual variation in resistance to parasitism and as a large number of the previous experiments employed so few animals that the results might be attributed to such individual variation, the author planned to undertake a series of experiments on a sufficiently large group of animals to permit of biometrical treatment of the data.

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HISTORICAL REVIEW

Chandler in his book on "Animal Parasites and Human Disease" (1922) sums up the general attitude and belief of many students of Parasitology as follows: "Although up to the present time the successful use of vaccinations or inoculations for cure of, or protection from, disease germs has been applied chiefly to bacterial diseases, the same principles of immunity apply to diseases caused by animal parasites and we may confidently expect in the not distant future a great extension of this relatively new field of medicine to diseases caused by animal parasites. It has already been applied successfully to some spirochaete diseases, and to some trypanosome diseases".

Blacklock and Gordon (1927) say: "Yet while immunity against metazoan parasites acquired as a result of previous invasion is recognized in a general way, experimental proof of its existence is singularly lacking".

During the last few years workers in the field of immunology have turned their attention toward the question of parasitological immunity and have undertaken the application of bacteriological and serological tests in cases of parasitic infection with more or less positive results.

That most animal parasites have a toxic effect on the host is generally recognized although the exact toxin or pathological factor is unknown. Flury and Leeb (1926) in their work on toxicology of liver flukes report that Fasciola hepatica and Dicrocoelium lanceatum contain no single specific toxin. "The disease consists in a poisoning caused by a series of various injurious substances, formed in part by the physiological activities of the parasites themselves."

Schwartz (1921) has shown that the serological reaction of the hosts harboring parasites afford evidence that parasitic worms liberate products against which hosts develop defense or immunity reactions.

Goldschmidt (1910), Ransom (1924) and others have reported the sensitization of workers to contact with vapors and fluids from horse and swine ascarids.

Since the body normally develops antibodies against bacterial infections considerable work has been done in testing for precipitin reactions and complement-fixation in parasitic infestations.

Kolmer, Trist and Heist (1916) according to results of complement-fixation tests with the sera of infested dogs had reason to believe that production of antibodies may occur after infestation of the intestine with certain common parasites.

Isbecque (1924) found positive complement-fixation in patients infested with Taenia, Dibothriocephalus and Ascaris, but LeBas (1924) was unable to obtain positive complement-fixation or precipitation tests in Dibothriocephalus cases. Nor did the intradermal injection of worm materials into infested persons result in a skin reaction in any way different from the irritation produced in a normal person.

Fairley (1926, 1927) working on goats and using protein-free, alcohol-soluble extracts of cercariae found that "In infected animals and in those receiving a second series of injections, there was a decisive rise of antibody content within a few days of the injection and a high maximum antibody response". Since the extracts used were protein free there was no anaphylaxis.

Taliaferro (1928) has been successful in securing antigens specific for malaria from extracts of an infested placenta.

Nicolau and Banciu (1926) found that the Bordet complement-fixation reaction was positive in 20 out of 35 cases of sarcoptic mange in human beings.

Bachman (1928a) in his work on Trichiniasis reports that Strödbel (1911), Romanovitch (1912), and Ducas were successful in detecting trichinella infested hogs by means

of complement-fixation, but failed to demonstrate the presence of precipitin antibodies. He attributes their failure to the methods used in preparing the test antigens. Satisfactory antigen solutions of Trichinella were prepared by first freeing the larvae from infected meat by artificial digestion and later hydrolyzing the dried trichinella powder in 0.1% Hcl. This antigen was successfully used for artificial immunization of rabbits. Precipitins were demonstrated in a case of human infection. In a subsequent paper, Bachman (1928b), using a similar antigen, found intradermal tests on Trichinella infected rabbits uniformally positive while preliminary tests before infection had been uniformly negative.

In the search for better clinical methods for diagnosis of helminthiasis intradermal tests with parasitic extracts have frequently been employed, generally with positive results. Ransom in 1920 reported on a series of experiments using various domestic animals as hosts and many different types of parasites. He found that in some cases the reaction was specific for the parasite from which the antigen was obtained while in other cases there was no relation between reaction and specific infection. Some animals even reacted to parasites with which they are not liable to infection.

Fülleborn (1926a, 1926b) reports specific skin reactions to Ascaris and Trichinella antigens in infected persons. This reaction may remain positive for years after the parasite has been expelled.

Ramsdell (1927) working with Taenia finds a non-specific dermal reaction which persists after the removal of the parasite.

Casoni (1911) and Rackemann and Stevens (1927) found positive specific reactions when hydatid cyst fluid was injected intradermally in cases of echinococcus disease. Passive transfer of local hypersensitiveness to the skin of normal individuals was uniformly successful. Reaction seemed limited to local injection sites and diminished after a time.

Brunner (1928) made intradermal tests with saline extracts of Ascaris, Dibothriocephalus latus, and Taenia solium. Reactions were similar to those of atopic hypersensitiveness (hay fever and asthma). Specificity of Dibothriocephalus and T. solium reactions were demonstrated, although one case of Dibothriocephalus infestation would not give a positive reaction to the antigen. Individuals infested with other nematodes gave reactions to Ascaris antigen which indicate a group reaction. Brunner

was also able to transfer this sensitivity passively to normal skin in all attempts.

From the above work it is evident that the body of the host does have a definite physiological reaction to the presence of a parasite within it. Experimental observations proving that immunity or resistance can be acquired against metazoon parasites as a result of previous infestation, apart from age, are very few. Also as previously stated much of the work is based on experiments in which the number of individuals concerned is too small to justify any positive conclusions.

Fuginami (1916) records a case of a horse, which had recovered from Schistosomiasis (blood fluke disease), and which was subsequently exposed to canal water containing numerous larval flukes (these enter the blood through the skin). Two other supposedly non-infested animals were likewise exposed. Upon later examination thousands of flukes were found in the portal systems of both control animals, but not a single fluke was found in the first animal. This is commonly cited as a true case of immunity due to previous parasitism.

Kritchevsky and Autonomoy (1926) working with trypanosomes report a non-sterile immunity resulting from previous infection in only a few animals. In the majority of cases immunity fails to develop.

Kliger and Weitzman (1926) found that injections of dead and attenuated trypanosomes, instead of immunizing the animal, actually lowered resistance.

Rivas (1927) on the other hand reported that immunization against trypanosomiasis in rats was readily obtained by the application of subcutaneous injections of inactivated trypanosomes.

Blacklock and Thompson (1923) proved experimentally that an immunity against the larvae of certain bot-flies can be acquired by both man and animals by previous attacks of the larvae rather than any age immunity of the host.

Blacklock and Gordon (1927) continued this work and were able to show that there was a positive immunity developed and that this immunity was governed by laws which differ widely from those governing bacterial immunity. From the experiments, it is shown that the immunity was confined to areas of the skin into which a parasite had previously penetrated and from these primary foci spread with diminishing intensity in all directions. They were unable to demonstrate any evidence of anaphylaxis and obtained consistent negative results in precipitin, complement-fixation and blood transfer tests.

Fülleborn (1926c) working on the behavior of hookworm larvae found that infections with Uncinaria disappear in about five months, and his experiments suggest that the worms produce an immunity to future infection.

Sandground (1927) working on Strongyloides stercoralis, one of the roundworms of the intestine, was unable to superimpose a secondary infection upon cats and dogs previously infested. The resistance developed independently of the age of the host.

Herrick (1928) working with the dog hookworm, Ancylostoma caninum, found that no increase in resistance could be demonstrated in dogs which had been previously infected, when all the worms from the previous infections were removed with anthelmintics, although in certain cases where worms were present from previous infections there seemed to be increased resistance to the superimposed infection.

Gordon (1925), Weinburg and Julien (1911) and others have recorded experiments and clinical cases where the body seemed to have developed a tolerance or resistance to the effects of the parasite rather than to the parasite itself. Although the parasites are very numerous in such cases the host does not seem to be detrimentally affected by them.

Ransom (1921, 1922) Yokogawa (1923) and others concluded, from circumstantial rather than experimental evidence, that there is also an age resistance developed to parasitism.

Herrick (1925) in his work on chickens and Ascaridia lineata definitely established the fact that there is a resistance or immunity against metazoan parasites acquired as a result of age alone. This was further proved by his work on dogs and Ancylostoma caninum (1928).

Ackert and Herrick (unpublished) attempted to superimpose an infestation upon a group of chickens already parasitized in order to test the effect of heavy parasitism on egg production but they were unable to observe any pathological effects of a secondary infestation. Whether this resistance to a later parasitism was due to the previous infestation or to age alone is not known.

The following studies were undertaken at the suggestion of Dr. J. E. Ackert with the purpose of ascertaining whether or not chickens develop resistance to infestation of the common roundworm of poultry, Ascaridia lineata Schneider, as the result of previous infestations of this parasite.

MATERIALS AND METHODS

The studies of which these experiments are a part are being conducted at the Experiment Station of the Kansas State Agricultural College under the direction of Dr. J. E. Ackert of the Zoology Department. All experiments were conducted at the animal house provided for the Experiment Station work.

The methods used were as follows:

The chicks used were pure bred white leghorns from a certified flock. They were obtained as day old chicks and reared under control conditions so that there was no opportunity of their becoming infested with parasites, except as desired. All were fed a ration experimentally proved to be adequate for confined growing chicks and were kept under conditions of equal floor space, ventilation, light, etc. (Herrick, Ackert and Danheim, 1923).

The nematode eggs used were obtained from mature worms of the species A. lineata secured from entrails of chickens killed at a retail poultry house where large numbers of chickens were drawn regularly. In order to make satisfactory cultures of the eggs, the anterior end of the worm was removed and the internal organs pressed out into a clean petri dish. The lower parts of the uteri, which were

usually well filled with fertilized eggs were then transferred to another dish where they were ground up with the aid of a spatula. The eggs from several worms were placed in the same dish and cultured in a medium of distilled water or tap water plus a few drops of 2% formalin (Herrick, 1925, p. 155). The cultures were incubated at about 30° C. and were aired for a few minutes every day or two. As the eggs usually reached the coiled embryo stage after ten days to two weeks of incubation, the cultures were used when about three weeks old.

The most satisfactory method of administering the eggs was to count them out on a slide under the low power of the microscope and then wash them off on to a small filter paper. A pinch or two of ground corn was then placed on the filter paper and a small pellet made of the whole. This was force-fed to the chick to be parasitized.

Before administering the worm eggs the chicks, now five weeks old, were divided into two groups so selected as to be equally balanced in weight and apparent vigor. One group, which I shall designate as "parasitized" was fed embryonated eggs from cultures as described above. The other group which I shall call "controls" was not parasitized at this time. The chicks of both groups were weighed each week of the experiment and their weights and

gains recorded (Graph I).

When the chickens were ten weeks old, five weeks after the parasitized birds had been subjected to infestation, each bird of both lots (parasitized and controls) was fed 300 embryonated eggs. The controls were then of the same age as the parasitized, but the latter had been subjected to a previous infestation and had had five weeks of parasitism in which to develop resistance to subsequent infestations.

The experiments were continued three weeks after the second parasitizing, until the birds were thirteen weeks of age, at which time all were killed and autopsied. The intestine of the host was removed and the intestinal contents washed out with warm water under pressure by means of a large rubber syringe or pipette. The intestine was then slit open and the mucus scraped off by drawing the opened intestine, inner side down, against the sharp edge of a Mason jar. The intestinal contents and mucus were placed together in glass jars partially filled with warm water and allowed to stand some three or four hours, during which time the worms freed themselves from the debris. Enough 40% formalin was then added to make a 4% fixing solution. After several days the fixing solution was carefully poured off and the sedimented material washed

with tap water to remove flocculent matter. The remaining debris was then stained with a solution of methylene blue which colored the foreign material and mucus but did not stain the worms. It was then examined under low power binoculars and the worms removed and placed in vials containing 4% formalin. Count was kept of all worms as they were removed.

Later each worm was measured and recorded. Worms measuring 20 mm. or longer were measured on a millimeter rule: those measuring between six and 20 mm. were measured by means of fine calipers adjusted to a width of one mm. This was done by placing the moist worm on a piece of paper and stepping off the length with the calipers. Camera lucida drawings were made of all worms under six mm. in length and accurate measurements secured from these.

In the parasitized group, the worms from the primary infestation were separated from those of the secondary parasitism by their length. Only the worms from the secondary parasitism were used in making comparisons with control infestations.

The number of worms and the total lengths of worms in each chicken were used as criteria for determining the presence of an increased or decreased resistance to the parasites. The method of the author differed from that of

Herrick (1925) in that the total length rather than the average length of worms from each intestine was used as an index of the resistance of the chick.

Three experiments were conducted as described. The first two were made with 40 chicks each, 20 parasitized and 20 controls. The third was made using 90 birds, 45 parasitized and 45 controls. In the second experiment one of the parasitized birds died and in the third experiment one of the controls died. Thus, combining the three experiments there were 84 chicks in the parasitized groups and 84 in the controls.

EXPERIMENTAL RESULTS

Experiment I. If we compare the control and parasitized chicks of the first experiment (Table I, Graph I), we see that in the controls, out of the 20 birds, one had no worms at all, four had only one worm each, four had only two worms, and one had 15 worms. The remaining ten contained from three to 11 worms each. The average for the whole group was 4.3 worms. The sizes of these worms varied from 1.95 mm. to 25 mm. in length; the average being 11.36 mm. per worm. The average total length of worms in each chicken was 50.65 mm.

In the parasitized group in all except five cases, the worms were easily separated by size into the two ages, those from the primary and those from the secondary parasitism. In these five cases, 26 mm. (the maximum length of worms in the control group) was taken as the maximum length of worms in the secondary parasitism. The general appearance of these worms and of worms secured from chicks of the same age and hatch in connection with another experiment all seemed to indicate this criterion as satisfactory. Two chicks had no worms at all, two had none from the second parasitizing and two had none from the first. Considering only the second parasitism, the range of variability was from 0 to 18 worms in number and from 1.67 mm. to 26 mm. in length, with an average of 5.9 worms per bird and an average length of 11.26 mm. per worm. The average total length of worms in each chicken was 64.12 mm. From the primary parasitism the average number of worms per bird was 8.4 with an average length of 48.32 mm. The range of variability was from 0 to 38 worms in number and 27 to 91 mm. in length.

Comparing the two groups; the parasitized birds have an average of 1.6 more worms per bird than do the controls. However, when divided by its probable error ($\pm$ 1.0) this difference gives a quotient of only 1.6 in favor of

lessened resistance. This is not recognized as being significant biometrically. The difference in total length of worms in the two groups was less than its probable error; consequently, these results give no evidence of increased or decreased resistance to parasitism.

Experiment II. The results of this experiment differ considerably from those of Experiment I, although the two experiments differed very little in the methods used. In Experiment I approximately 500 eggs were fed each bird of the parasitized group in the primary parasitizing. In Experiments II and III exactly 300 embryonated eggs per chick were fed for both primary and secondary parasitisms. The ration being fed was changed somewhat in the middle of Experiment II, but both rations were adequate and both the parasitized and the control groups were fed the same rations at all times.

Examining the data from the control group (Table II, Graph I) we find that out of the 20 chicks considered one had no worms at all, two had only one worm each, five had only two worms, one had 17 worms, two had 16 and the remaining nine birds had from three to 13 worms each. The average was 5.85 worms. These worms varied from one to 27 mm. in length, the average being 8.07 mm. per worm. The average total length of worms per chicken was 28.245 mm.

The worms from the primary and secondary parasitisms were easily separated on the basis of length in the parasitized group. Of the 19 birds considered, four had no worms at all, six others had none from the second parasitizing and four had none from the primary parasitizing. Thus, out of the 19 birds parasitized only nine showed a successful reinfestation upon autopsy. The range of variability for the secondary parasitism was from zero to eight worms and from 1.65 mm. to 21.5 mm. in length, with an average of 1.68 worms per bird and an average length of 5.67 mm. per worm. The average total length of worms per chicken was 11.654 mm.

From the primary parasitism the average number of worms per bird was 2.79 with an average length of 59.09 mm. The range of variability was from 0 to 13 worms in number and 43 to 88 mm. in length.

Comparing the data from the two groups: the controls have an average of 4.17 more worms per chick than do the parasitized. This difference when divided by its probable error ($\pm$ .898) gives a quotient of 4.64 which is generally considered significant. Considering total lengths of worms per bird, the controls have an average total length of 16.591 mm. per bird greater than the parasitized group. This difference when treated biometrically (probable error

± 5.193) gives a result of 3.195 which is considered as significant by some workers.

Thus, in this experiment there is significant evidence of an increased resistance on the part of the parasitized birds to a secondary infestation, as indicated by the number of worms and the greater preponderance of parasitized birds that showed no secondary worms present upon autopsy. The total lengths of the worms also give, at least, an indication of an increased resistance.

However, comparing the results of the two experiments, we find that the birds of Experiment II, as a whole, were much more vigorous than those of Experiment I. This is shown by their greater growth (Graph I), the fewer numbers of worms in both parasitized and controls, and the smaller sizes of the worms. This greater virility on the part of the chicks in Experiment II may be due to the fact that they were early spring hatched and had inherited a greater resistance. That this group of chickens was extraordinarily vigorous and resistant was also found by Mr. L. O. Nolf who used birds from the same hatch in a series of experiments (Ackert and Nolf, unpublished).

Experiment III. In this experiment instead of using only 40 birds, as in the two previous ones, 89 birds were used. These were obtained from the same hatchery as those of the other experiments and were hatched seven weeks

later (March 21, 1928) than the chicks in Experiment II. The results from this experiment (Table III, Graph I) were very similar to those of Experiment I.

In the controls, of the 44 birds in the group, three had no worms at all, six had only one worm each, one had 24 worms, one had 23, and the other 33 had from two to 20 worms each. The average was 6.25 worms per bird. The worms varied in length from 1.2 to 36 mm., the average being 8.41 mm. per worm. The average total length of worms per chicken was 52.68 mm.

In the parasitized group there was no difficulty in separating the worms from the primary and secondary parasitisms. Of the 45 birds, two had no worms at all, 14 had none from the primary parasitism and two none from the secondary. Thus, 41 of the 45 birds parasitized showed worms from the second parasitizing. The range of variability for the secondary parasitism was from 0 to 69 worms in number and from 1.3 mm. to 26.5 mm. in length of worms, with an average of 8.47 worms per bird and an average length of 4.97 mm. per worm. The average total length of worms per chicken was 41.33 mm.

In the primary parasitism the average number of worms per bird was 5.53 with an average length of 59.62 mm. The range of variability was 0 to 52 worms in number and 38 to 87 mm. in length.

Examining the data from the two groups: the parasitized chicks have 2.216 more worms per chick than the controls. This is not significant, however, since divided by its probable error (± 1.4) the result is only 1.58. Comparing the total lengths of worms per bird, the controls have an average of 11.35 mm. greater than the parasitized. But when treated biometrically (probable error of the difference ± 8.19) the result (1.38) is not at all significant. Therefore, the data from this experiment as those from Experiment I give no evidence of increased or decreased resistance as a result of previous parasitism.

Combined Data. Since all three experiments were conducted under similar conditions and since the chicks of all experiments were of the same ages when parasitized and killed, the data from the three experiments were combined and treated biometrically as a single experiment (Table IV, Graph I).

Comparing the data as to numbers of worms, in the control groups we find an average of 5.69 worms per chick, while in the parasitized groups (worms from secondary parasitisms only) the average was 6.321 worms. Thus, the parasitized birds had an average of .631 more worms each than did the controls. This difference is less than its probable error (± .838) and is, therefore, not significant (quotient = .753).

Combining the data on the total lengths of worms in all three experiments and treating biometrically: the controls have an average of 46.3765 mm., total length of worms per bird, compared to an average of 40.0457 mm. per bird for the parasitized groups. This difference (6.3308 mm.) when divided by its probable error (± 5.84) gives a result of 1.084 in favor of increased resistance. However, this is not sufficient to be recognized as significant.

Thus, the three experiments when combined show no significant evidence of an increased or decreased resistance in numbers of worms present or in total length of worms per bird.

DISCUSSION OF DATA

From the evidence in the above experiments it is obvious that at least some of the chickens subjected to subsequent parasitisms are highly resistant to infestation by A. lineata. That this resistance is not due to age, as shown by Herrick (1925), is also evident because all were of the same age when infested.

That there is a great variation in the natural resistance of different individuals to parasitism is well known. Part of the variation in resistance shown by the

animals in these experiments may be thus accounted for. This variation in individual resistance is further evidenced by the fact that, in general, where few worms of the secondary infestation were found there were also few worms of the primary parasitism, although the reverse was not necessarily true. Not only is there a great variation in the individual resistance of the host, but there is also a wide variation in the viability and growth of the individual worms. This is shown by the variability in the size of the worms in an individual host. A similar variation in size of worms of the same age and in the same host was found by Herrick (1925).

Since there is such a great individual difference in resistance to parasitism among chickens one of the problems suggested by this research is that of determining if there is such a thing as a genetic factor for resistance.

As indicated in the graph showing the growth curves for the various experiments the pathological effects of the parasitism were not more noticeable in one group than in the other. Ackert (1923) in his work on the habitat of A. lineata determined that the larvae penetrate the wall of the intestine to some extent and become embedded in the villi. They are found there until about the seventeenth day after infection. It is during this period

that the most apparent pathological effects are observed. As shown by the graph, in most every case there is a slight drop in the weight curve immediately following parasitism which indicates that the worms had begun to establish themselves. In all experiments the pathological effects of the parasitism were apparent in the ruffling of the feathers and general appearance of the birds for a few days following infestation, some chicks showing the presence of the parasites much more than others.

Much of the work previously done has produced evidence that intestinal helminthiasis does have a physiological reaction on the host but very little conclusive experimental evidence has been produced that this physiological effect is an immunity reaction. Considerable work has been done on intradermal and serological reactions to extracts of intestinal parasites. However, as stated by Kritschewski and Heronimus (1927), "the presence or absence of antibody is not related to the possibility or impossibility of producing superinfection of infected animals".

In examining the data from the present experiments, it is apparent that if conclusions had been drawn from Experiment II alone the presence of an acquired resistance would have been postulated at once, but additional ex-

periments involving a larger number of birds and identical to Experiment II in methods employed substantiate no such conclusion. In as much as the conditions of all three experiments were identical, except as to the individual chickens and worm eggs employed, the discrepancy in results between the experiments is apparently attributable to one or both of these factors, the individual resistance of the group of chickens used or the individual lack of viability of the worms. Both the growth curves for the chickens and observations made throughout the period of the experiments give evidence that the chickens used in Experiment II were unusually vigorous which would indicate a probability that the results were due to the unusually high resistance of this group of birds. From this it is further apparent that individual variation might account for some of the results accepted as evidence of acquired resistance in experiments of previous workers where only a few animals were used.

Considering all of the data from the above experiments, it is apparent that there is no biometrically significant evidence of an acquired resistance to parasitism by chickens because of previous infestation. Since the individual resistance varied so widely in the above experiments it is suggested that in future work of this

nature, a considerable number of host animals secured from different localities or parents be employed so as to eliminate any possible genetic factor for resistance, and that a sufficient number of animals be used to allow of biometric treatment of the data so as to offset the effect of individual variations in resistance. A series of experiments upon smaller groups using similar methods is preferable to the use of one large group because of the possibility of an entire group being significantly influenced by some factor not accounted for by the experimental conditions.

SUMMARY

1. Three experiments were conducted on 168 white leghorn chickens, all of the same age, to determine if they would develop resistance to a secondary infestation of Ascaridia lineata because of a previous parasitism.

2. The first experiment, involving 40 chicks, showed no significant resistance either in number of worms, length of worms, or in effect of parasitism on growth of the chickens. Although not biometrically significant the number of worms pointed toward a possibly lower resistance.

3. The second experiment, including 40 chicks, showed a biometrically significant acquired resistance in both number of worms and in length of worms. This may possibly be accounted for as due to a genetically resistant strain of chicks, since this entire lot of birds were unusually strong and vigorous.

4. The third experiment, involving 89 chicks, showed no significant evidence of acquired resistance and resembled Experiment I in practically all results.

5. Considering all the experiments together there is no biometrically significant evidence that chickens acquire resistance to superimposed infestations of A. lineata because of previous infestations with that parasite.

6. The individual variations in natural resistance of the host and in the viability of the parasite are so great as to demand that a considerable number of individuals be employed in experiments of this nature.

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TABLE I

Data from Experiment I

PARASITIZED								CONTROLS							
Chick No.	Sex	Gm. wt. begin'g exp't. age 5 weeks	Gm. wt. end exp't. age 13 weeks	Primary infestation Number of worms	Secondary infestation No. of worms	Secondary infestation *Total length in mm.	Secondary infestation *Average length in mm.	Chick No.	Sex	Gm. wt. begin'g exp't. age 5 weeks	Gm. wt. end exp't. age 13 weeks	No. of worms	*Total length in mm.	*Average length in mm.
2956	♀	173	633	2	0	0.0	0.0	2953	♂	171	906	11	91.05	8.28
2960	♂	205	800	10	3	50.90	16.97	2954	♀	163	630	4	34.40	8.60
2961	♂	176	757	12	8	91.49	11.44	2955	♂	208	924	6	79.05	13.18
2962	♂	225	915	0	1	11.00	11.00	2957	♀	206	774	0	0.0	0.0
2964	♀	200	774	12	4	62.00	15.50	2958	♀	182	684	6	54.10	9.02
2968	♂	194	792	12	6	66.83	11.14	2959	♂	176	900	1	9.00	9.00
2970	♀	236	782	3	4	*43.00	*21.50	2963	♂	184	870	2	32.00	16.00
2971	♂	202	850	11	5	42.70	8.54	2965	♂	218	943	4	33.40	8.35
2973	♂	215	876	13	18	141.43	7.86	2966	♂	235	980	2	32.50	16.25
2974	♀	217	785	0	8	32.63	4.08	2967	♀	213	870	7	111.65	15.95
2975	♀	220	797	0	0	0.0	0.0	2969	♀	236	874	2	28.00	14.00
2977	♂	253	892	11	16	187.82	11.74	2972	♀	209	778	1	21.00	21.00
2978	♀	241	842	0	0	0.0	0.0	2976	♂	240	957	1	4.00	4.00
2979	♂	234	819	3	9	122.57	13.62	2980	♀	216	817	1	4.55	4.55
2981	♂	178	844	11	17	*291.65	*18.23	2983	♂	225	830	5	19.10	3.82
2982	♂	245	922	22	10	99.00	9.90	2984	♀	222	769	10	*139.95	*15.55
2987	♀	172	663	3	3	8.60	2.87	2985	♂	202	940	15	240.03	16.02
2988	♂	215	931	2	0	0.0	0.0	2986	♂	272	1166	3	7.65	2.82
2991	♀	185	697	38	5	18.83	3.77	2989	♂	253	1069	2	33.50	16.75
2992	♂	260	1129	3	1	12.00	12.00	2990	♂	246	1064	3	38.00	12.67
Total		4246	16,500	168	118	1282.45	180.16	Total		4277	17,745	86	1012.93	215.81
Average		212.3	825.0	8.4	5.9	64.12	11.26	Average		213.9	887.25	4.3	50.647	11.36

SUMMARY

	Mean	Standard deviation
No. of worms	5.9 ± .84	5.567
Length of worms	64.12 ± 11.064	73.359

	Mean	Standard deviation
No. of worms	4.3 ± .577	3.822
Length of worms	50.65 ± 8.57	56.808

$$\text{Number of worms } \frac{\text{difference}}{\text{Error of difference}} = \frac{1.6}{1.0} = 1.6$$

$$\text{Length of worms } \frac{\text{difference}}{\text{Error of difference}} = \frac{13.4760}{13.9936} = 0.963$$

*Total length and average length compiled from worms not broken in removal.

TABLE II

Data from Experiment II

PARASITIZED								CONTROLS						
Chick No.	Sex	Gm. wt. begin'g exp't. age 5 weeks	Gm. wt. end exp't. age 13 weeks	Primary infestations Number of worms	Secondary infestation No. of worms	Secondary infestation *Total length in mm.	Secondary infestation *Average length in mm.	Chick No.	Sex	Gm. wt. begin'g exp't. age 5 weeks	Gm. wt. end exp't. age 13 weeks	No. of worms	*Total length in mm.	*Average length in mm.
3004	♀	230	816	5	0	0.0	0.0	3001	♀	244	907	3	5.68	1.89
3008	♀	240	857	3	0	0.0	0.0	3002	♂	240	983	4	13.10	3.27
3010	♀	238	835	0	0	0.0	0.0	3003	♂	250	801	17	33.13	2.21
3011	♂	266	1103	1	0	0.0	0.0	3005	♀	230	894	16	29.42	1.84
3016	♂	244	1020	4	2	5.1	2.55	3006	♂	264	1126	5	23.42	4.68
3020	♀	216	691	13	5	6.03	3.02	3007	♀	280	920	2	18.65	9.32
3024	♀	276	1011	6	0	0.0	0.0	3012	♂	234	1061	11	66.28	6.63
3026	♂	260	1090	0	1	2.2	2.2	3013	♂	228	978	2	4.47	2.23
3027	♀	242	805	0	2	16.65	8.32	3015	♂	247	977	3	7.7	2.56
3028	♂	249	989	10	5	52.4	10.48	3017	♀	205	678	8	31.87	4.55
3029	♂	290	1135	2	3	10.40	3.46	3018	♂	238	1082	16	28.04	1.87
3032	♀	231	890	0	0	0.0	0.0	3021	♂	290	1102	1	19.00	19.00
3033	♂	285	1110	0	0	0.0	0.0	3022	♂	215	1021	2	28.75	14.37
3034	♂	246	1178	0	5	43.40	8.68	3025	♀	249	825	0	0.0	0.0
3035	♀	255	920	1	0	0.0	0.0	3031	♀	236	715	13	36.53	3.04
3039	♀	248	895	0	0	0.0	0.0	3038	♂	275	1127	6	118.00	19.66
3046	♀	273	1040	3	0	0.0	0.0	3041	♀	273	870	3	46.30	15.43
3047	♀	202	812	5	8	83.37	10.46	3044	♀	257	900	1	22.00	22.00
3048	♀	220	840	0	1	1.87	1.87	3045	♂	245	1075	2	5.07	5.07
								3050	♀	220	836	2	27.50	13.75
Total		4711	18,037	53	32	221.42	51.04	Total		4920	18,878	117	564.91	153.37
Average		247.95	949.32	2.79	1.68	11.654	5.67	Average		246.00	943.90	5.85	28.245	8.07

SUMMARY

	Mean	Standard deviation
No. of worms	1.68 ± 0.359	2.318
Length of worms	11.654 ± 3.452	22.308

	Mean	Standard deviation
No. of worms	5.85 ± 0.824	5.461
Length of worms	28.245 ± 3.88	25.726

Number of worms $\frac{\text{difference } 4.17}{\text{Error of difference } 0.898} = 4.643$

Length of worms $\frac{\text{difference } 16.591}{\text{Error of difference } 5.193} = 3.195$

*Total length and average length compiled from worms not broken.

TABLE III
Data from Experiment III

PARASITIZED								CONTROLS							
Chick No.	Sex	Gm. wt. begin'g exp't. age 5 weeks	Gm.wt. end exp't. age 13 weeks	Primary infestations Number of worms	Secondary infestation No. of worms	*Total length in mm.	*Average length in mm.	Chick No.	Sex	Gm. wt. begin'g exp't. age 5 weeks	Gm.wt. end exp't. age 13 weeks	No. of worms	*Total length in mm.	*Average length in mm.
3111	♂	230	1055	0	0	0.0	0.0	3110	♂	235	915	8	106.20	13.28
3112	♀	165	690	4	2	8.90	4.45	3114	♂	211	923	2	5.00	2.50
3116	♂	138	755	0	1	7.30	7.03	3115	♀	159	599	1	6.90	6.90
3117	♂	141	750	16	23	77.80	3.38	3118	♀	181	675	2	19.20	9.60
3119	♂	211	890	5	13	140.80	10.83	3120	♀	185	670	6	68.90	11.48
3122	♀	236	737	1	3	12.05	4.02	3124	♀	137	605	1	29.00	29.00
3123	♀	194	630	0	0	0.0	0.0	3127	♂	143	728	9	78.25	8.69
3125	♂	158	762	0	2	4.91	2.45	3129	♀	176	651	4	39.40	9.85
3126	♂	174	783	5	12	*36.11	*3.61	3130	♂	216	976	17	66.15	3.89
3128	♂	196	787	18	6	105.70	17.61	3131	♀	142	573	7	208.00	29.71
3132	♂	164	765	0	1	3.2	3.2	3133	♂	152	890	3	11.50	3.83
3134	♀	170	685	7	3	24.50	8.17	3137	♂	193	817	12	*67.90	*6.17
3135	♀	178	710	1	1	1.90	1.90	3139	♂	175	684	8	61.10	7.51
3136	♀	155	699	1	1	10.00	10.00	3140	♀	165	620	0	0.0	0.0
3138	♀	184	775	1	1	3.80	3.80	3141	♀	192	580	24	*294.60	*12.80
3142	♀	192	657	8	13	115.20	8.86	3143	♀	147	578	1	12.00	12.00
3144	♀	153	580	1	4	19.56	4.89	3147	♂	182	886	3	7.10	2.37
3145	♂	145	712	1	29	87.75	3.03	3152	♀	160	680	2	12.10	6.05
3146	♂	211	816	0	3	8.41	2.80	3154	♀	155	648	7	137.70	19.67
3148	♀	170	557	10	4	23.70	5.92	3155	♀	139	563	7	*22.65	*3.77
3149	♀	200	765	0	5	22.30	4.46	3156	♀	159	545	10	116.20	11.62
3150	♂	120	605	8	69	248.62	3.65	3158	♀	153	654	1	12.00	12.00
3151	♀	144	580	4	7	58.25	8.32	3159	♀	189	665	3	40.50	13.50
3153	♀	136	565	9	11	56.55	5.14	3163	♂	196	920	17	185.05	10.88
3160	♂	225	955	2	24	*75.85	*3.79	3166	♂	196	902	2	5.30	2.65
3161	♂	150	836	1	18	*117.08	6.88	3167	♂	233	830	4	*12.30	*4.10
3162	♀	160	645	0	1	3.30	3.30	3169	♂	208	936	9	25.94	2.88
3164	♂	188	825	0	2	8.20	4.10	3170	♂	148	713	2	12.60	6.30
3165	♀	166	680	0	1	8.00	8.00	3171	♀	180	638	2	6.20	3.10
3168	♀	200	774	0	1	*0.0	*0.0	3208	♀	165	605	5	50.90	10.18
3175	♀	139	530	19	19	*128.55	*7.56	3173	♀	151	742	1	10.50	10.50
3177	♀	152	560	1	35	*122.85	*4.24	3174	♀	160	600	0	0.0	0.0
3179	♂	188	865	0	2	11.50	5.75	3176	♂	183	895	14	66.80	4.77
3180	♀	184	698	2	0	0.0	0.0	3178	♂	218	905	3	18.90	6.30
3181	♂	192	611	52	17	*44.78	*2.80	3182	♂	172	778	5	16.75	3.35
3183	♂	128	625	38	13	40.60	3.12	3184	♂	190	958	13	69.90	5.38
3188	♀	174	690	4	0	0.0	0.0	3185	♀	170	690	1	8.00	8.00
3189	♀	150	615	3	1	2.20	2.20	3186	♀	135	580	3	35.10	11.70
3191	♀	181	645	0	2	*3.90	*3.90	3187	♀	184	726	2	21.00	10.50
3193	♀	179	642	0	2	10.60	5.30	3190	♀	167	660	2	18.00	9.00
3195	♀	132	625	10	14	*103.00	7.92	3197	♀	139	640	0	0.0	0.0
3194	♀	182	772	0	2	2.70	1.35	3198	♂	169	735	20	45.10	2.25
3200	♀	162	573	14	5	68.60	13.72	3201	♂	125	591	23	149.10	6.48
3202	♀	140	635	0	1	9.00	9.00	3203	♂	133	680	9	138.00	15.33
3204	♀	159	636	3	7	21.95	3.13							
Total		7696	31,747	249	381	1859.97	223.85	Total		7568	31,849	275	2317.79	369.84
Average		171.02	705.49	5.53	8.47	41.33	4.97	Average		172.00	723.84	6.25	52.68	8.41

SUMMARY

	Mean	Standard deviation
No. of worms	8.47 ± 1.25	12.41
Length of worms	41.33 ± 5.29	52.58

	Mean	Standard deviation
No. of worms	6.25 ± 0.63	6.20
Length of worms	52.68 ± 6.25	61.48

Number of worms $\frac{\text{difference } 2.216}{\text{Error of difference } 1.40} = 1.58$

Length of worms $\frac{\text{difference } 11.35}{\text{Error of difference } 8.19} = 1.38$

* Total length and average length compiled from worms not broken in removal.

TABLE IV

Data of Experiments I, II and III Combined

	PARASITIZED			CONTROLS		
	No. of chicks	No. of worms	*Total length of worms in mm.	No. of chicks	No. of worms	*Total length of worms in mm.
Experiment I	20	118	1282.45	20	86	1012.93
Experiment II	19	32	221.42	20	117	564.91
Experiment III	45	381	1859.97	44	275	2317.79
Total	84	531	3363.84	84	478	3895.63
Mean	6.321 ± .73		40.046 ± 4.16	5.69 ± .412		46.377 ± 4.1
Standard Deviation	9.922		56.536	5.599		55.71

Number of worms $\frac{\text{difference}}{\text{Error of difference}} = \frac{.631}{.838} = .753$

Length of worms $\frac{\text{difference}}{\text{Error of difference}} = \frac{6.3308}{5.84} = 1.084$

*Only worms from secondary parasitisms considered.