PART I.   FURTHER STUDIES OF THE EFFECT OF HELMINTHIASIS
ON THE RESISTANCE OF CHICKENS TO PARASITISM

PART II.   DEVELOPMENT OF AN ANTHELMINTIC FOR GROWING
CHICKENS

by

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Introduction

Much of the evidence of acquired resistance, or immunity to parasites is found to be insufficient when statistical methods are used. The occurrence of bacterial immunity, on the other hand, has been well established in such disease processes as smallpox, typhoid, tetanus and diphtheria. The mechanism of such immunity is a physicochemical reaction involving the multitudinous reactions of colloidal systems such as certain proteins show.

It is this basic fact of antigen - anti-body reaction of colloidal systems which affords the best foundation for consideration of the possible immunity or resistance which may be exhibited by organisms against invading metazoan parasites. The tacit assumption that the physiological activity of a metazoan organism may produce metabolic products

of a protein nature against which the host organism may defend itself, is not unwarranted. The physiological processes of bacteria are indeed as complicated as those of more highly organized metazoans, and conversely, metazoans should be equally as able to produce as a result of their metabolism, substances which would engender certain specific immunity reactions when introduced into or absorbed by another organism. However, the demonstration of such reactions is seemingly more difficult in the case of metazoan parasites than in the case of bacteria.

Much work has been done, none of which conclusively demonstrated the mechanism by which the immunity was brought about. In very few instances has there been clear cut evidence that an active, acquired immunity or resistance, independent of age or other complicating factors, has been developed. In certain instances, the experimental animals used were too few in number to justify positive conclusions, although there was some evidence indicating acquired resistance.

An indication of the development of acquired resistance to parasitism was obtained in this laboratory by Ackert and Jones (1928). As the results were not wholly conclusive, it seemed desirable to extend and modify these experiments in the hope of securing convincing evidence of either an increased or decreased resistance.

Acknowledgements

Historical Review

That there is a similarity between the reactions of host animals to bacterial and metazoan invasions is held by many helminthologists. Chandler (1922) states that the same principle applies to both types of infections. Stoll (1929), working with the nematode, Haemonchus contortus, of sheep under conditions of natural reinfestations, seeks to explain the immunity which developed on an empirical basis: "It is probably a matter of consequence that the fact of more or less continual reinfection with worm parasites, which typically do not multiply in the host, permits an accumulation of parasitic material analogous to bacterial multiplication in vivo. This point of view would bring the helminthologist and bacteriologist to common ground. And if the persistent massing of organisms within the host is responsible for va-

ried manifestations of clinical damage and host resistance, ought the helminthologist not expect to find self-curative and protective mechanisms developed in the host perhaps as commonly with worms as do his bacteriologist confreres with more minute micro-organisms?"

It is thus seen that it is commonly believed that bacteriological and serological methods may be used to demonstrate or substantiate claims of metazoan immunity, and investigations of parasitic infestations by serological methods have been widely undertaken and not without success in many instances. Schwartz (1921) demonstrated hematoxins formed by parasites by serological methods. It is evident that the toxins liberated by the worms were absorbed, and that they engendered immunity reactions in the host.

In some parasitic infections, the damage done to the host is not specific, but is caused by the liberation of toxic products which are not antigenic. Flury and Leéb (1926) working with Fasciola hepatica and Dicrocoelium lanceatum were unable to demonstrate any specific toxin. Anaphylaxis due to contact with vapors or fluids from various ascarid worms have been reported by several workers, among them Goldschmidt (1910), Ransom (1924), and Emery and Herrick (1929). The latter show that the extract of Ascaris has a decided physiological effect on the respiration and circulation.

Complement-fixation and precipitin tests have been widely used with a variety of parasites and host animals. Positive complement-fixation was found by Isbecque (1924) in patients infested with Taenia, Dibothriocephalus and Ascaris. Le Bas (1924), however, was unable to demonstrate either complete fixation or precipitin tests in Dibothrio-cephalus cases. Intradermal tests on infected persons were unsuccessful. Kolmer, Trist and Heist (1916) were able to secure positive complement-fixation in the sera of dogs harboring common intestinal parasites.

In studies of sarcoptic mange in human beings, Nicolau and Banciu (1926) were successful in demonstrating complement-fixation in only 20 out of 35 cases. Results such as these are open to considerable question as to whether they could be used to give a true clinical diagnosis of a diseased condition.

Bachman (1928a) prepared an antigen from Trichinella infested meat which was successfully used in precipitin tests on the sera of infected rabbits. The antigen was also successfully used to immunize animals and precipitins were demonstrable in the blood after 30 days, increasing in titer up to 90 days and were still capable of being detected a year after being immunized. In a later paper, Bachman (1928b) shows a specific intradermal test which was detect-

able before precipitins showed in the serum. There was no group reaction as the tests were not positive when Ascaris proteins were used.

Ciculesco-Mavromati (1927) was able to demonstrate anaphylaxis in guinea pigs with Ascaris antigen when the guinea pigs had been previously injected with the blood of an Ascaris infected patient. Fülleborn (1926a, 1926b) reports specific skin reactions to Ascaris and Trichinella antigens in infected persons. Ramsdell (1927), Casoni (1911), and Rackemann and Stevens (1927) showed dermal reactions with cestode infestations. Passive transfer of local hypersensitiveness was uniformly successful in the attempts of the latter workers.

It is evident from the above work that there is a definite physiological reaction of the host to parasites within it. From the many clear evidences from serological tests, it would seem that immunological reactions must take place. However, they are difficult to demonstrate and cases of immunity to infestation due to previous infection are few in number. Sandground (1929) is of the opinion that there is a high degree of correlation between host-specificity and acquired resistance. In other words, low specificity between the host and the parasite tends to give a high order of immunity and conversely, high specificity induces only a low

order of immunity.

Blacklock and Thompson (1923) showed an immunity against botfly larvae developed by man and animals due to previous attacks of the larvae rather than by any age immunity developed by the host. Continuing this work, Blacklock and Gordon (1927) showed that the immunity was local and confined to areas of the skin attacked and decreased in intensity at a distance from these primary immune areas. No serological tests were demonstrable.

Blacklock, Gordon and Fine (1930) reported that the haemocoele fluid and excreta of the third instar larvae of Cordylobia anthropophaga shows precipitin reactions with the serum of previously infected guinea pigs and is uniformly negative to sera from animals that have never been infected. Death of the larvae in immune animals is closely associated with the reaction between the gut contents of the larvae and the serum of the immune animal.

Fujinami (1916) recorded a case in which a horse, having acquired an infection of the blood fluke, Schistosoma japonicum, recovered and was subsequently immune. Sandground (1929) points out that this is an instance where a polyxenous parasite is not in its most favorable host.

Donham, Simms and Miller (1926) reporting on the so-called "salmon-poisoning" of dogs in Oregon have secured

evidence which indicates a true immunity. The disease,
which is caused by a small intestinal fluke, Nanophyes
salminicola, is usually fatal even in a small infection. A
small percentage of the animals recovered and were decidedly
immune to further infestations. This is another instance of
a polyxenous parasite which is endemic in the area, para-
sitizing the fox, raccoon and coyote, in which heavy infes-
tations do not cause the severe clinical picture seen in
the dog as reported by Cram (1926).

Ducas (1921) reporting on Trichinella spiralis and the
immunity which develops following infection in rats points
out that parasitism is not successful to the same degree in
various hosts. Cameron (1927) says: "Doubt has been ex-
pressed whether these animals (rats) are normal hosts at
all, or merely accidental agents by which the parasite may
be further disseminated."

Sandground (1927) working with Strongyloides ster-
coralis, an intestinal roundworm, demonstrated a resistance
to a superimposed infestation in dogs and cats that was in-
dependent of age. Herrick (1928) was unable to show re-
sistance due to previous infestation of the dog hookworm,
Ancylostoma caninum following the use of an anthelmintic,
although there was evidence of increased resistance when an
attempt was made to superimpose an infestation on previously

parasitized dogs. Fülleborn (1926c) showed that infections of Uncinaria disappeared in about five months and that an immunity was developed to subsequent infections. Gordon (1925), Weinberg and Julien (1911) and others reporting experiments and clinical cases showed that the body develops a tolerance to the effect of the parasite rather than a resistance to the parasite itself. This appears to bear out the contention of Sandground (1929) that there is only a low grade resistance developed where the host-parasite relationship is highly specific.

Joyeux (1925) working with Hymenolepis nana-fraterna showed a resistance developing in mice and rats within an age limit. Age resistance plays an important part in protecting animals from certain parasites. It is widely known that young animals are more susceptible to certain types of parasites than older animals and Sandground (loc. cit.) would account for this on the basis of abnormal host-parasite relationship.

An interesting case is that reported by Reuling (1919) in which the glochidia of the fresh water mussel, Lampsilus luteola, induced an immunity in the large-mouthed black bass, Micropterus salmoides. In vitro tests showed the serum of immune fish to contain a lysin which destroyed the glochidia shortly after they became encysted. Apparently,

this is not an abnormality in the specific relationship be-tween the host and the parasite.

Stoll (loc. cit.) working with H. contortus, the stom-ach of sheep, kept parasitized animals under conditions where normal conditions of reinfestation occurred and by use of a worm-egg count method was able to demonstrate a resistance which developed independent of age.

Herrick (1925, 1928) showed an age resistance developed in the case of Ascaridia lineata in chickens and A. caninum in dogs. Scott (1928) working in conjunction with Herrick noted inhibited development of larvae when introduced into the intestine of the dog. Maturing worms were rapidly e-liminated from the body. Sarles (1928) showed an age re-sistance in infection with A. braziliense which develops in the cat.

Ackert and Herrick (1928) were unable to superimpose a pathological infestation of A. lineata on a group of previ-ously parasitized chickens. No pathological effects were noticed and as it was unknown whether the failure of the second parasitism was due to the previous infestation or to age, the series of experiments by Ackert and Jones (1928) and by the present writer were undertaken.

Materials and Methods

Day old, purebred, white leghorn chicks from an accred-

ited flock were obtained and raised under confined condi-
tions which were adequate for normal growth (Herrick, Ackert
and Danheim, 1923). The parasitizing was done with the em-
bryonated eggs of the nematode, A. lineata. These were se-
cured by cutting the anterior end from gravid female worms
and pressing the internal organs into clean Petri dishes.
The uteri were separated and the proximal portions which
usually contain a high percentage of fertilized eggs were
macerated in another clean Petri dish. These were covered
with distilled water to which was added three to four drops
of two per cent formalin to inhibit the growth of mold and
bacteria which otherwise caused clumping and often the de-
struction of the eggs. These egg cultures, incubated in an
electric incubator at 28°-34°C. developed to the infective
(embryonated) stage in from 12 to 20 days. At the time of
parasitizing, the dose of embryonated eggs was counted out
on a slide by means of a compound microscope and mechanical
stage. The eggs were then washed off onto a filter paper,
a pinch of corn meal added and the whole rolled into a pel-
let to be force-fed to the chick.

At the beginning of each experiment the chicks were
banded and weighed. Groups of equal number were selected
so that the total weight of each group was the same. Ex-
ceptionally small and large birds were rejected, only those

representing the average of the group being used. In Experiments I and II, the primary parasitizing was done when the chicks were five weeks of age and the anthelmintic administered when they were nine weeks and five days old in Experiment I and when they were nine weeks old in Experiment II. The secondary parasitism was administered when the birds were 10 weeks old in Experiments I and II, and when they were $11\frac{1}{2}$ weeks old in Experiment III. In all four experiments, the birds were killed and the worms collected from the intestines after three weeks of the secondary parasitism. In Experiment IV, the chicks received the primary parasitism when four weeks old, the anthelmintic treatment when eight weeks old, and the secondary parasitism when they were $10\frac{1}{2}$ weeks old. Weekly weight records of each bird were kept and the average gain of each group calculated. By comparisons, the effect of the parasitism and the anthelmintic treatment could be detected in most instances from these weight records.

In each experiment, one of the previously parasitized lots was treated with carbon tetrachloride (vide Part Two) as an anthelmintic to remove the roundworms that might be present; thus if any resistance to the secondary parasitism were indicated, it clearly could not be due to the physiological or mechanical factors which might be held accountable if worms of the primary parasitism were present. The

lot receiving only the secondary parasitism, designated as the controls were also treated with the anthelmintic to eliminate all uncontrolled factors which might enter in determining the degree of parasitism.

In Experiments II, III and IV, the work was done in duplicate, two experimental groups being anthelmintic treated and two not treated. This afforded data in determining the possible error which was liable to occur in arbitrarily separating worms of the secondary and primary parasitism on the basis of the maximum length found in the control. In Experiments I and II, a single group which received only a single parasitism, the primary, were allowed to run in order to determine the minimum size of worms which might be obtained from the primary parasitism.

In Experiment I, four weeks and five days elapsed between the time of primary parasitism and secondary parasitism in which the previously parasitized groups could have developed a resistance to subsequent infestations due to the presence of the parasites. In Experiments II, III and IV, the time in which the previously parasitized groups could have developed a resistance was four weeks.

At the time of autopsy, the birds were killed and the small intestine removed, being detached at the gizzard and the junction of the ceca. The gut was then broken into

three or four pieces and the contents flushed into a flask
by means of warm water under pressure (Ackert and Nolf,
1929). This material was placed in Mason jars; sufficient
formalin being added to inhibit bacterial growth. Later,
the worms were removed, a binocular dissecting microscope
mounted on a swinging arm being used to detect small worms
in the water diluted debris. The worms were opaque and eas-
ily distinguishable from feathers, etc. They were then
placed in vials with two to four per cent formalin along
with the leg band of the chicken for identification.

In measuring the worms, use was made of a photomicro-
scopic apparatus, which was raised in a vertical position
and adjusted over a lighted plate so that it magnified ex-
actly six diameters. The worms were placed in water in
Petri dishes over the lighted plate, and the enlarged shad-
ows traced on onion skin paper. These tracings were then
measured by the use of a specially prepared brass tracing
wheel from which a direct reading of the worm length in
millimeters could be made. This method of measurement as-
sured a high degree of accuracy.

In Experiments II, III and IV, the worms of the primary
and secondary infestations in the previously parasitized
lot of the non-anthelmintic group were separated from each
other on the basis of length, the maximum length found in

the control lot being used as the criterion. Only the
worms of the secondary infestation in the previously para-
sitized lots were used in making comparisons with those from
the control lots. In the anthelmintic treated groups, no
such separation was necessary, since all of the worms pres-
ent in the previously parasitized lots were of the secondary
infestation, as the efficacy of the anthelmintic used had
been previously established and demonstrated to be 100 per
cent (_vide_ Part Two).

The number of worms, i. e., the degree of infestation
and the length of the worms, i. e., the rate of growth were
used as the criteria in making the statistical comparisons
to determine the increased or decreased resistance of the
previously parasitized chickens to the parasites. The aver-
age length of the worms, rather than the average or total
lengths per bird, was used since it seemed to give a more
accurate figure for the actual rate of growth of the worms.
No account has been taken of the genetic difference of the
parasites or of the chickens. Both may introduce uncon-
trolled factors, but the use of young, vigorous egg cultures
in parasitizing, and the utilization of rather large numbers
of chickens of nearly uniform size seem to minimize the pos-
sible effects of these factors.

Four experiments were conducted as described. Experi-

ment I was made with three lots of 25 chicks each, one being the previously parasitized and one the control lot. Both lots were anthelmintic treated. Experiment II was made with five lots of 25 chicks each, two of which were non-anthelmintic treated and two anthelmintic treated. Experiments III and IV were made with four lots each, each lot containing 20 chicks, the experiments closely resembling Experiment II.

Not counting the chickens that died of various causes, 44 chickens have been used in determining the minimum size of worms of the primary parasitism, 77 as previously parasitized anthelmintic treated, 78 as control anthelmintic treated, 56 as previously parasitized non-anthelmintic treated and 55 as control non-anthelmintic treated, making a total of 310 chickens used in the various experiments.

An important divergence should be noted at this point as it may be due to this that the difference in correlation between the work of Ackert and Jones and that presented here occurs. Whereas, they gave each chick 300 embryonated eggs of the parasite, only 50 were given in the experiments herein reported. When the larger number is used, birds that do not have a high natural resistance to the parasite are apt to be heavily parasitized. Although the average obtained may be low, a few heavily parasitized birds may yield suf-

ficient worms to distort it enough that any resistance
which may be present will not be revealed either upon an
examination of the mass data, correlation tables or statis-
tical treatment. Thus the use of a fewer number of eggs in
parasitizing may be of a decided advantage. Ackert, Graham
and Nolf (unpublished) have conducted experiments in which
no significance could be detected between the use of 300
and 100 eggs, 100 and 50 eggs, and 50 and 25 eggs although
a significance in numbers of worms recovered was found in a
comparative experiment using 100 and 25 eggs.

Experimental Results

This problem was undertaken to secure further evidence
of either an increased or decreased resistance, as it seemed
desirable to demonstrate this point conclusively if possible.
Ackert and Jones presented evidence which was more or less
indicative of increased resistance in one experiment and an
effort has been made to adhere as closely as possible to
the general methods which they employed. Two points of di-
vergence have been made, one of which was for the purpose
of eliminating the arbitrary method of separating worms of
the secondary and primary parasitism. To this end, an an-
thelmintic was used for one group of each experiment, there-
by removing the worms of the primary parasitism. The second

point of difference was in the numbers of embryonated eggs fed in the parasitizing. Whereas they fed 300 eggs, the number was reduced to 50 in these experiments. This had the advantage of limiting the degree of parasitism in susceptible birds and tended to reduce the error of the means found in the data when statistical treatment was employed.

Experiment I. In this experiment which contained three lots of chickens, only the anthelmintic treated groups were used. The carbon tetrachloride was administered (vide Part Two) when the chickens were nine weeks and five days of age, just two days before they were reparasitized. A mortality of 25 per cent resulted from this treatment and upon autopsy, it was evident that the attempt to reparasitize had been made too soon after this severe treatment.

No worms were found in the 17 remaining chickens in the previously parasitized lot and of the 18 birds left in the control lot, three harbored a total of five worms which ranged in length from 3.2 to 23.4 mm. The untreated lot which had received only the primary parasitism contained 21 chickens. In these were found 128 worms or an average of 6.1 worms per bird. Five of the chickens had no worms at all, three had only one worm, three had two worms and one had 25 worms. The worms ranged in length from 33.4 to 95.2 mm., the average being 73.48 mm. (Table I). No attempt was

made to treat any of the data statistically since it was obviously abnormal in that the reparasitizing was done too soon after treatment with a heavy dose of an anthelmintic.

Experiment II. Five lots of chickens were used in this experiment as has been previously stated. The dosage rate for the anthelmintic treatment was reduced and administered one week previous to the secondary parasitizing. Examination of the data from the anthelmintic control lot showed that 21 birds yielded 62 worms or an average of 2.95 worms which ranged in size from 2.7 to 32.8 mm. The average length of the worms was 12.35 mm. (Table I). In the previously parasitized anthelmintic treated lot which contained 22 birds, 26 worms were found, or an average of 1.1 worms per chicken. Thirteen contained no worms at all, nine worms being the largest number found in any one bird. The worms ranged in length from 3.4 to 21.3 mm., the average being 8.29 mm.

Comparing the data from the two groups, the controls had an average of 1.77 more worms per chick than did the previously parasitized lot. This difference was not significant. Considering lengths of worms, the average length of the worms from the controls was 4.07 mm. greater than that of the previously parasitized lot. This difference was 4.4 times its probable error when treated biometrically

TABLE I. NUMBERS OF WORMS PER CHICKEN

	Control Primary Parasitism	Anthelmintic Group Previously Parasitized (Secondary)	Control (Secondary)	Non-Anthelmintic Group Previously Parasitized Primary Parasitism	Secondary Parasitism	Control (Secondary)
Expt. I	6.1	0	0.29	-	-	-
Expt. II	5.3	1.18	2.95	4.28	0.88	3.75
Expt. III	-	0.15	0.45	2.55	0.25	0.65
Expt. IV	-	0.32	0.42	8.00	0.37	0.11

AVERAGE LENGTH OF WORMS IN MILLIMETERS

Expt. I	73.48	0	13.52	-	-	-
Expt. II	58.27	8.29	12.35	68.16	8.32	12.36
Expt. III	-	20.43*	9.13	64.22	12.44	13.94
Expt. IV	-	13.97	13.38	77.03	14.09	14.85

*3 worms -- one of which was 40.8 mm. in length.

which is considered to be significant. (Table II).

In the non-anthelmintic treated groups, it was necessary to separate the worms of the primary and secondary parasitism in the previously parasitized lot. This was done arbitrarily, 27mm. being taken as the largest worm of the secondary parasitism. A total of 15 worms was found in the 18 remaining chickens. One-half of them had no worms at all and the rest had from one to three worms each, the average being 0.88 worms per bird. The worms of the secondary parasitism ranged from 3.3 to 27.0 mm. in length, the average being 8.32 mm. The worms of the primary parasitism, numbering 77 or an average of 4.28 worms per chicken, ranged from 40.2 to 95.6 mm. in length, averaging 68.16 mm. (Table I).

In the non-anthelmintic treated controls, 16 birds yielded 60 worms or an average of 3.75 worms per bird. Only two of the chickens had no worms, six had only one worm, eight had from two to six worms each and one had 20 worms. The worms ranged in length from 4.4 to 23.3 mm. and averaged 12.36 mm.

Comparing the data of the secondary parasitism, it was found that the controls had an average of 2.87 more worms per bird than did the previously parasitized lot. This difference was 3.52 times its probable error, indicating an acquired resistance. The worms of the controls averaged

TABLE II. BIOMETRICAL COMPARISON OF DATA FROM EXPERIMENTS II AND III

Group	Average Number	Actual Difference	P. E. of Difference	Significance
Anthelmintic Treated		Numbers of Worms		
II previously parasitized	1.18			
		1.77	0.637	2.78
II control	2.95			
III previously parasitized	0.15			
		0.3	0.388	0.77
III control	0.45			
Non-anthelmintic Treated				
II previously parasitized	0.88			
		2.87	0.814	3.52
II control	3.75			
III previously parasitized	0.25			
		0.4	0.203	1.97
III control	0.65			

Table II-Continued:

Lengths of Worms

Anthelmintic Treated

II previously parasitized	8.29			
		4.07	0.923	4.4
II control	12.35			
III previously parasitized	20.43			
		11.3	7.99	1.41
III control	9.13			

Non-anthelmintic Treated

II previously parasitized	8.32			
		4.03	1.17	3.44
II control	12.36			
III previously parasitized	12.44			
		1.5	2.35	0.64
III control	13.94			

4.025 mm. greater in length than did those of the previous-
ly parasitized lot. This difference which was 3.44 times
its probable error may also be considered significant.
(Table II).

The single lot which received only the primary para-
sitism contained 122 worms in 23 chickens or an average of
5.3 worms per bird. The worms ranged from 26.2 to 91.6 mm.,
averaging 58.27 mm. Eleven of the worms in this group were
smaller than the largest worm of the anthelmintic treated
control which was 32.8 mm. in length. This proves conclu-
sively that in non-anthelmintic treated groups, extreme cau-
tion must be exercised in separating worms of primary and
secondary parasitisms. The error which would be introduced
would be quite sufficient to throw the results of statisti-
cal treatment off enough to prevent any indication of an
increased resistance if it were to occur in an experimental
group.

It seems to be clearly indicated by this experiment
that a previous parasitism materially affects the degree of
subsequent parasitism as well as the rate of growth of the
worms. The statistical treatment of the data is clearly
favorable to this conclusion as is also the averages of
numbers and lengths of worms.

Experiment III. This experiment contained four lots of

20 chicks each that averaged 212.6 grams in weight. The chicks were exceedingly large and vigorous and were $6\frac{1}{2}$ weeks old before an egg culture was secured that was satisfactory for parasitizing them. The previously parasitized lots of both the anthelmintic treated and non-anthelmintic treated groups were parasitized at this time. All four lots were parasitized when $11\frac{1}{2}$ weeks old; the anthelmintic treated group having received the carbon tetrachloride one week before. Autopsy was made when the chickens were $14\frac{1}{2}$ weeks of age.

In the anthelmintic treated group, the previously parasitized lot was found to harbor only three worms, one in each of three chickens. The worms measured 6.8, 13.7 and 40.8 mm. respectively. It is evident that extremely large size may occasionally be acquired by a small number of worms of the secondary parasitism. In the anthelmintic treated control lot, 14 of the chickens had no worms at all, four had only one worm, one had two worms and one had three worms. The worms ranged in length from 3.5 to 13.2 mm., averaging 9.13 mm. in length.

Concerning numbers of worms, it was found that the previously parasitized lot averaged 0.15 worm per bird and the controls 0.45 worms, giving a difference of 0.3 worm which is not significant. (Table II).

No significant difference occurred between the lengths of the previously parasitized lot and the controls as the difference of 11.3 mm. was only 1.41 times its probable error. (Table II). It seems probable that the increased age of the chickens coupled with the fact that they were exceedingly large and vigorous is sufficient to account for the ill success of the secondary parasitism in establishing itself.

In the non-anthelmintic treated group, the 19 birds in the previously parasitized lot harbored five worms of the secondary parasitism. These were found in three birds. The average number of worms per bird was 0.25. The five worms measured 4.6, 9.1, 9.7, 14.6 and 24.2 mm., respectively, an average of 12.44 mm. There were 51 worms of the primary parasitism present, 30 of which were in one bird. The average number per bird was 2.55. They ranged in length from 42.6 to 90.6 mm. and averaged 64.22 mm.

The 20 birds of the control lot had 13 worms, an average of 0.65 worm per bird. Fourteen had no worms at all and the rest had from one to four. The worms ranged in size from 3.7 to 29.4 mm., averaging 13.94 mm.

Considering numbers of worms, it is seen that the controls have 0.4 more worms per bird than the previously parasitized lot. This figure, when divided by its probable

error ($\pm$ 0.2033) yields a quotient of 1.97 in favor of in-
creased resistance. This is not significant.

The difference of the mean lengths of the worms from
the two lots is only 1.5 mm. and the probable error of
$\pm$ 2.35 gives a quotient of 0.639 which is not significant.

No significance in favor of an increased resistance can
be detected in this experiment. It is obvious that the
small numbers of worms obtained makes the error of the means
extremely high and this is sufficient to effectively mask
any positive results.

Experiment IV. In this experiment, the previously par-
asitized lots of both groups of chicks were parasitized when
four weeks of age. The younger age may have been the factor
which influenced the increased number of worms of the prima-
ry parasitism found in the non-anthelmintic treated group.
Nineteen chickens harbored 152 mature worms or an average of
eight worms per bird. The worms ranged in length from 51.8
to 101.6 mm., averaging 77.03 mm.

The chickens did not receive the secondary parasitism
until they were $10\frac{1}{2}$ weeks of age. As very few worms of this
parasitism were recovered, it was obvious that some factor
had entered which rendered chickens that were previously
rather highly susceptible to infestation, relatively resist-
ant. As the birds were not especially vigorous for their

age, it seemed probable that this factor was age resistance.

From the anthelmintic treated group, only 0.32 worm per bird was recovered from the previously parasitized lot and 0.42 worm per bird from the control lot. Obviously, the difference is not significant. The worms averaged 13.97 and 13.38 mm. in length, respectively. No significance was indicated by these figures. The non-anthelmintic treated group yielded similar data. The previously parasitized lot had 0.37 worm per bird from the secondary parasitism and the controls 0.11 worm per bird. The average length was 14.09 and 14.85 mm., respectively. No significance was indicated by these figures.

Discussion of Data

The evidence from Experiments I, II, III and IV showed that some chickens, when subjected to a subsequent parasitism, possessed a high resistance to infestation with A. lineata. This resistance could not have been due to age since all of the chickens under comparison were of the same age. Much individual variation occurred among different chickens and it seemed probable that this would account for at least a part of the fluctuation found in the data. It seemed to be a constant observation that there was a great variation in the numbers of worms found in different birds. This va-

riation was more striking as it concerned rate of growth.
The worms from individual birds tended to be of the same ap-
proximate size regardless of the number of worms in the
chickens or the range of variation for the group. This fac-
tor, undoubtedly, was one of the most important in effecting
an arbitrary variable which influenced the results of the
biometrical treatment of the data, especially where the num-
bers of worms were small.

A critical examination of the average numbers and aver-
age lengths of worms in the various groups showed a high de-
gree of correlation indicating an acquired resistance to
superimposed infestation (Table I). These data cannot be
ignored in spite of the fact that a resistance was indicated
in only one experiment when biometrical methods were em-
ployed. The rate of growth in both groups in Experiment II
afforded evidence that some factor had affected both groups
to the same degree, thereby inducing increased resistance
in the previously parasitized lots. That this process was
not influenced by the administration of an anthelmintic was
obvious.

When the results of the various experiments were con-
sidered from the standpoint of age of chickens at the time
of parasitism (Table III), it was clear that age resistance
began to develop early and increased as the chickens grew

TABLE III. AGE OF CHICKENS IN WEEKS AT -

	Primary Parasitism	Anthelmintic Treatment	Secondary Parasitism	Autopsy
Experiment I	5	9^5	10	13
Experiment II	5	9	10	13
Experiment III	$6\frac{1}{2}$	$10\frac{1}{2}$	$11\frac{1}{2}$	$14\frac{1}{2}$
Experiment IV	4	8	$10\frac{1}{2}$	$13\frac{1}{2}$

Note: exponent (5) indicates days.

older. Considering the worms of the primary parasitism from the various lots, it was seen that parasitism at four weeks of age resulted in eight worms per bird at autopsy; at five weeks of age, 6.1, 5.3 and 4.28 worms per bird; and at $6\frac{1}{2}$ weeks of age, 2.55 worms per bird. This tends to confirm the findings of Herrick (1925) and Ackert (1930) that age is an important factor in the resistance of young chickens to parasitism.

In Experiment II, the secondary parasitism was successful in becoming established but in Experiments III and IV, the worms recovered were too few in number and too variable in size to lend themselves to statistical consideration. Undoubtedly, the increased age in the last two experiments was the disrupting factor. Although age resistance may not have been at its maximum in the chickens 10 weeks of age, it was obvious that for successful parasitism, chickens no older than this should have been used.

The use of the smaller number of eggs in parasitizing resulted in certain differences of visible effects. No pathological symptoms were noted in the chickens and only minor fluctuations were noticeable in the growth curves. This factor apparently was of no consequence in the resulting data yielded by the various groups.

From the work done, it has been shown rather definitely

that parasitism does have a physiological reaction on the host, but there is no overwhelming mass of evidence that it is immunological in nature. Kritschewski and Heronimus (1927) say, "....the presence or absence of antibody is not related to the possibility or impossibility of producing super-infection in infected animals". This appeared to be the case in the present instance; where resistance was demonstrated satisfactorily in one experiment, and apparently it was of the nature of an immunity reaction, an entirely different factor was responsible for the inhibition of successful parasitism in the other two experiments.

The evidence from these experiments seems to substantiate the conclusions of Sandground (loc. cit.) that the development of age resistance may be taken as ipso facto evidence of a low order of host-parasite specificity with the concomitant fact of high acquired resistance. The fact that large numbers of host animals are necessary for its demonstration and other various complicating factors which may enter all tend to make it difficult to demonstrate as satisfactorily as could be desired. Nevertheless, the evidence presented is positive and does not contradict that of previous experimenters in this field.

In view of these facts, the conclusion that chickens may develop an acquired resistance to the intestinal nematode,

Ascaridia lineata (Schneider) due to previous infestation and prior to the development of an active age resistance, does not seem unwarranted.

Summary

1. Four experiments were conducted on 310 white leghorn chickens to determine if they would develop resistance to a secondary infestation of Ascaridia lineata due to a previous parasitism with this worm.

2. The secondary parasitism was unsuccessful in Experiment I due to the fact that it occurred too soon after the administration of an anthelmintic. Hence, no data of importance were obtained.

3. In Experiment II, 99 chickens were involved. Duplicate experiments yielded biometrically significant results in favor of acquired resistance to A. lineata. Evidence was also obtained on the rate of growth of the worms, proving that the arbitrary method of separating worms of the primary and secondary parasitism on the basis of length was not without danger of considerable error.

4. Experiments III and IV, involving 79 and 76 chickens, respectively, yielded no data of biometrical significance, but indicated that parasitism after chickens are 10 weeks of age was subject to a rather highly developed age resistance

which effectively masked any acquired resistance which may
have been developed. The results were more or less indica-
tive of acquired resistance on the basis of correlation
alone.

5. The individual variations in natural resistance of
the host make it evident that large adequately controlled
series should be used in experiments of this nature. It
was also obvious that all parasitizing should be done before
age resistance becomes so great as to mask the evidence of
acquired resistance.

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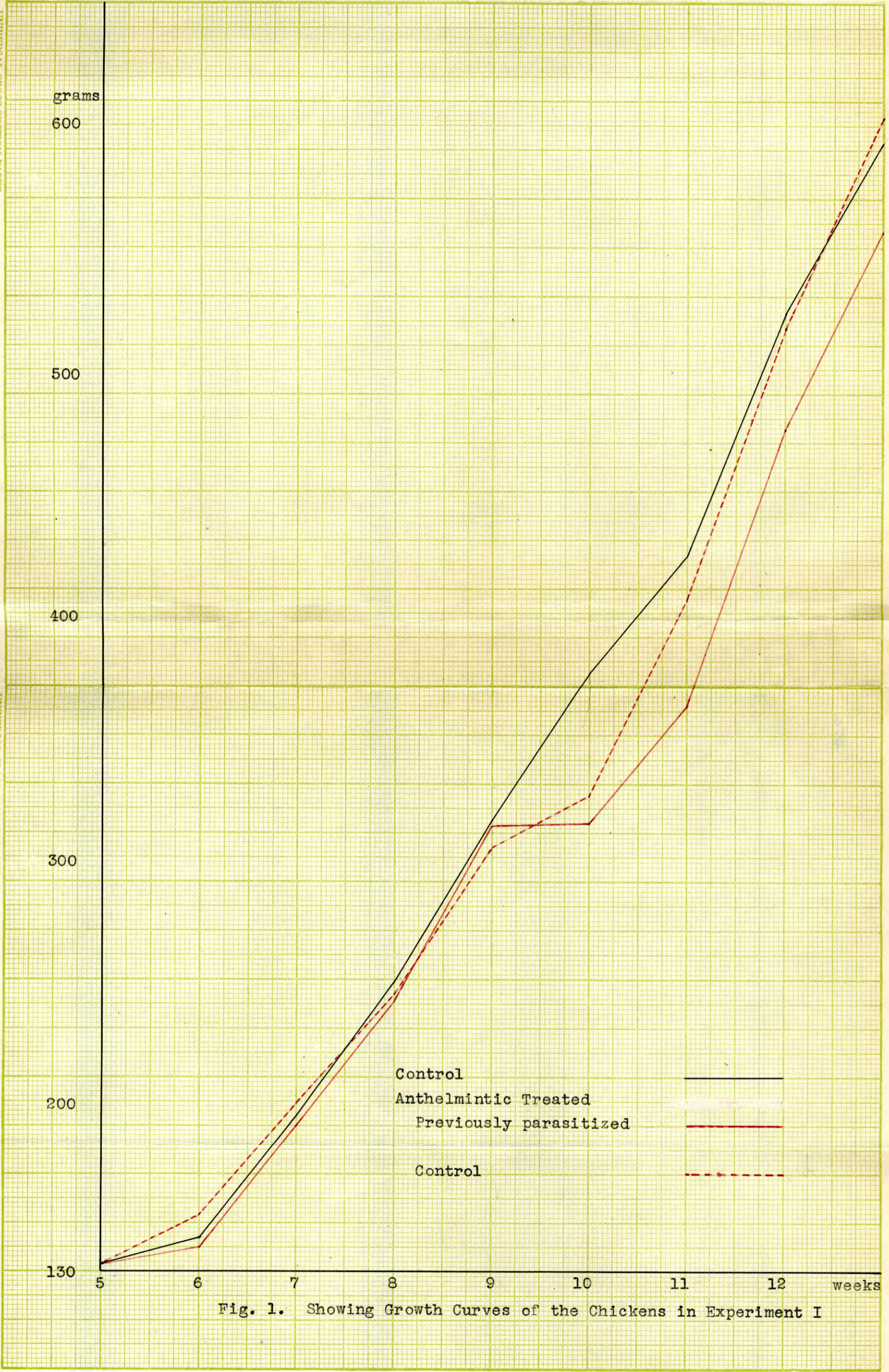   Jour. Hyg., V. 8 (4), pp. 507-538.

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grams

600

500

400

300

200

130

Control
Anthelmintic Treated
Previously parasitized

Control

5   6   7   8   9   10   11   12   weeks
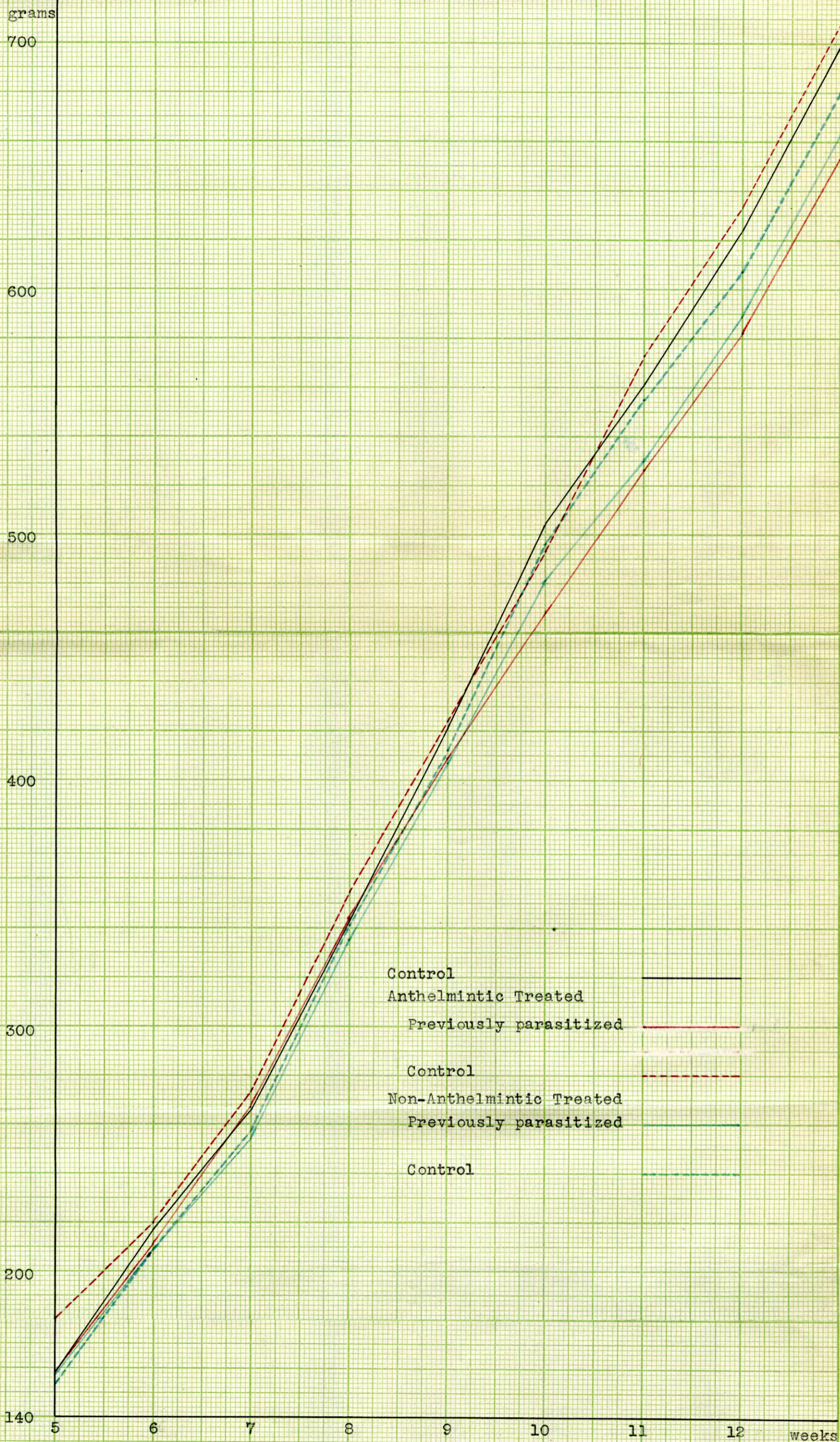
Fig. 1.   Showing Growth Curves of the Chickens in Experiment I

Fig. 2. Showing Growth Curves of the Chickens in Experiment II

Control
Anthelmintic Treated
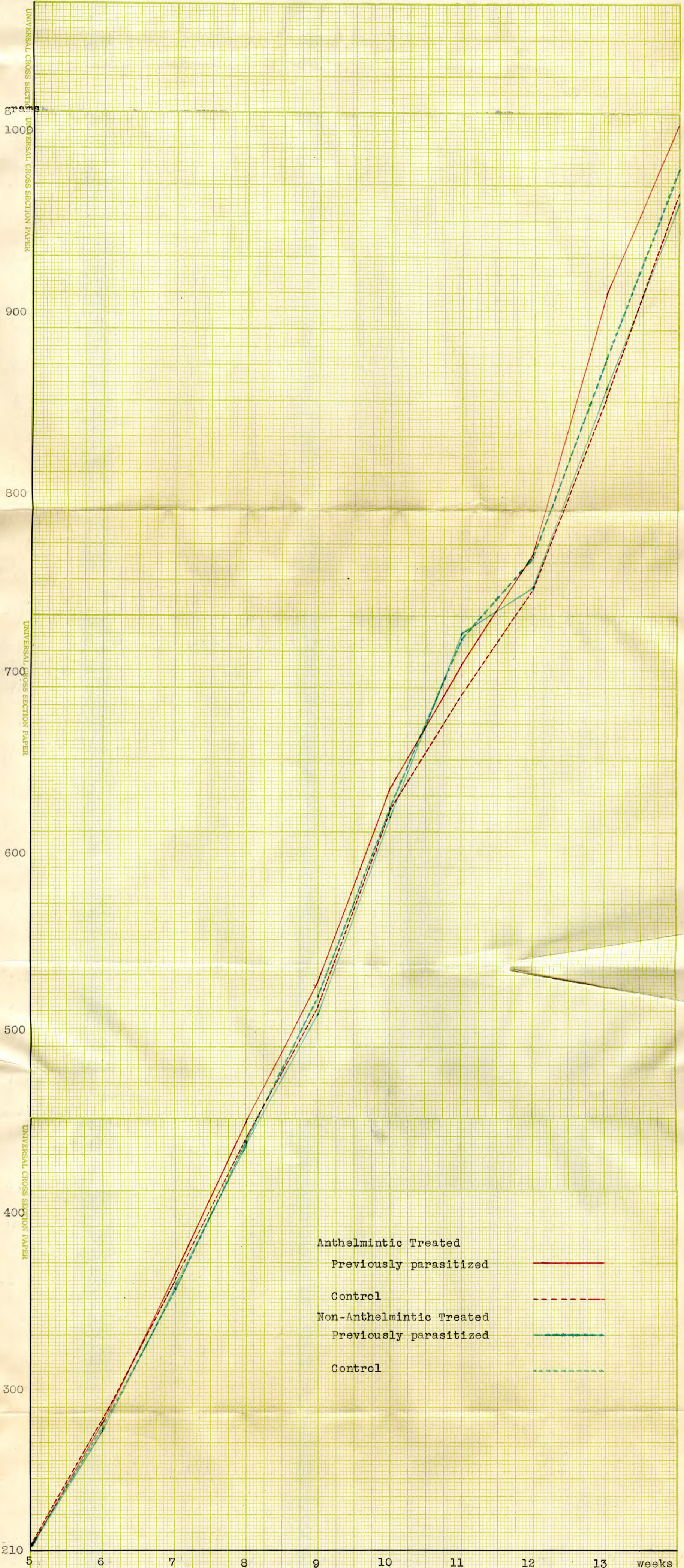Previously parasitized

Control
Non-Anthelmintic Treated
Previously parasitized

Control

grams
700
600
500
400
300
200
140

5   6   7   8   9   10   11   12   weeks

Fig. 3. Showing Growth Curves of the Chickens in Experiment III

Anthelmintic Treated
Previously parasitized
Control
Non-Anthelmintic Treated
Previously parasitized
Control

Fig. 4. Showing Growth Curves of the Chickens in Experiment IV

Legend:
- Anthelmintic Treated
  - Previously parasitized
  - Control
- Non-Anthelmintic Treated
  - Previously parasitized
  - Control
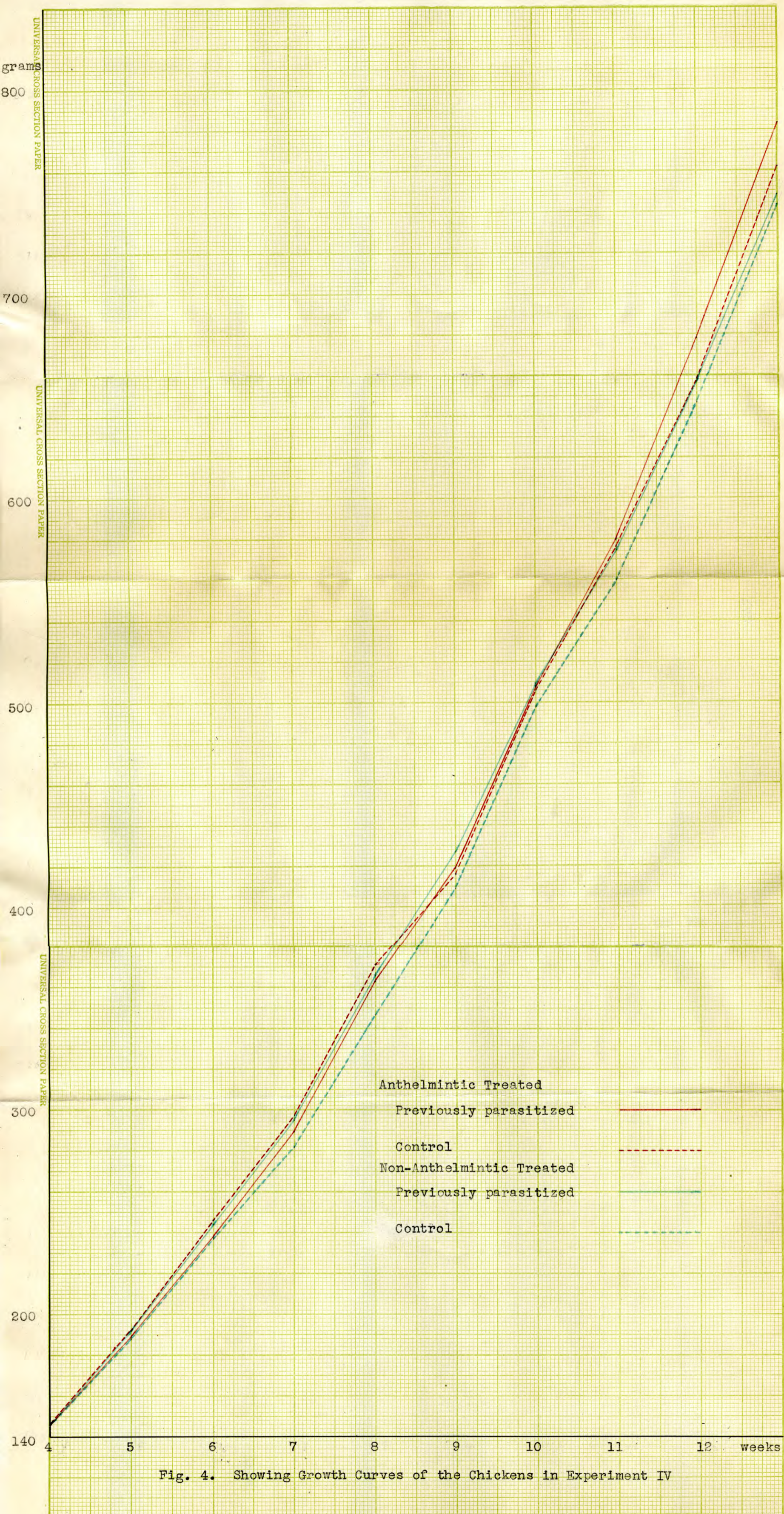
PART II. THE DEVELOPMENT OF AN ANTHELMINTIC FOR GROWING CHICKENS

Introduction

The work herein reported grew out of the attempt to find an adequate anthelmintic which would safely remove the nematode, <u>Ascaridia</u> <u>lineata</u> (Schneider) from the intestines of young growing chickens in connection with the resistance experiments reported in Part One. Several anthelmintics were suggested and an effort was made to secure one that answered the purpose required. Adverse results obtain in Experiment I led to varied experimentation that served admirably in determining the necessary procedure to be followed in future experiments. The use of this anthelmintic, carbon tetrachloride, has suggested several fields of experimentation which have received but slight attention and within certain limits has shown some points of difference with the experience of other workers.

Acknowledgements

Dr. J. E. Ackert suggested the use of the anthelmintic in connection with the experiments reported in Part One, and to him the writer wishes to express his indebtedness for valuable suggestions and aid in securing materials and conducting the work.

Historical Review

Hall and Shillinger (1923) reporting on experiments made upon a variety of domestic animals in which carbon tetrachloride as an anthelmintic state that it is a safe and efficient anthelmintic for chickens. Using it in doses ranging from one to 18 cc. per kilo of body weight they found that doses of 2 cc. and over per kilo of body weight were efficacious for the removal of Ascaridia lineata from chickens. They indicated no deleterious effects even in the largest doses used. Graybill and Beach (1925) found that doses of 2 cc., administered in hard gelatine capsules, were approximately 98 per cent efficacious in removing the large roundworms. Graham and Ackert (1929) reporting the results of the use of anthelmintics in the experiments of Part One stated that carbon tetrachloride used at a dose rate of 10 cc. per kilo of body weight was effective as an anthelmintic but decidedly toxic to young growing chickens. Used at a rate of 4 cc. per kilo, it was 100 per cent efficacious and relatively non-toxic.

Schlingman (1927) reported that tetrachlorethylene used at a rate of 1 cc. was effective in removing the roundworms from chickens up to 2 lbs. 12 oz. in weight when used alone, but that its efficacy was reduced when used at the same rate

with 1 gram of kamala. The same author in 1929 made further tests on the anthelmintic efficacy of tetrachlorethylene and found that it was not particularly effective in doses of less than 1 cc. on birds weighing up to 2 lbs. and that at a rate of 1 cc., it was not always reliable.

In view of the fact that a 100 per cent efficacious anthelmintic was necessary for adaptation to the experiments being conducted by the writer, it was decided that carbon tetrachloride in gelatin capsules be administered to the chickens at a dose rate of 10 cc. per kilo of body weight.

Materials and Methods

Carbon tetrachloride (technical quality) was placed in gelatin capsules of three sizes holding 0.5, 0.9 and 1.3 cc., respectively. The minimum dosage for each bird was calculated and the capsules were forced through the oesophagus into the crop. Care was necessary in this procedure to prevent the collapse of the capsule and the escape of fluid into the throat. When this occurred, inhalation-intoxication was noted which persisted for several minutes. No fatalities occurred because of this, however. The procedure has been the same in all of the experiments, regardless of the dosage used or the nature of the experiments.

Experimental Results

Experiment I. Forty-eight chickens were treated with
carbon tetrachloride at a dose rate of 10 cc. per kilo. All
birds soon showed visible effects and a mortality of 25 per
cent occurred within five days as a result of this treat-
ment. The heaviest mortality occurred from 36 to 72 hours
after treatment. The birds that died were examined and in
no cases were any worms found. The day following the ad-
ministration of the anthelmintic, nearly all of the treated
birds were dull and listless; they showed a distinct loss of
appetite, and the oral emission of a thin ropy sputum.

The chickens that died within three days after treat-
ment were found at autopsy to have deep crimson colored
livers which were abnormal; the gall bladders were greatly
enlarged, sometimes being from two to three times the normal
size. The pancreas had a white waxy appearance, showing
considerable bleaching when compared with the cream colored
pancreas of normal chickens. Areas of petechial hemorrhage
were seen in the intestine and the contents of the posterior
intestine were greenish in color due to the excessive secre-
tion of bile. Four days after treatment, the above symptoms
were the same except that the liver was not congested with
blood, was blotched with greyish-yellow and was very fragile,

indicating necrotic areas.

At the conclusion of the experiment, no worms of the primary parasitism were found in any of the treated birds. Evidently, the efficacy was 100 per cent. However, carbon tetrachloride at this dose rate is obviously not a good anthelmintic in view of the heavy mortality which it induced in chickens 10 weeks of age. Figure 1 (Part I) shows a growth curve of the three groups in this experiment and shows considerable depression in the two groups receiving the anthelmintic.

Experiment Ib. Thirty-one chickens were used in a comparative study of the anthelmintic efficacy of carbon tetrachloride and tetrachlorethylene. Three groups of parasitized chickens were treated with carbon tetrachloride in gelatin capsules at dose rates of 8, 6 and 4 cc. per kilo respectively. Two birds from each group were given a feeding of A. lineata eggs three days and five days after treatment, respectively. At autopsy three weeks later, no worms of the primary parasitism were found and parasitism after the treatment was successful in several instances. Two of the chickens receiving the anthelmintic at the rate of 8 cc. per kilo showed visible symptoms for several days but no ill-effects were noted in any of the other birds, other than transitory effects caused by inhalation-intoxication.

Three groups of three chickens each were treated with
tetrachlorethylene in gelatin capsules at dose rates of 6,
4 and 2 cc. per kilo, respectively. The birds treated at the
rate of 6 cc. per kilo showed visible effects of the treat-
ment for five days, thus proving that tetrachlorethylene is
a more toxic compound for chickens than is carbon tetrach-
loride. Re-parasitism was successful and the efficacy of
the compound as an anthelmintic was 100 per cent. A group
of eight chickens kept as controls had sufficient large
worms to assure the presence of worms in a high percentage
of the treated birds.

Experiments II, III and IV. These experiments which
correspond to those of the same number given in Part I mere-
ly confirmed the choice of anthelmintic used. It was decid-
ed from the results of Experiment Ib that carbon tetrachlor-
ide be used at a dose rate of 4 cc. per kilo. This decision
was made in view of the fact that this dosage was apparently
100 per cent efficient as an anthelmintic and the compound
appeared to be less toxic than tetrachlorethylene as well
as much cheaper.

The results of these experiments showed an efficiency
of 100 per cent for the anthelmintic and no ill effects other
than the transitory inhalation-intoxication noted in a few
instances. Growth curves showed only minor drops following

anthelmintic treatment (Figures 2, 3 and 4, Part I)

Experiment Ic. Two lots of ten laying pullets each were used in an effort to determine the effect of carbon tetrachloride upon egg production. Accurate check was kept upon the egg production of the two groups over a 21 day period when one of the groups ranging from 1.13 to 1.49 kilos in weight was treated with carbon tetrachloride at a dose rate of 4 cc. per kilo.

The day following treatment all of the treated pullets were more or less inactive and did not eat as heartily as usual. The droppings under the roost were decidedly diarrheic. Obviously, the treatment of laying pullets with carbon tetrachloride at a dose rate of 4 cc. per kilo of body weight is not unaccompanied by symptoms of toxicity. Within two days, however, they were apparently normal and quite as active as the untreated group. Their egg production showed a marked decrease under that of the untreated group for over a week following treatment and their total production for the three week period was lower than during the previous period. A second anthelmintic treatment at the end of three weeks resulted in a decrease in egg production similar to that from the first anthelmintic administration.

The egg production of group I, over the three week period prior to the administration of the anthelmintic to

group II, was 50 48 per cent and for group II, 62.38 per
cent. Over the three week period following treatment, group
I produced 61.43 per cent and group II, 53.81 per cent.
Over the period following the second anthelmintic treatment,
the production of group I was 70.48 per cent and of group
II, 52.38 per cent. It seemed obvious that the anthelmintic
lowered the egg production of group II appreciably and was
not free of deleterious effects.

Discussion of Data

It was obvious from the results of Experiment I that
the conclusions of Hall and Shillinger (loc. cit.) were not
confirmed. A difference of opinion may be held as to just
what constitutes "deleterious effects" but the author does
not think that the symptoms of toxicity observed in the
pullets in Experiment Ic can be otherwise interpreted, hence
a difference of opinion exists as to the safety of carbon
tetrachloride as an anthelmintic. A weight-dose ratio may
be safe for young growing chicks, but there seems to be a
point at which older and heavier birds will receive a dosage
which is not non-toxic if used.

The results of Experiment Ib showed carbon tetrach-
loride to be relatively as efficient as tetrachlorethylene
and somewhat less toxic. Since it is a much cheaper com-

pound, its use as an anthelmintic for roundworms seemed advisable. When used in non-toxic doses, preferably not less than 4 cc. per kilo of body weight, it was 100 per cent efficient for <u>Ascaridia</u> <u>lineata</u> in chickens. However, when it was used at this dose rate on laying pullets, symptoms of toxicity were exhibited and an appreciable decrease in egg production was noted.

Summary

1. Carbon tetrachloride used at a dose rate of 10 cc. per kilo of body weight caused a mortality of 25 per cent in a group of young chickens, 10 weeks of age.

2. Carbon tetrachloride was demonstrated to be less toxic than tetrachlorethylene and 100 per cent efficient as an anthelmintic for <u>Ascaridia</u> <u>lineata</u> in young chickens, 12 weeks of age.

3. A dose-weight ratio may be safely used only on young chickens from 8 to 12 weeks of age. When used on older, heavier birds, the dose rate must be reduced to escape toxic symptoms.

4. The use of carbon tetrachloride as an anthelmintic on laying pullets materially reduces the egg production for a period of 7 to 10 days.

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