# EFFECTS OF BLOOD LOSS UPON THE RESISTANCE OF CHICKENS TO VARIABLE DEGREES OF PARASITISM

by

DALE ALBERT PORTER

A. B., Kalamazoo College, 1930

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A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1932

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# INTRODUCTION

The role played by the blood in resisting infectious bacteria and protozoa has long been a field of active research. The part taken by this fluid tissue in physiological resistance to metazoan parasites is less easily demonstrated. Carrel (1923) showed the presence of a growth promoting substance and of a more active growth inhibiting substance in the blood of adult chickens. Ackert and Titus (1924) found that heavy parasitism with Ascaridia perspicillum* significantly reduced the blood sugar content of chicks six weeks of age. From these and other observations Ackert (1925) concluded that when one-half to two cubic centimeters of blood were taken weekly from month old chicks, over a period of five weeks they did not eliminate their young worms (A. lineata)as was normally the case.

Herrick (1926) when working on a few individuals obtained results that indicated that the resistance of adult chickens to A. perspicillum was lowered when large quantities of blood were removed. As the experiments of Herrick were carried out upon adult chickens and as Ackert's observations on the effects of bleeding were secondary to another experiment it seemed desirable to make an extensive study of the

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*Refers to Ascaridia lineata.

effects of blood letting upon the resistance of chickens to the intestinal nematode, Ascaridia lineata (Schneider).

## ACKNOWLEDGMENTS

The writer wishes to thank Dr. James E. Ackert and Mr. George L. Graham for suggesting and organizing this work and for their able help and timely suggestions in carrying out the investigation. Thanks are also due Mr. George E. Cauthen Mrs. Naomi Zimmerman Crawford, and Mr. T. D. Beach for their help with the routine work of the experiments.

## MATERIALS AND METHODS

Purebred white leghorn chicks one day old were obtained from an accredited hatchery and raised parasite-free in confinement on a diet adequate for normal growth (Herrick, Ackert and Danheim, 1923). When the chicks in each experiment were four weeks of age they were banded and weighed. Lots of equal number in each group in all three experiments were selected so that the total weight of each lot was the same. Extremely large and small birds were rejected, only those representing the average of the flock being used in the experiments. The rejected birds were kept as a reserve group to take the place of casualties during the early stages of experimentation.

Except for Experiment 3 where only two groups of thirty chicks each were used, the experiments were conducted in the following manner: One hundred fifty chicks were divided into five groups of thirty each and then each group subdivided equally into an A lot and a B lot. Lot A of each group were bled at weekly intervals. This was done by using a Leur 5 cc. syringe and a 20 gauge hypodermic needle to make a cardiac puncture. About 0.1 cc. of a 0.5 per cent sodium citrate solution was used as an anti-coagulant. The volume of blood taken from each bird was increased from ½ cc at the fourth week to 1 cc. at the fifth week and then increased 1 cc. each week until the ninth week when 5 cc. of blood were taken. A volume of 15½ cc. of blood was thus removed from each bird during a period of five weeks. Lot B of each group in all experiments served as controls. All groups were run in the same double pen and fed a ration adequate for normal growth as follows: Yellow corn meal 40 gm., alfalfa leaf meal 4 gm., meat meal 10 gm., powdered milk 6.4 gm., oatona 17 gm., cracked wheat 15 gm., and cod liver oil 1.6 gm. Weekly weight records of each bird were kept and the average gain of each lot was calculated. The average gain of Lot A was plotted against that of Lot B in each group in each experiment.

The embryonated eggs of the intestinal nematode A. lineata were used for parasitizing. These were obtained by cutting the anterior ends from gravid female worms and pressing the internal organs into clean Petri dishes. The uteri were teased away from the other internal organs with needles and the proximal portions of each branch which, according to Ackert (1931), contains the highest percentage of fertilized eggs were macerated in another clean sterile Petri dish. These were covered with distilled water to which was added three or four drops of four per cent formalin to inhibit the growth of bacteria and molds which apparently may kill the eggs. These cultures of ova incubated in an electric incubator at 24° to 33° C. developed to the embryonated stage (infective) in from 14 to 21 days (Ackert, 1931. Plate I). When parasitizing, the dose of eggs to be fed each chicken was counted out on a slide by using a compound microscope with a mechanical stage. When the proper number of eggs were on the slide they were washed off into a filter paper, a pinch of yellow cornmeal added, the paper rolled into a pellet and then forced into the crop of the chicken by using a blunt nosed pipette filled with water. Egg doses of variable sizes were given to the different lots under comparison as Ackert, Graham, Nolf and Porter (1931) found that the percentage of survival of A. lineata larvae

increased as the egg dose decreased in size and that smaller infestations were more conducive to the growth of the nematode than larger infestations.

The parasites were given three weeks in which to develop because at the end of this time the worms have withdrawn from the intestinal wall where they have been partially buried and have taken up their abode in the lumen of the intestine (Ackert, 1923). Also a rapid elimination of young worms occurs at about the third week of parasitism (Ackert and Herrick, 1928). At the time of autopsy the birds were killed and the small intestine removed, being detached at the gizzard and the junction of the caeca. 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). The amount of intestinal debris was lessened by removing the feed hoppers from 12 to 14 hours before autopsy. The material flushed out was placed in Mason jars and preserved in 10 per cent formalin. Later, the worms were removed, a Spencer binocular on a swinging arm being used to detect the minute worms in the debris which was greatly diluted with tap water. The worms could be distinguished readily from fragments of feathers and other debris because of their opalescence. They were counted out into vials containing four per cent formalin, the leg band of the chicken serving as a label.

In measuring the worms a photomicroscopic bellows was so adjusted over a lighted plate as to throw on the ground glass worm images magnified exactly six diameters. The worms were placed in a dish over the lighted plate and their images traced on onion skin paper. These tracings were measured by means of a milled wheel from which a direct reading of the worm length in millimeters could be made. This method has proved to be very accurate for this purpose.

The number of worms (degree of infestation) and the length of worms (rate of growth) were used as criteria for making the statistical comparisons to determine the decreased resistance of the birds bled and to ascertain the effects of feeding variable numbers of worm eggs. The average lengths of the worms, rather than the total lengths per bird, were used since they give a truer index of the rate of growth of the worms. Any genetic differences of the chickens or of the parasites were not taken into account. The use of rather large numbers of chickens of the same age, size and breed and the use of vigorous egg cultures in parasitizing seemed to minimize the effects of any uncontrolled factors that might be introduced.

## EXPERIMENTAL DATA

Experiment I. One hundred fifty chickens were divided into five groups of thirty chicks and then subdivided into

two lots of fifteen chicks each. Lot B of each group served as controls while each bird in Lot A was bled every week as described. At six weeks of age the chickens were parasitized with embryonated eggs of A. lineata as follows: 500 eggs to Group I, Lot A (IA bled) and Group I, Lot B (IB unbled); 300 to Group II, Lots A and B; 100 to Group III, Lots A and B; 50 to Group IV, Lots A and B; and 25 eggs to each bird of Group V, Lots A and B.

The growth curves of the A and B lots of the five groups under comparison are plotted in Figures 1 to 5. It may be seen that the growth of the A lots (bled) is retarded greatly. The gain in weight of both lots is materially affected between the 42nd and the 49th day (2nd week of parasitism) when the larvae are burrowing in the intestinal wall. At the end of three weeks the chickens were killed and the worms collected and measured. During the course of the experiment sixteen chickens from the various lots died. Upon autopsy the hearts of the birds that were bled showed no malformations except a small amount of scar tissue where the punctures had been made.

Examination of the data from Lot IA showed that the 14 chickens yielded 133 worms or an average of 9.50 worms (Table I). These nematodes ranged in size from 1.8 to 35.2 mm.; the mean length being 18.88 mm. (Table II). In Lot IB which likewise had 14 birds, 102 worms were found or an

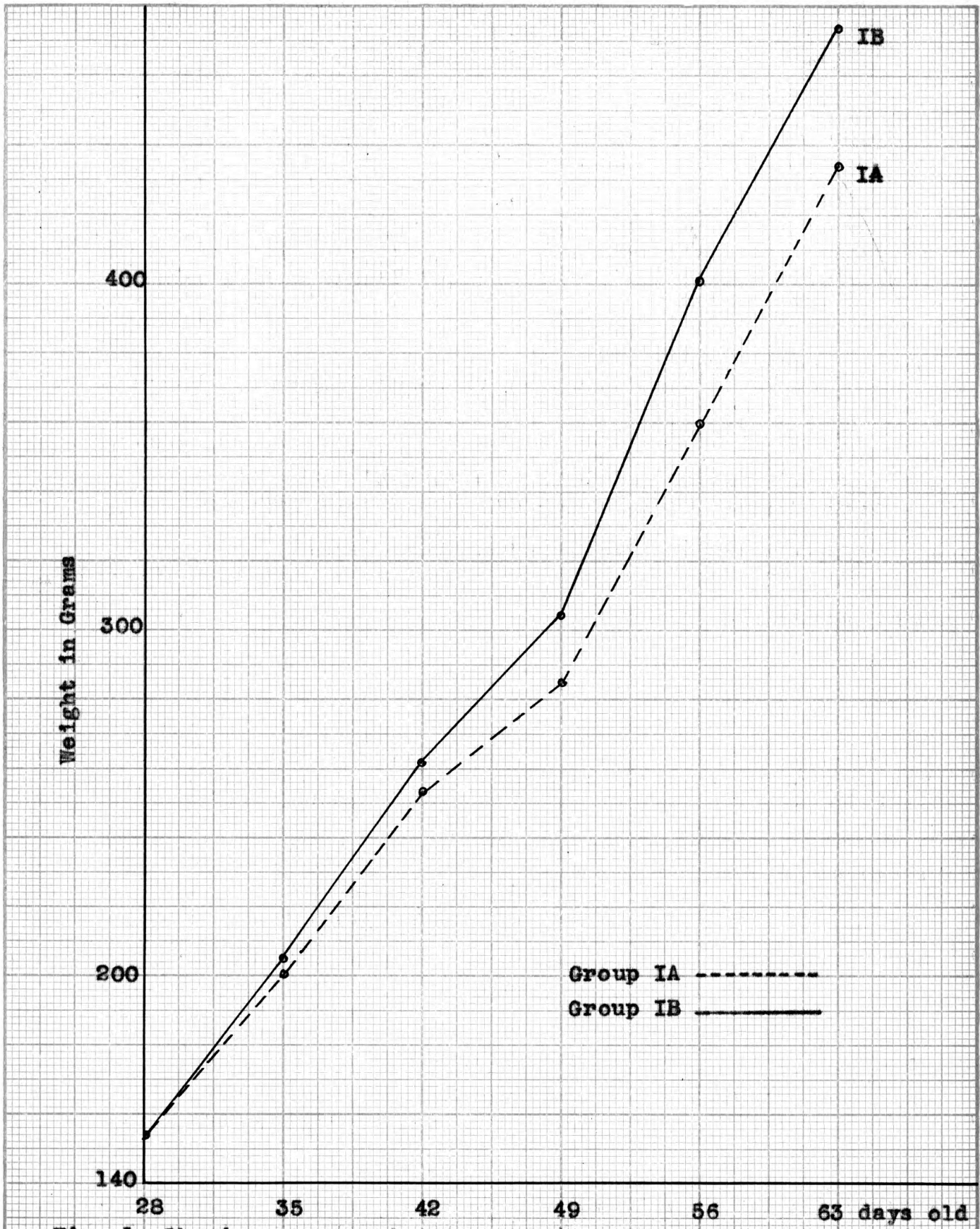


Fig. 1. Showing comparative growth rates of chickens in Lot IA (bled weekly) and Lot IB (not bled). Parasitized (500 A. lineata eggs) when 42 days old.

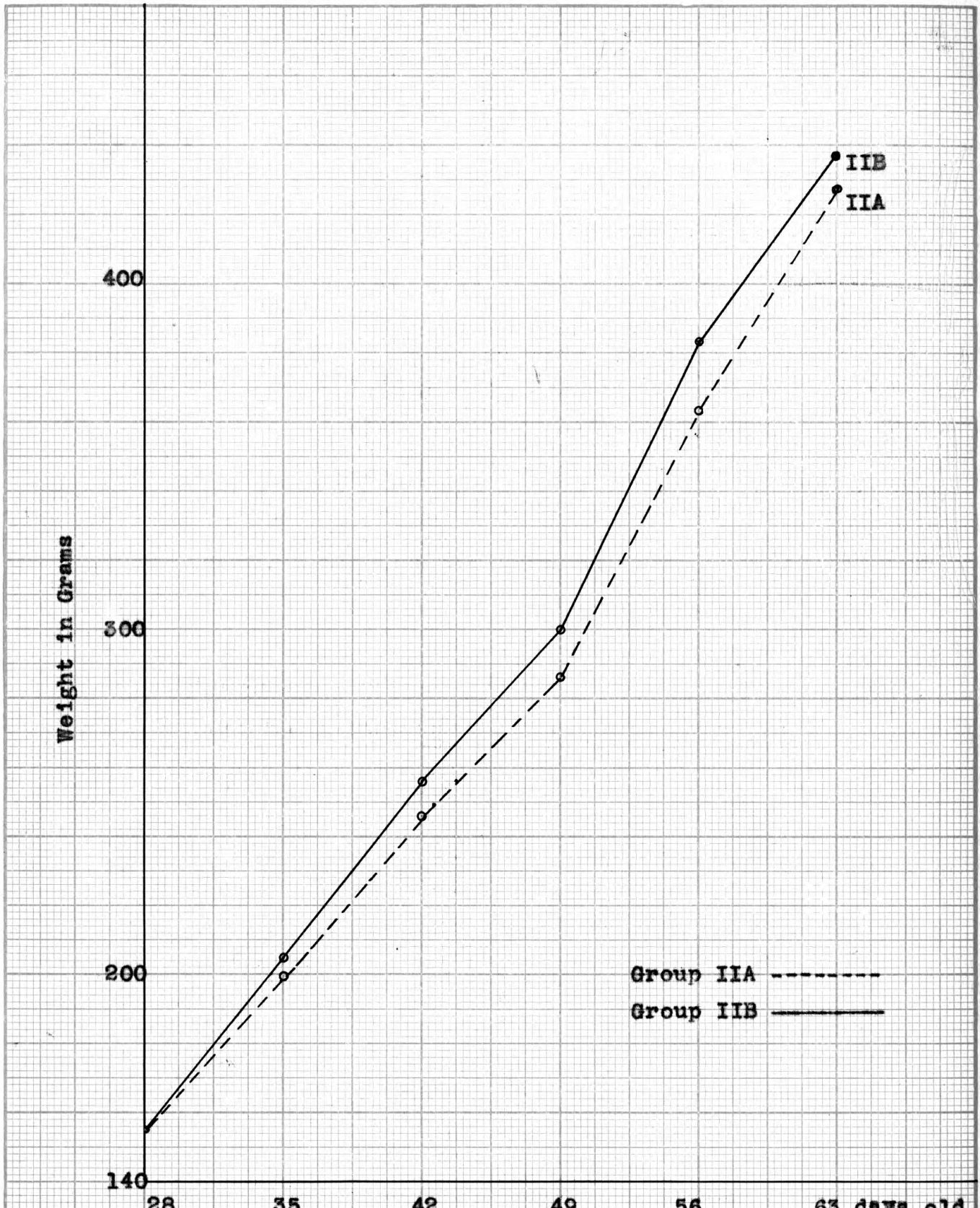


Fig. 2. Showing comparative growth rates of chickens in Lot IIA (bled weekly) and Lot IIB (not bled). Parasitized (300 A. lineata eggs) when 42 days old.

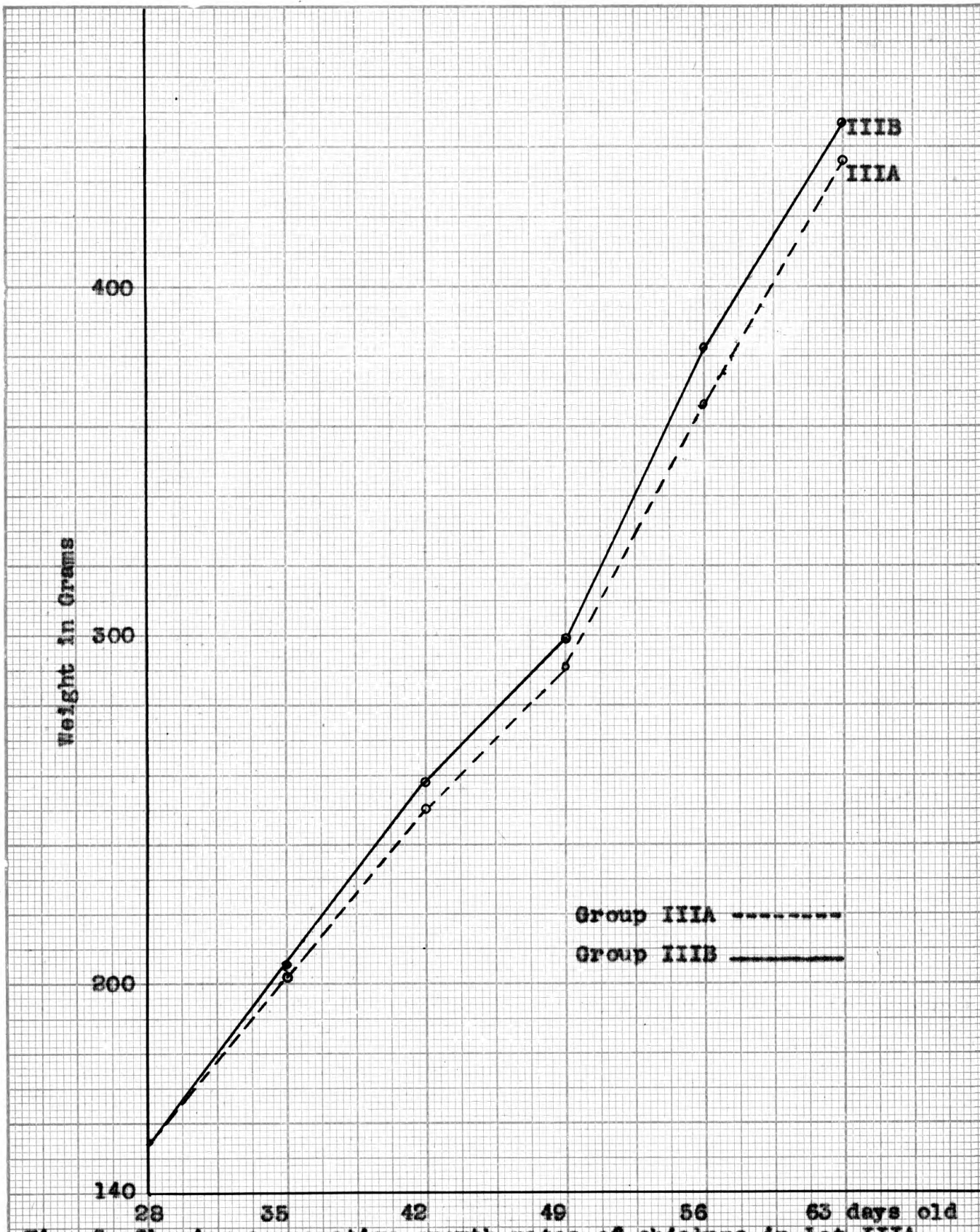


Fig. 3. Showing comparative growth rates of chickens in Lot IIIA (bled weekly) and Lot IIIB (not bled). Parasitized (100 A. lineata eggs) when 42 days old.

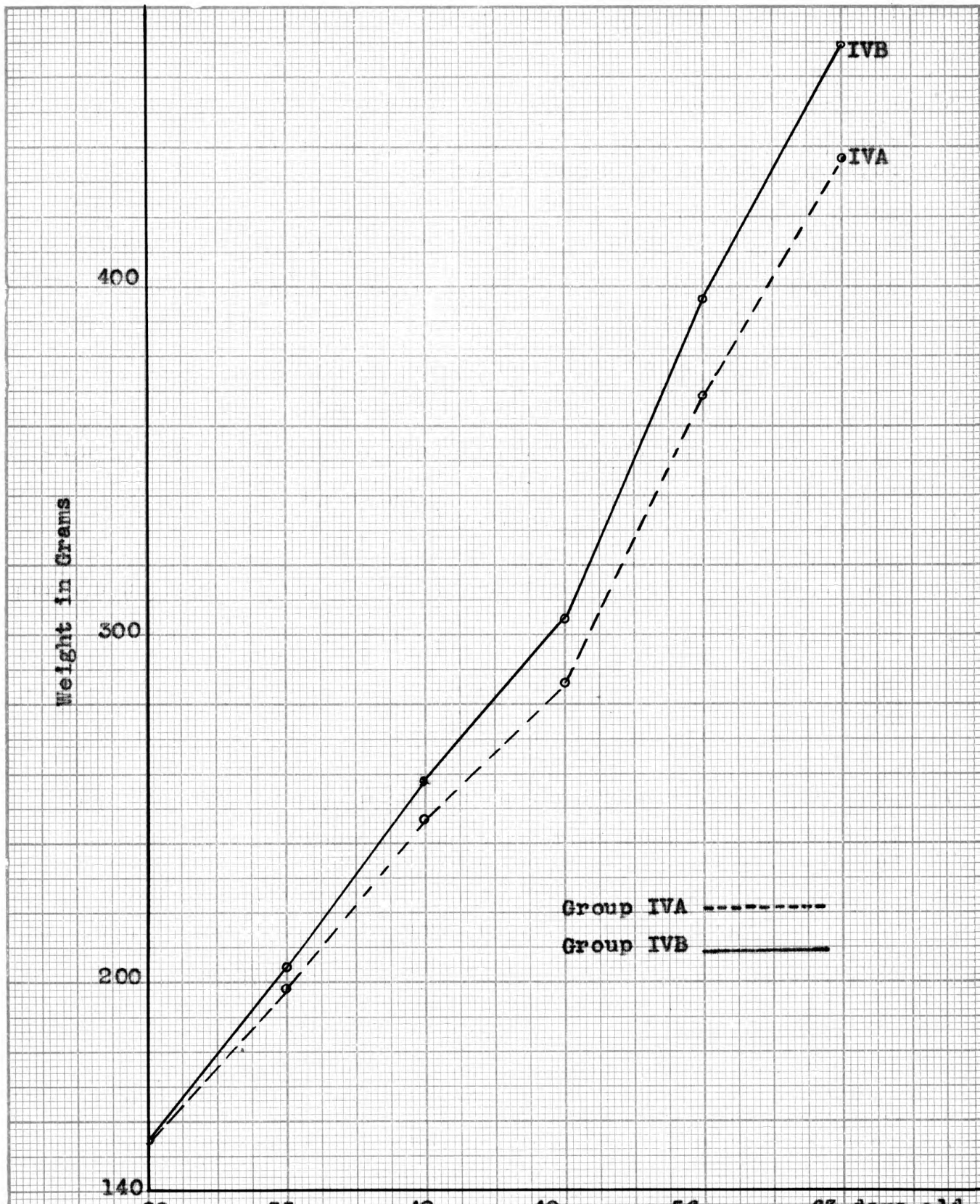


Fig. 4. Showing comparative growth rates of chickens in Lot IVA (bled weekly) and Lot IVB (not bled). Parasitized (50 A. lineata eggs) when 42 days old.

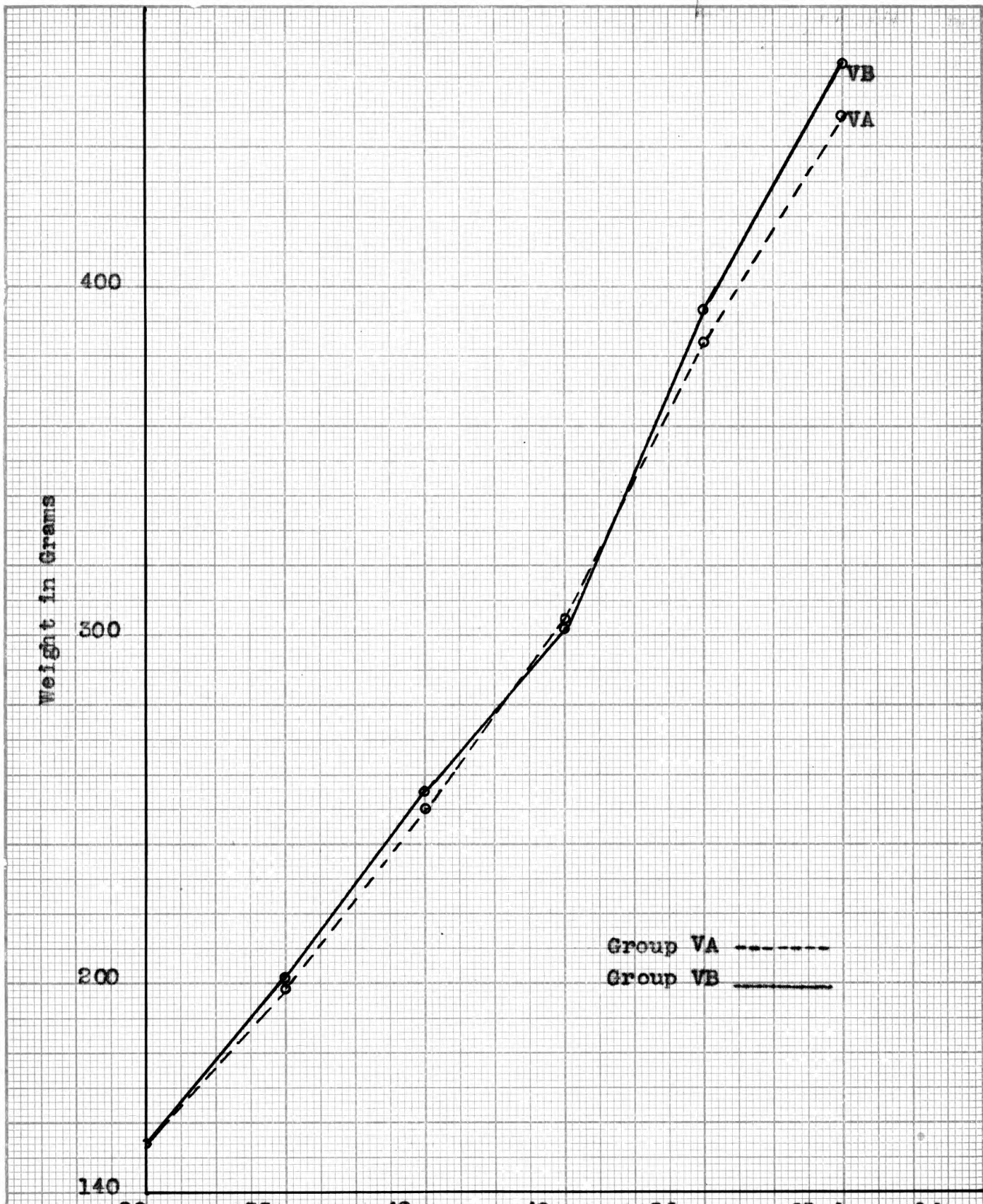


Fig. 5. Showing comparative growth rates of chickens in Lot VA (bled weekly) and Lot VB (not bled). Parasitized (25 A. lineata eggs) when 42 days old.

Table 1. Showing relation of dose size of Ascaridia lineata eggs to size of infestation and percentage of survival of A. lineata in chickens. Lots A, bled; Lots B, unbled

| Lot | Number of chickens | Size of Egg Dose | Range of variability in numbers of A. lineata | Mean number of A. lineata | Error of Mean | Standard deviation | Percentage of survival of A. lineata |
|---|---|---|---|---|---|---|---|
| | | | Experiment 1 | | | | |
| IA | 14 | 500 | 0-42 | 9.50 | 1.84 | 10.23 | 1.9 |
| IIA | 13 | 300 | 0-48 | 11.31 | 2.89 | 15.44 | 3.8 |
| IIIA | 11 | 100 | 0-24 | 8.91 | 1.73 | 8.51 | 8.9 |
| IVA | 13 | 50 | 0-12 | 4.62 | 0.68 | 3.63 | 9.2 |
| VA | 13 | 25 | 0-12 | 4.00 | 0.72 | 3.82 | 16.0 |
| IB | 14 | 500 | 0-27 | 7.43 | 1.51 | 8.35 | 1.5 |
| IIB | 15 | 300 | 0-18 | 4.13 | 0.82 | 4.72 | 1.4 |
| IIIB | 13 | 100 | 0-28 | 8.15 | 1.46 | 7.80 | 8.2 |
| IVB | 14 | 50 | 0-32 | 8.79 | 1.68 | 9.31 | 17.6 |
| VB | 14 | 25 | 0-14 | 4.43 | 0.78 | 4.35 | 17.7 |
| | | | Experiment 2 | | | | |
| VIA | 15 | 500 | 0-39 | 6.60 | 1.66 | 9.58 | 1.3 |
| VIIA | 13 | 300 | 0-29 | 7.46 | 1.81 | 9.69 | 2.5 |
| VIIIA | 15 | 100 | 0- 7 | 2.46 | 0.35 | 2.03 | 2.5 |
| IXA | 15 | 50 | 0- 7 | 3.07 | 0.14 | 2.32 | 6.0 |
| XA | 15 | 25 | 0- 8 | 1.60 | 0.39 | 2.24 | 6.0 |
| VIB | 15 | 500 | 0-11 | 3.00 | 0.59 | 3.42 | 0.6 |
| VIIB | 15 | 300 | 0-13 | 4.73 | 0.84 | 4.84 | 1.6 |
| VIIIB | 15 | 100 | 0-15 | 4.30 | 0.84 | 4.85 | 4.3 |
| IXB | 15 | 50 | 0-13 | 3.60 | 0.65 | 3.63 | 7.4 |
| XB | 15 | 25 | 0- 3 | 0.06 | 0.21 | 1.19 | 2.6 |
| | | | Experiment 3 | | | | |
| XIA | 14 | 500 | 0-18 | 5.21 | 0.94 | 5.24 | 1.1 |
| XIIA | 15 | 300 | 0-16 | 5.26 | 0.69 | 3.98 | 1.8 |
| XIB | 15 | 500 | 0-16 | 5.53 | 0.76 | 4.39 | 1.1 |
| XIIB | 14 | 300 | 0-31 | 5.85 | 1.42 | 7.86 | 1.9 |

Table II. Showing relation of dose size of Ascaridia lineata eggs to rate of growth (length) of A. lineata in chickens. Lots A, bled; Lots b, unbled

| Lot | Number of Chickens | Size of Egg Dose | Range of variability in numbers of A. lineata | Mean number of A. lineata in mm. | Error of Mean | Standard deviation |
|---|---|---|---|---|---|---|
| | | | Experiment 1 | | | |
| IA | 14 | 500 | 1.8 - 35.2 | 18.88 | 0.47 | 7.97 |
| IIA | 13 | 300 | 3.0 - 41.8 | 23.14 | 0.55 | 9.92 |
| IIIA | 11 | 100 | 2.9 - 39.3 | 21.73 | 0.56 | 8.25 |
| IVA | 13 | 50 | 5.3 - 34.5 | 25.00 | 0.52 | 6.00 |
| VA | 13 | 25 | 5.7 - 44.0 | 25.45 | 0.59 | 6.36 |
| IB | 14 | 500 | 2.2 - 30.3 | 13.38 | 0.64 | 8.36 |
| IIB | 15 | 300 | 2.4 - 29.3 | 13.39 | 0.80 | 8.17 |
| IIIB | 13 | 100 | 3.3 - 36.0 | 20.91 | 0.72 | 10.92 |
| IVB | 14 | 50 | 4.2 - 40.6 | 24.77 | 0.39 | 6.46 |
| VB | 13 | 25 | 4.4 - 36.5 | 23.05 | 0.63 | 7.36 |
| | | | Experiment 2 | | | |
| VIA | 15 | 500 | 2.9 - 27.6 | 15.39 | 0.46 | 6.90 |
| VIIA | 13 | 300 | 3.0 - 33.8 | 16.72 | 0.46 | 6.73 |
| VIIIA | 15 | 100 | 3.7 - 36.8 | 17.62 | 0.96 | 8.74 |
| IXA | 15 | 50 | 6.0 - 31.8 | 18.14 | 0.68 | 6.88 |
| XA | 15 | 25 | 5.0 - 29.6 | 17.94 | 0.92 | 6.71 |
| VIB | 15 | 500 | 2.0 - 24.0 | 10.23 | 0.53 | 5.24 |
| VIIB | 15 | 300 | 3.8 - 30.0 | 17.11 | 0.56 | 7.06 |
| VIIIB | 15 | 100 | 2.3 - 26.8 | 13.10 | 0.59 | 7.12 |
| IXB | 15 | 50 | 5.3 - 23.5 | 15.56 | 0.48 | 5.27 |
| XB | 15 | 25 | 8.8 - 31.5 | 16.87 | 1.48 | 6.97 |
| | | | Experiment 3 | | | |
| XIA | 14 | 500 | 1.1 - 26.0 | 11.74 | 0.59 | 7.47 |
| XIIA | 15 | 300 | 2.7 - 26.6 | 13.53 | 0.53 | 7.04 |
| XIB | 15 | 500 | 1.2 - 29.9 | 10.22 | 0.59 | 8.05 |
| XIIB | 14 | 300 | 2.0 - 26.2 | 16.12 | 0.48 | 6.47 |

average of 7.43 per bird (Table I). The worms ranged in length from 2.2 to 30.3 mm., the average being 13.38 mm. (Table II).

Comparing the data for the two groups, Lot IA (bled) averaged 2.07 more worms per bird than did Lot IB (unbled). This difference was not significant. However, the mean lengths of the worms from Lot IA was 5.50 mm. greater than that of Lot IB. This difference was 6.93 times its probable error and is considered to be significant (Table III). In Lot IIA there were 13 chickens having a total of 147 worms or an average of 11.31 worms per chicken (Table I). The average length of the worms was 23.14 mm. (Table II). In its groupmate, Lot IIB there were 62 worms in 15 chickens or an average of 4.13 worms per chicken. The mean length of these worms was 13.39 mm. (Table II). Although there were more worms per bird in Lot IIA than in Lot IIB the difference is only 2.37 times its probable error, and therefore within the limits of experimental error. The worms in Lot IIA average 9.75 mm. longer than those in Lot IIB and as this difference is 10.01 times its probable error, the worms in Lot IIA are considered significantly longer (Table III).

The data for Lot IIIA show that there were 98 worms recovered from 11 individuals. The mean number for each chick was 8.91 (Table I), and they ranged in size from 2.9 to 39.3

Table III. Biometrical comparison of mean numbers and lengths of Ascaridia lineata resulting from egg doses of various sizes fed to the chickens in the A and B lots in Experiments 1, 2 and 3

| Lot | Size of Egg Dose | Difference of Means (= D) of A lineata | | Probable Error of Difference (= E) | Ratio, $\frac{D}{E}$ | Group Having Largest Mean |
|---|---|---|---|---|---|---|
| **Experiment 1** | | | | | | |
| IA vs: IB | 500 | Numbers | 2.07 | 2.32 | 0.87 | IA |
| | 500 | Lengths | 5.50 | 0.79 | 6.93 | IA |
| IIA vs: IIB | 300 | Numbers | 7.17 | 3.03 | 2.37 | IIA |
| | 300 | Lengths | 9.75 | 0.79 | 10.01 | IIA |
| IIIA vs: IIIB | 100 | Numbers | 0.76 | 2.26 | 0.33 | IIIA |
| | 100 | Lengths | 0.82 | 0.91 | 0.90 | IIIA |
| IVA vs: IVB | 50 | Numbers | 4.17 | 1.81 | 2.31 | IVB |
| | 50 | Lengths | 0.24 | 0.65 | 0.36 | IVA |
| VA vs: VB | 25 | Numbers | 0.43 | 1.05 | 0.41 | VB |
| | 25 | Lengths | 2.39 | 0.87 | 2.75 | IVB |
| **Experiment 2** | | | | | | |
| VIA vs: VIB | 500 | Numbers | 3.60 | 1.77 | 2.03 | VIA |
| | 500 | Lengths | 5.15 | 0.70 | 7.31 | VIA |
| VIIA vs: VIIB | 300 | Numbers | 2.73 | 1.19 | 1.36 | VIIA |
| | 300 | Lengths | 0.40 | 0.73 | 0.54 | VIIB |
| VIIIA vs: VIIIB | 100 | Numbers | 1.83 | 0.92 | 2.00 | VIIIB |
| | 100 | Lengths | 4.48 | 1.13 | 3.94 | VIIIA |
| IXA vs: IXB | 50 | Numbers | 0.54 | 0.65 | 0.84 | IXB |
| | 50 | Lengths | 2.58 | 0.84 | 3.08 | IXA |
| XA vs: XB | 25 | Numbers | 1.53 | 0.44 | 3.46 | XA |
| | 25 | Lengths | 1.07 | 1.75 | 0.61 | XA |
| **Experiment 3** | | | | | | |
| [illegible] | [illegible] | [illegible] | [illegible] | 1.22 | 0.26 | XIB |
| | 300 | Lengths | 1.52 | 0.84 | 1.82 | XIA |
| XIIA vs: XIIB | 300 | Numbers | 0.59 | 1.58 | 0.37 | XIIB |
| | 300 | Lengths | 2.58 | 0.72 | 3.59 | XIIB |

mm. The mean length of the nematodes was 21.73 mm. (Table II). In Lot IIIB, 106 worms were recovered from 13 birds, giving an average per chicken of 8.15 worms. The average length of the worms which ranged from 3.3 to 36.0 mm. long was 20.91 mm. Comparing these two lots it was found that the number of worms in Lot IIIA was not significantly greater than in Lot IIIB. The mean length of the worms per bird in Lot IIIA was 0.82 mm. longer than in Lot IIIB. As this difference is only 0.90 times its probable error it is not significant (Table III).

In Lot IVA the 13 chickens yielded 60 worms or a mean of 4.62 worms per individual. These ranged in length from 5.3 to 34.5 mm., the average length being 25.00 mm. per worm. The average number of worms in Lot IVB which was the control group run with Lot IVA was 8.79 worms for each chick. These worms varied from 4.2 to 40.6 mm. in length and averaged 24.77 mm. each. The difference of the mean numbers of worms in these two lots was 4.17 mm., which was 2.31 times its probable error (Table III). This difference approaches significance in favor of the IVB lot. The difference between the mean lengths of worms in the two groups was not significant (Table III).

The data on Lot VA and Lot VB showed that 52 worms were obtained from Lot VA containing 13 chickens or a mean number of four worms per chick (Table I). These worms

ranged from 5.7 to 44.0 mm. long with a mean length of 25.43 mm. (Table II). In the VB lot which had 13 experimental chickens 62 worms were recovered or an average of 4.43 worms per bird. These worms were from 4.4 to 36.5 mm. long and averaged 23.05 mm. in length.

The mean number of worms per chick in Lot VB was 0.43 larger than the mean number per chick in Lot VA. This difference was not significant. The mean length of the worms per chick in the VB group, however, was 2.39 mm. larger than that of the worms in VA. This difference is 2.75 times the probable error and therefore not significant (Table III).

A biometrical comparison of the mean numbers and lengths of A. lineata recovered from the A lots of all groups in this experiment is found in Table IV. When the A lots that were fed large numbers of eggs (500, 300 and 100 eggs) are compared with the A lots fed small numbers of eggs (50 and 25) the mean numbers of worms found in the lots fed large numbers of eggs were greater than the mean numbers of worms found in those fed small numbers of worm eggs. The differences approached significance. Examination of Table IV also shows that whenever two lots fed different numbers of worm eggs are compared, the lot fed the smaller number of eggs has worms whose mean length is larger than those fed

the larger number of eggs. The only exception to this is in the case of IIA vs IIIA (300 vs 100 eggs) where the mean length of the worms in the lot fed 300 eggs is larger than the mean length of those worms from the lot fed 100 eggs. The difference, 1.41, however, is only 1.81 times its probable error which is not significant (Table IV).

The instances in which the differences between the mean lengths of A. lineata in the lots under comparison are so large as to lie outside of the limits of experimental error obviously include Lots IA and IIA (500 vs 300 eggs), IA and IIIA (500 vs 100 eggs), IA and IIIA (500 vs 50 eggs), IA and VA (500 vs 25), IIIA and IVA (100 vs 50), and IIIA and VA (100 vs 25 eggs).

Likewise in a comparison of the mean lengths of worms in the B lots of all groups in this experiment it was found that the lots fed smaller numbers of eggs had longer worms when compared with lots fed larger doses of eggs. The only exception to this case was in Lot IVB (fed 50 eggs) where the mean length of the nematodes was 1.72 mm. larger than in Lot VB (fed 25 eggs). This difference was not significant (Table V). Table V also shows many cases in which the differences between the mean lengths of the worms in the B lots under comparison are significant. Such cases are Lots IB and IIIB (500 vs 100 eggs), IB and IVB (500 vs 50), IB and VB (500 vs 25), IIB and IIIB (300 vs 100), IIB and IVB

Table IV. Biometrical comparison of mean numbers and lengths of A. lineata resulting from egg doses of various sizes fed to the chickens in the A lots in Experiments 1, 2 and 3

| Lot | Size of Egg Dose | Difference of Means (= D) of A lineata | Probable Error of Difference (= E) | Ratio, $\frac{D}{E}$ | Group having largest mean |
|---|---|---|---|---|---|
| **Experiment 1** | | | | | |
| IA vs: IIA | 500 : 300 | Numbers 1.81 : Lengths 4.26 | 3.43 : 0.72 | 0.53 : 5.92 | IIA : IIA |
| IA vs: IIIA | 500 : 100 | Numbers 0.59 : Lengths 2.85 | 2.53 : 0.73 | 0.23 : 3.90 | IA : IIIA |
| IA vs: IVA | 500 : 50 | Numbers 4.88 : Lengths 6.12 | 1.96 : 0.70 | 2.49 : 8.74 | IA : IVA |
| IA vs: VA | 500 : 25 | Numbers 5.50 : Lengths 6.55 | 1.98 : 0.75 | 2.78 : 8.73 | IA : VA |
| IIA vs: IIIA | 300 : 100 | Numbers 2.40 : Lengths 1.41 | 3.37 : 0.78 | 0.71 : 1.81 | IIA : IIA |
| IIA vs: IVA | 300 : 50 | Numbers 6.69 : Lengths 1.86 | 2.97 : 0.76 | 2.25 : 2.45 | IIA : IVA |
| IIA vs: VA | 300 : 25 | Numbers 7.31 : Lengths 2.29 | 2.98 : 0.81 | 2.45 : 2.83 | IIA : VA |
| IIIA vs: IVA | 100 : 50 | Numbers 4.29 : Lengths 3.27 | 1.86 : 0.76 | 2.31 : 4.30 | IIIA : IVA |
| IIIA vs: VA | 100 : 25 | Numbers 4.91 : Lengths 3.70 | 1.87 : 0.81 | 2.63 : 4.57 | IIIA : VA |
| IVA vs: VA | 50 : 25 | Numbers 0.62 : Lengths 0.43 | 0.99 : 0.79 | 0.63 : 0.54 | IVA : VA |
| **Experiment 2** | | | | | |
| VIA vs: VIIA | 500 : 300 | Numbers 8.62 : Lengths 1.33 | 2.46 : 0.66 | 0.35 : 2.02 | VIIA : VIIA |
| VIA vs: VIIIA | 500 : 100 | Numbers 4.13 : Lengths 2.24 | 1.70 : 1.08 | 2.42 : 2.07 | VIA : VIIIA |
| VIA vs: IXA | 500 : 50 | Numbers 3.53 : Lengths 2.76 | 1.67 : 0.83 | 2.11 : 3.33 | VIA : IXA |
| VIA vs: XA | 500 : 25 | Numbers 5.00 : Lengths 2.56 | 1.71 : 1.04 | 2.92 : 2.47 | VIA : XA |
| VIIA vs: VIIIA | 300 : 100 | Numbers 4.99 : Lengths 0.91 | 1.85 : 1.07 | 2.70 : 0.84 | VIIA : VIIIA |
| IIA vs: IVA | 300 : 50 | Numbers 6.69 : Lengths 1.86 | 2.97 : 0.76 | 2.25 : 2.45 | IIA : IVA |
| VIIA vs: XA | 300 : 25 | Numbers 5.86 : Lengths 1.23 | 1.85 : 1.03 | 3.16 : 1.19 | VIIA : XA |
| VIIIA vs: IXA | 100 : 50 | Numbers 0.60 : Lengths 0.52 | 0.38 : 1.19 | 1.56 : 0.44 | IXA : IXA |
| VIIIA vs: XA | 100 : 25 | Numbers 0.87 : Lengths 0.32 | 0.53 : 1.34 | 1.64 : 0.24 | VIIIA : XA |
| IXA vs: XA | 50 : 25 | Numbers 1.46 : Lengths 0.20 | 0.42 : 1.15 | 3.51 : 0.18 | IXA : IXA |
| **Experiment 3** | | | | | |
| XIA vs: XIIA | 500 : 300 | Numbers 0.05 : Lengths 1.79 | 1.17 : 0.79 | 0.04 : 2.25 | XIIA : XIIA |

Table V. Biometrical comparison of mean numbers and lengths of A. lineata resulting from egg doses of various sizes fed to the chickens in the B lots in Experiments 1, 2 and 3

| Lot | Egg Dose | A. lineata | Difference of Means (= D) of A. lineata | Probable Error of Difference (= E) | Ratio, $\frac{D}{E}$ | Group Having Largest Mean |
|---|---|---|---|---|---|---|
| **Experiment 1** | | | | | | |
| IB vs: IIB | 500 : 300 | Numbers | 3.30 | 1.72 | 1.91 | IB |
| | | Lengths | 0.01 | 1.02 | 0.01 | IIB |
| IB vs: IIIB | 500 : 100 | Numbers | 0.72 | 2.10 | 0.34 | IIIB |
| | | Lengths | 7.53 | 0.96 | 7.84 | IIIB |
| IB vs: IVB | 500 : 50 | Numbers | 1.36 | 2.26 | 0.60 | IVB |
| | | Lengths | 11.39 | 0.75 | 15.19 | IVB |
| IB vs: VB | 500 : 25 | Numbers | 3.00 | 1.70 | 1.76 | IB |
| | | Lengths | 9.67 | 0.90 | 10.74 | VB |
| IIB vs: IIIB | 300 : 100 | Numbers | 4.02 | 1.67 | 2.41 | IIIB |
| | | Lengths | 7.52 | 1.08 | 6.96 | IIIB |
| IIB vs: IVB | 300 : 50 | Numbers | 4.66 | 1.87 | 2.49 | IVB |
| | | Lengths | 11.38 | 0.89 | 12.79 | IVB |
| IIB vs: VB | 300 : 25 | Numbers | 0.30 | 1.13 | 0.27 | VB |
| | | Lengths | 9.66 | 1.02 | 9.47 | VB |
| IIIB vs: IVB | 100 : 50 | Numbers | 0.64 | 2.23 | 0.29 | IVB |
| | | Lengths | 3.86 | 0.82 | 4.71 | IVB |
| IIIB vs: VB | 100 : 25 | Numbers | 3.72 | 1.66 | 2.24 | IIIB |
| | | Lengths | 2.14 | 0.96 | 2.23 | VB |
| IVB vs: -VB | 50 : 25 | Numbers | 4.36 | 1.85 | 2.36 | IVB |
| | | Lengths | 1.72 | 0.74 | 2.32 | IVB |
| **Experiment 2** | | | | | | |
| VIB vs: VIIB | 500 : 300 | Numbers | 1.73 | 1.08 | 1.67 | VIIB |
| | | Lengths | 6.88 | 0.77 | 8.90 | VIIB |
| VIB vs: VIIIB | 500 : 100 | Numbers | 1.30 | 1.03 | 1.26 | VIIIB |
| | | Lengths | 2.87 | 0.79 | 3.61 | VIIIB |
| VIB vs: IXB | 500 : 50 | Numbers | 0.60 | 0.87 | 0.69 | IXB |
| | | Lengths | 5.33 | 0.72 | 7.44 | IXB |
| VIB vs: XB | 500 : 25 | Numbers | 2.93 | 0.63 | 4.64 | VIB |
| | | Lengths | 6.64 | 1.58 | 4.21 | XB |
| VIIB vs: VIIIB | 300 : 100 | Numbers | 0.43 | 1.19 | 0.36 | VIIB |
| | | Lengths | 4.01 | 0.82 | 4.88 | VIIB |
| VIIB vs: IXB | 300 : 50 | Numbers | 1.13 | 1.05 | 1.07 | VIIB |
| | | Lengths | 1.54 | 0.74 | 2.08 | VIIB |
| VIIB vs: XB | 300 : 25 | Numbers | 4.66 | 0.87 | 5.37 | VIIB |
| | | Lengths | 0.24 | 1.59 | 0.15 | VIIB |
| VIIIB vs: IXB | 100 : 50 | Numbers | 0.70 | 1.06 | 0.66 | VIIIB |
| | | Lengths | 1.46 | 0.76 | 1.90 | IXB |
| VIIIB vs: XB | 100 : 25 | Numbers | 4.23 | 0.87 | 4.86 | VIIIB |
| | | Lengths | 3.76 | 1.60 | 2.35 | XB |
| IXB vs: XB | 50 : 25 | Numbers | 3.53 | 0.66 | 5.31 | IXB |
| | | Lengths | 1.31 | 1.56 | 0.83 | XB |
| **Experiment 3** | | | | | | |
| XIB vs: XIIB | 500 : 300 | Numbers | 0.32 | 1.16 | 0.27 | XIIB |
| | | Lengths | 5.90 | 0.76 | 7.69 | XIIB |

(300 vs 50), IIB and VB (300 vs 25), IIIB and IVB (100 eggs vs 25 eggs).

Contrary to the results from the A lots, a comparison of mean numbers in the B lots shows that the only cases where larger mean numbers of worms are found in the lots fed large number of eggs are in the following: Lot IB (500 eggs) vs Lot VB (25 eggs) and Lot IIIB (100 eggs) vs Lot VB (25 eggs). Moreover the differences in these cases were not significant (Table V).

It is seen in Table I that from feeding 500 eggs to each chicken in Lot IA and Lot IB the percentages of survival were 1.9 and 1.5, respectively; from feeding 300 eggs to Lot IIA and Lot IIB the percentages were 3.8 and 1.4; from giving 100 eggs (Lots IIIA and IIIB) the percentages of survival were 8.9 and 8.2; from administering 50 eggs (Lots IVA and IVB) the percentages were 9.2 and 17.6; from feeding 25 eggs to each bird in Lot VA and Lot VB the percentages of survival of nematodes were 16.0 and 17.7, respectively.

Experiment II. A second experiment was conducted in the same manner as the first. One hundred fifty chickens four weeks old were divided into five groups of thirty chicks and then subdivided into two lots of fifteen chickens each. The B lot of each group again served as controls while each bird in Lot A was bled every week as described.

When six weeks of age the chickens were parasitized with embryonated eggs of Ascaridia lineata as follows: 500 eggs to Group VI, Lot A (VIA bled) and Group VI, Lot B (VIB unbled); 300 to Group VII, Lots A and B; 100 to Group VIII, Lots A and B; 50 to Group IX, Lots A and B; and 25 eggs to each bird of Group X, Lots A and B. As in Experiment I the growth curves (Figures 6 to 10) show the retarding effect of the periodic bleeding upon the gain of the chicks in the A lots. After three weeks of parasitism the chickens were killed and the worms collected and measured. During the course of the experiment only two chickens died. Although this hatch of chickens weighed less than those of the hatch in the previous experiment, they appeared as healthy and active.

Study of the data from Lot VIA showed that 15 chickens harbored 99 worms or an average of 6.60 worms per bird (Table I). These worms ranged in size from 2.9 to 27.6 mm.; the mean length being 15.39 mm. (Table II). In the control lot (Lot VIB) which likewise had 15 chicks, 45 worms were found or an average of 3 worms per bird (Table I). These nematodes ranged in length from 2.0 to 24.0 mm., the average being 10.23 mm. (Table II).

By comparing the data of the two groups biometrically, it was seen that Lot VIA (bled) averaged 3.60 more worms

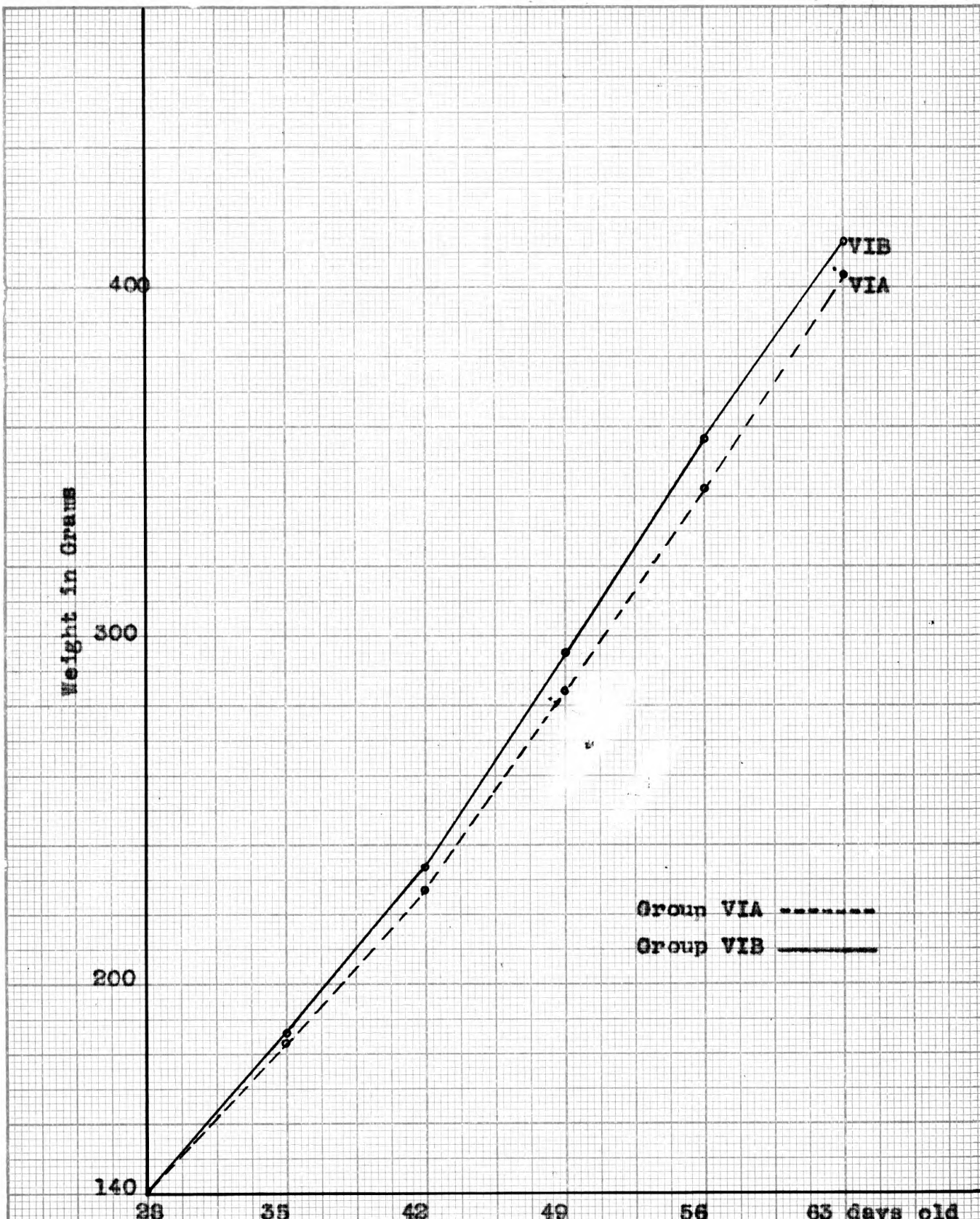


Fig. 6. Showing comparative growth rates of chickens in Lot VIA (bled weekly) and Lot VIB (not bled). Parasitized (500 A. lineata eggs) when 42 days old.

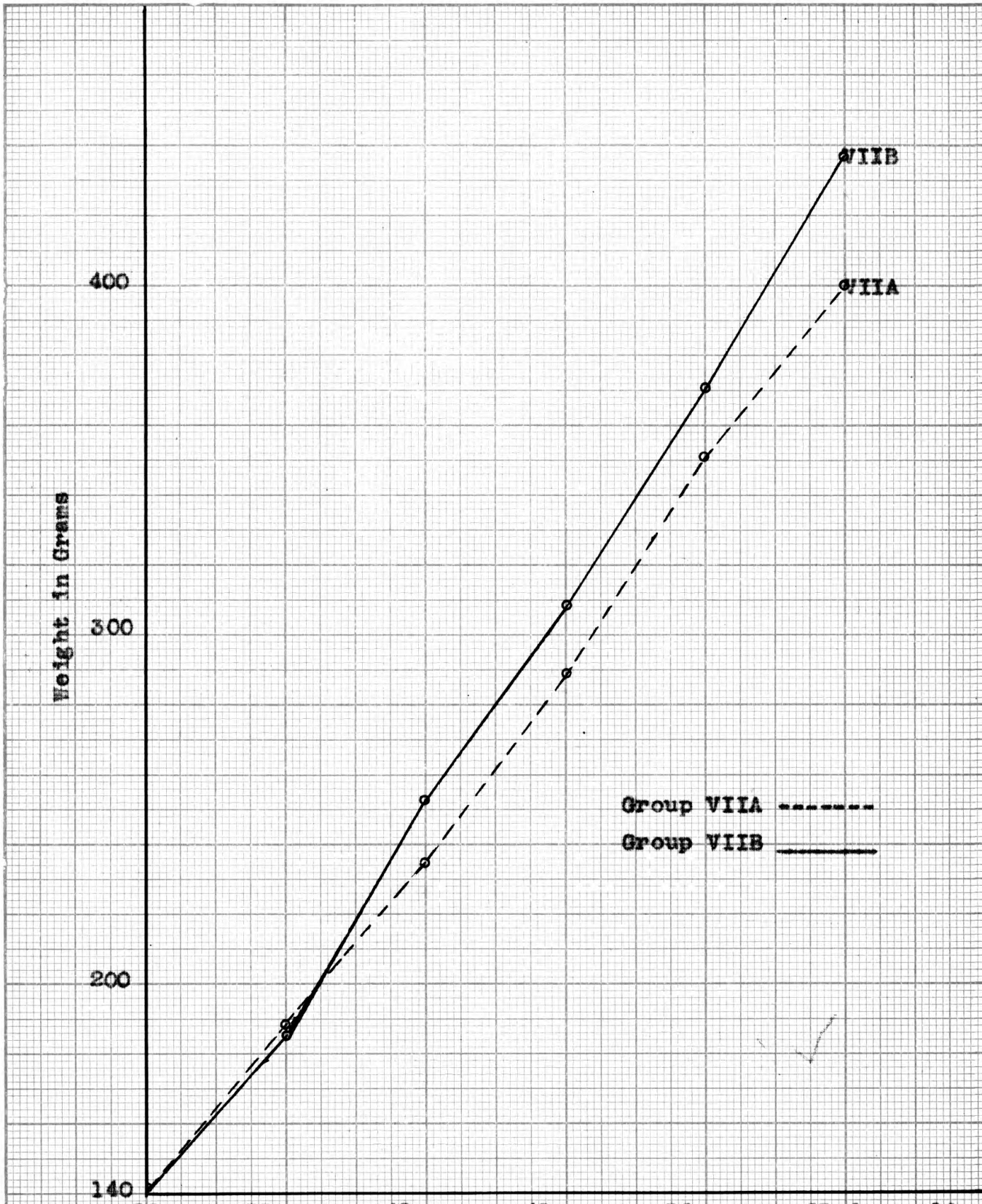


Fig. 7. Showing comparative growth rates of chickens in Lot VIIA (bled weekly) and Lot VIIB (not bled). Parasitized (300 A. lineata eggs) when 42 days old.

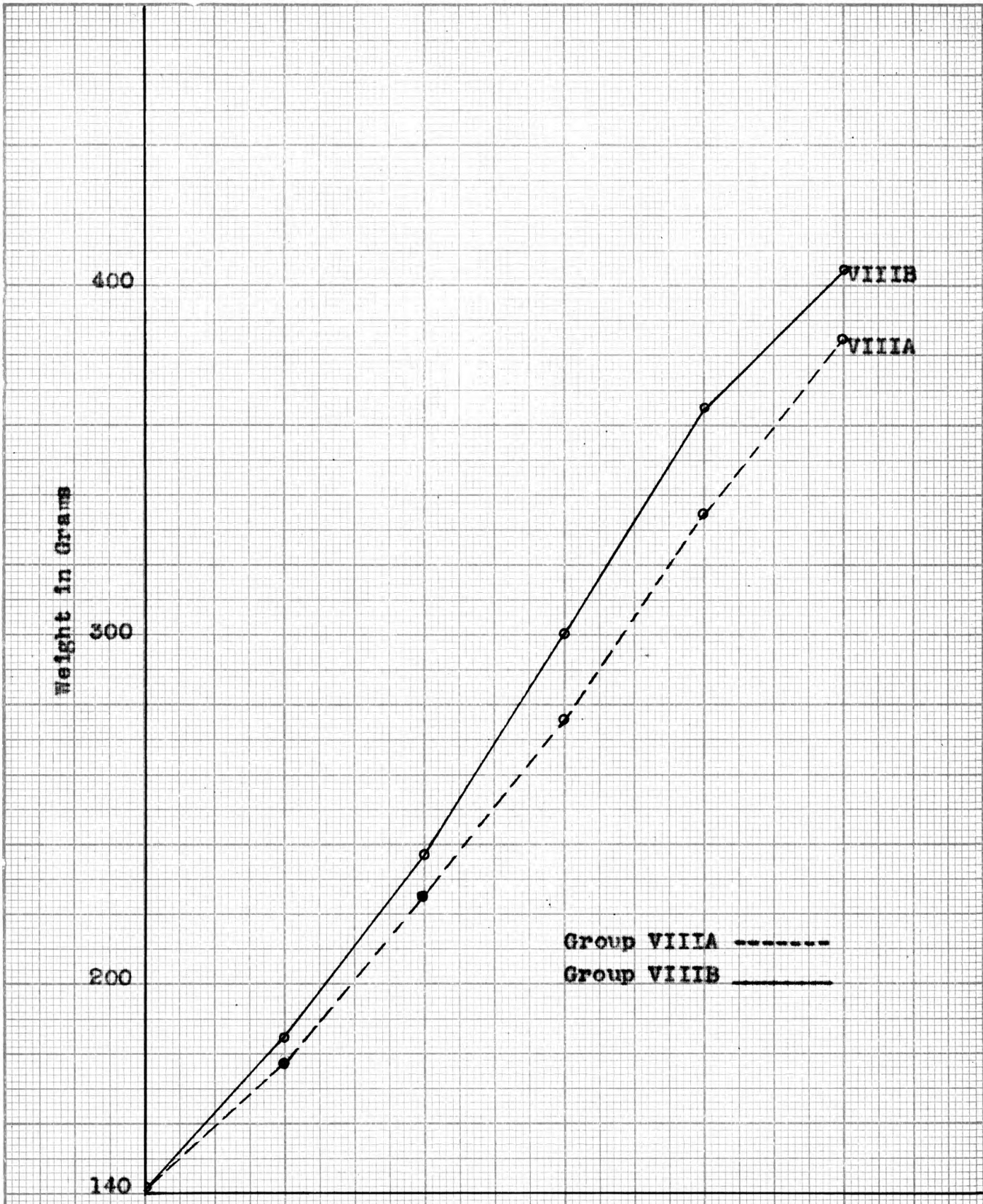


Fig. 8. Showing comparative growth rates of chickens in Lot VIIIA (bled weekly) and Lot VIIIB (not bled). Parasitized (100 *A. lineata* eggs) when 42 days old.

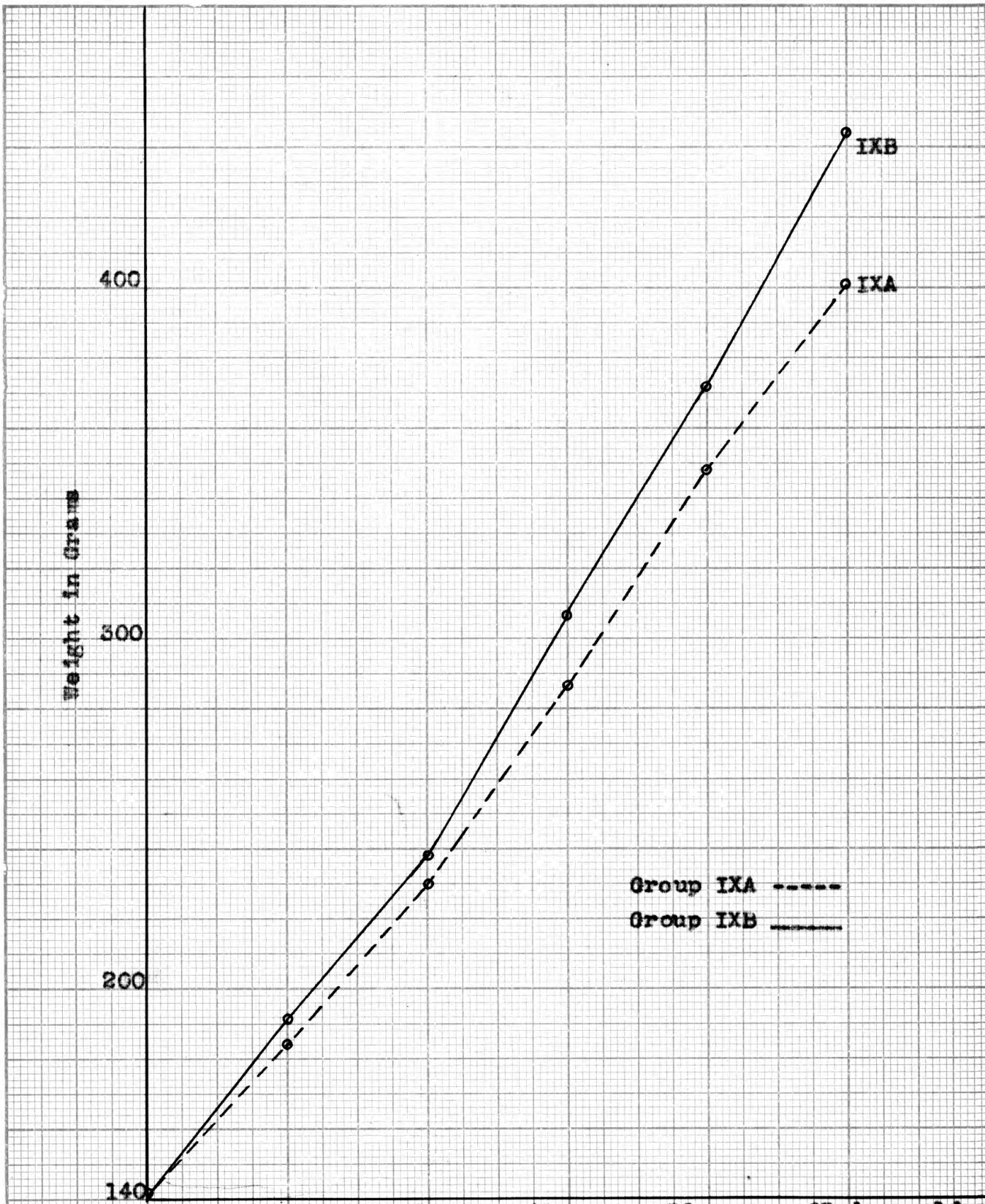


Fig. 9. Showing comparative growth rates of chickens in Lot IXA (bled weekly) and Lot IXB (not bled). Parasitized (50 A. lineata eggs) when 42 days old.

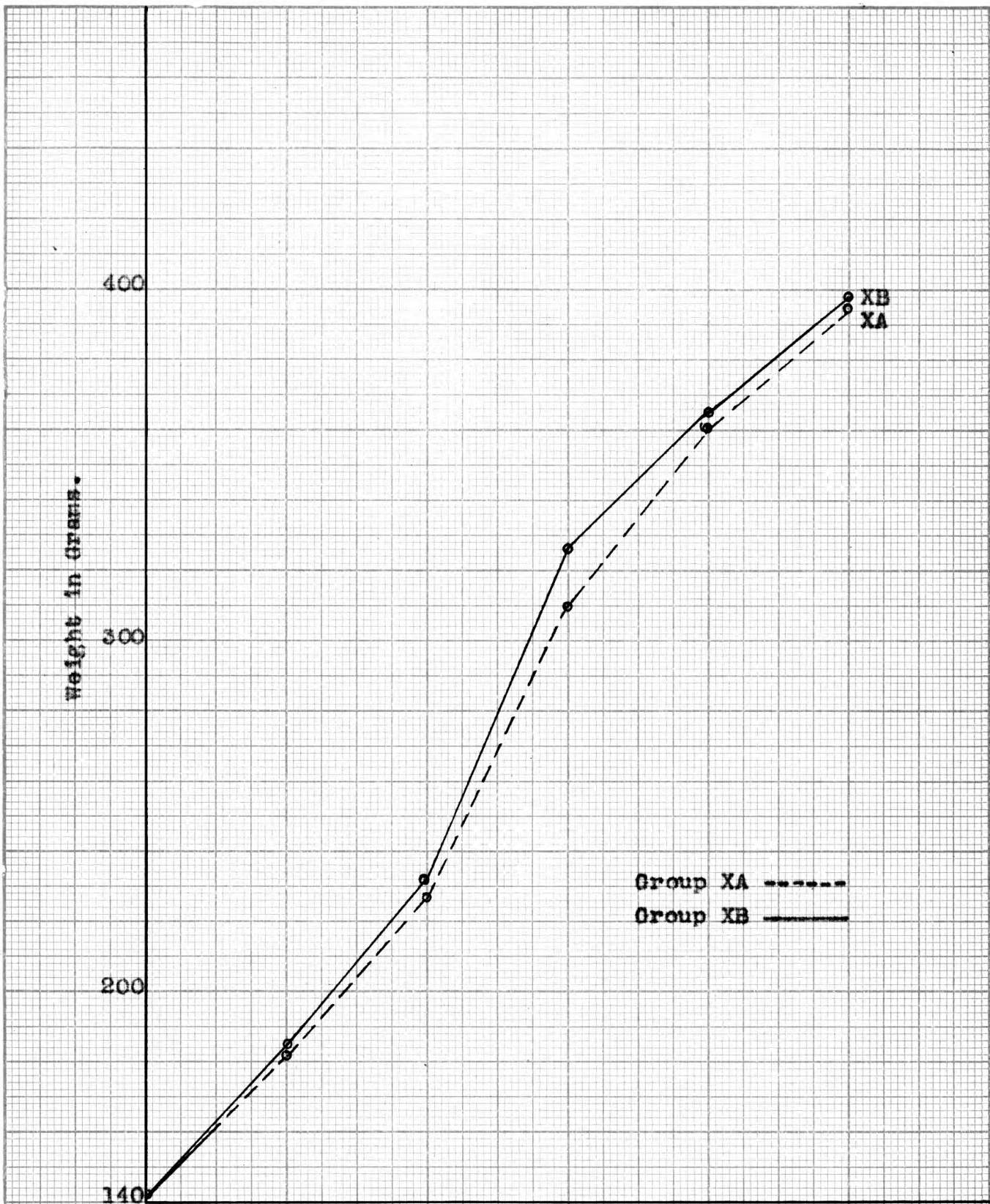


Fig. 10. Showing comparative growth rates of chickens in Lot XA (bled weekly) and Lot XB (not bled). Parasitized (28 A. lineata eggs when 42 days old.

per bird than did Lot VIB (control). This difference was 2.03 times its probable error which was not considered significant. But the mean lengths of the worms from Lot VIA was 5.15 mm. greater than that of Lot VIB. This difference was 7.31 times its probable error and is considered to be significant (Table III).

In Lot VIIA there were only 13 chickens having a total of 97 worms or an average of 7.46 worms per bird (Table I). The average length of these worms was 16.72 mm. (Table II). In its control lot, Lot VIIB, there were only 71 worms recovered from 15 chickens or an average of 4.73 worms per chicken. The mean length of these worms was 17.11 mm. (Table II). The difference in the mean numbers of worms found in Lot VIIA (bled) and Lot VIIB (control) is only 1.36 times its probable error and therefore is not significant. The worms in Lot VIIB, however, average 0.40 mm. longer than those in Lot VIIA. This difference however obviously lies well within the limits of experimental error (Table III)

In Lot VIIIA the 15 chickens yielded 37 worms or a mean number of 2.46 worms per chicken (Table I); they ranged in size from 3.7 to 36.8 mm. The mean length was 17.62 mm. per chick (Table II). In its groupmate, Lot VIIIB, where 65 worms were recovered from 15 individuals giving an average per bird of 4.30 worms, the average length of the worms which ranged in length from 2.3 to 26.8 mm. was 13.10 mm.

Contrary to the result obtained in the lots fed 100 eggs in Experiment I, it was seen that there were more worms in Lot VIIIB than in Lot VIIIA. The difference, however, was not significant as it was only twice the probable error (Table III). The mean length of the worms per bird in Lot VIIIA was 4.48 mm. longer than in Lot VIIIB. This difference is 3.94 times its probable error and is considered significant.

The data for Lot IXA show that 46 worms were recovered from 15 individuals, or an average of 3.07 worms per chicken (Table I). These nematodes ranged in size from 6.0 to 31.8 mm., their mean length being 18.14 mm. (Table II). In Lot IXB where 54 worms were recovered from 15 birds (average of 3.60 worms per chicken) the mean length of the worms, which ranged in length from 5.3 to 23.5 mm. was 15.56 mm. (Table II). There was 0.54 more worm in Lot IXB than in Lot IXA, but this difference is within the limits of experimental error. The mean length of the nematodes in Lot IXA was 2.58 mm. longer than the mean length of those in Lot IXB; this difference is 3.08 times its probable error and therefore probably significant (Table III).

The data for Group X Lots A and B showed that 24 worms were obtained from Lot X A containing the usual 15 chickens or a mean number of 1.60 worms per chick (Table I). These

worms ranged from 5.0 to 29.6 mm. long with an average length of 17.94 mm. (Table II). In the XB lot which likewise had 15 chicks there were only 10 worms recovered or an average of 0.06 worms per chicken. These worms ranged from 8.8 to 31.5 mm. in size or an average length of 16.87 mm. (Table II). The mean number of worms per bird in the XA lot was 1.53 larger than the mean number per bird in Lot XB. This difference was 3.46 times its probable error and is considered significant (Table III). Although the worms in Lot XA averaged longer than the worms in Lot XB the difference was well within the limits of experimental error (Table III).

As in Experiment I when the A lots fed large numbers of eggs (500, 300, and 100 eggs) were compared with the A lots fed smaller doses of eggs (50 or 25) the mean numbers of worms in the former lots were larger. Those cases were VIA and IXA (500 vs 50 eggs); VIA and XA (500 vs 25); VIIA and IXA (300 vs 50); VIIA and XA (500 vs 25); VIIA and XA (100 vs 25). The exception to these cases was VIIIA and IXA (100 vs 50) where the mean number of worms in Lot IXA (50 eggs fed) was 0.60 larger than the mean number of nematodes in Lot XIIIA (100 eggs fed). The difference however is not significant (Table IV).

In the B lots the cases where larger mean numbers of worms were found in the lots fed large numbers of eggs were in the following: Lot VIB and IXB (500 vs 50 eggs): VIIB

and IXB (300 vs 50); and VIIIB and IXB (100 vs 50). In the following comparisons (Table V) differences in mean numbers of larvae occur that probably lie outside the limits of experimental error; VIB and XB (500 vs 25); VIIIB and XB (300 vs 25) and VIIIB and XB (100 vs 25).

A biometrical comparison of the mean lengths in the A lots fed variable doses of nematode eggs demonstrated that the largest mean length was found in the group fed the smaller number of eggs except in the case of Lot IXA and XA (50 vs 25 eggs). In this comparison as in Lot IIA and IIIA (Experiment I), the largest worms would hardly be expected in the lot fed the smaller dose because there is not enough difference in the size of the egg doses. The only lots under comparison that showed significant difference in mean length was Lot VIA and XA (500 vs 50 eggs).

In Lot VIIB (fed 300 eggs) the mean length of the worms was 17.11 mm. (Table II). This mean length was much larger than those of the worms in Lot VIIIB (100 eggs), Lot IXB (50 eggs), and Lot XB (25 eggs). Obviously when Lot VIIB was compared with these lots, results were obtained that would not check the comparisons of related B lots in Experiment I (Table V). Table V shows a significant difference in mean lengths of worms in the following cases: VIB and VIIB (500 vs 300 eggs); VIB and VIIIB (500 vs 100); VIB

and IXB (500 vs 50) and VIB and XB (500 vs 25 eggs).

Percentages of survival of larvae of 1.3 and 0.6 are obtained in Lots VIA and VIB, each chick fed 500 eggs; 2.5 and 1.6 from Lots VIIA and VIIB (fed 300 eggs); 2.5 and 4.3 per cent from those lots in which each chick was fed 100 eggs (Lots VIIIA and VIIIB). When 50 eggs were administered to each chick in Lots IXA and IXB the percentages of survival were 6.0 and 7.4 but where 25 eggs were given each bird in Lots XA and XB 6.0 per cent of the larvae survived in Lot XA but only 2.6 per cent in Lot XB. The cause for this decrease in survival of larvae between Lots IXB fed 50 eggs and Lot XB fed 25 eggs, where in all other similar cases of comparison there had been an increase in percentage of survival, has not been explained.

Experiment III. After it was seen that the clear cut cases of lowered resistance occurred in Lot IA vs Lot IB (500 eggs) and Lot IIA vs Lot IIB (300 eggs) in Experiment I and in Lot VIA vs VIB (500 eggs) and Lot VIIA vs Lot VIIB (300 eggs) in Experiment II. A third experiment was conducted to check these results. Sixty chickens were divided into two groups of thirty each and each group was divided into two lots of fifteen chickens each. The chickens of the A lots of each group were bled each week as they were in the previous experiments. The B Lots served as controls.

The growth curves in Figures 11 and 12 show that the gains of the A lots were retarded by repeated bleeding. The gain in weight of neither group was affected by parasitism during the second week. This may have been due to the fact that the chickens in these groups were heavier and gained more readily during the experimentation than the chickens in either Experiment I or II. When the chicks were six weeks of age they were parasitized as follows: 500 eggs to each chick of Lot XIA (bled) and XIV (controls) and 300 eggs to each chick in Lot XIIA and Lot XIIB. After three weeks the chicks in all lots were killed and the worms recovered and measured.

Examination of data showed that in Lot XIA there were 74 worms recovered from 14 chickens or a mean number of 5.21 worms per bird. These worms ranged in length from 1.1 to 26 mm. The average length was 11.74 mm. Lot XIB which contained 15 chicks yielded 83 worms or an average of 5.53 worms per bird. These worms ranged from 1.2 to 29.9 mm. long and averaged 10.22 mm. in length.

Statistical comparison of these two lots showed that the number of worms in Lot XIB was larger than in Lot XIA. The difference in means was 0.32 which is only 0.26 times its probable error which is not significant. While the worms were slightly longer in Lot XIA than in Lot XIB, the

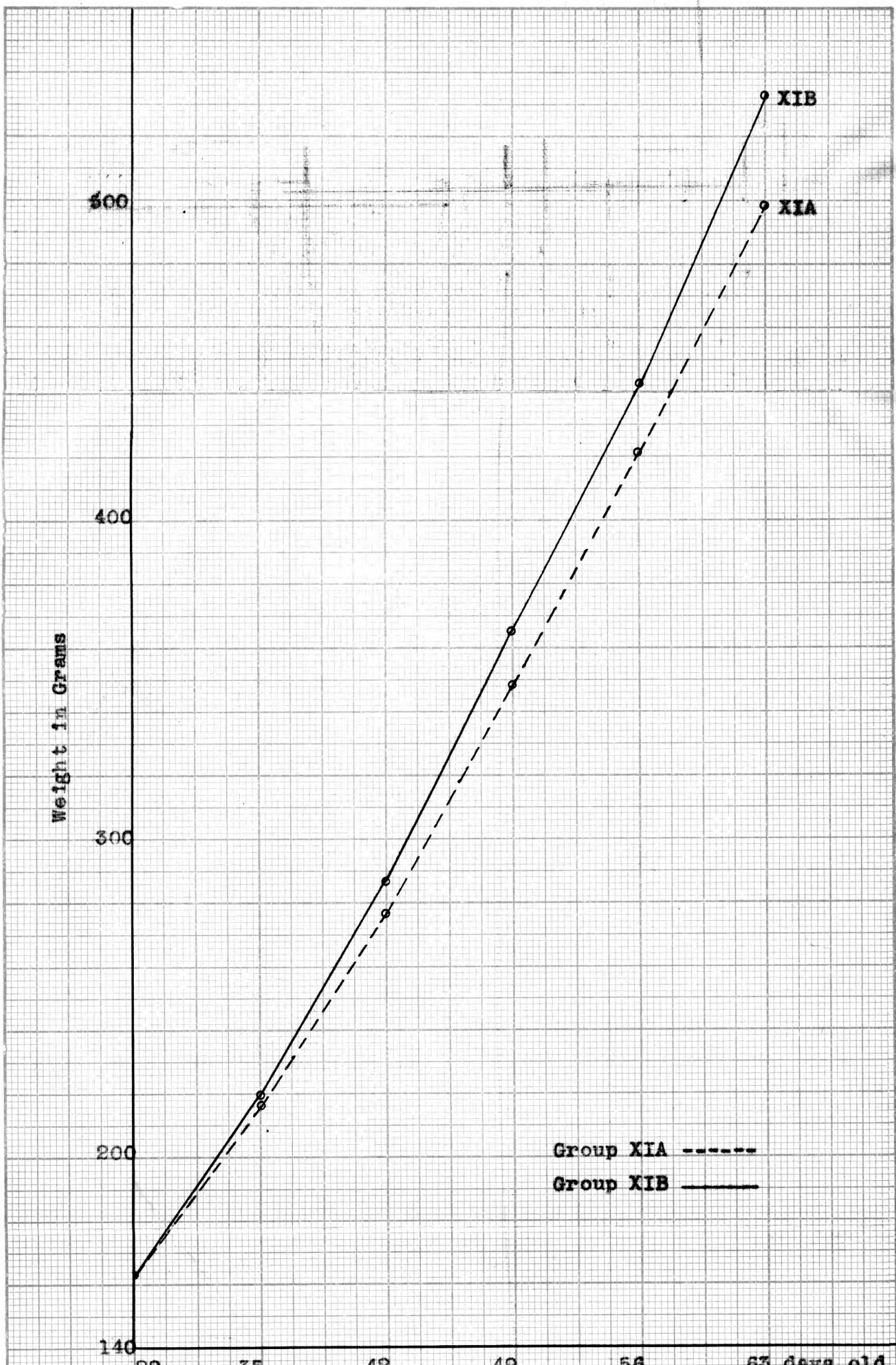


Fig. 11. Showing comparative growth rates of chickens in Lot XIA (bled weekly) and Lot XIB (not bled). Parasitized (500 A. lineata eggs) when 42 days old.

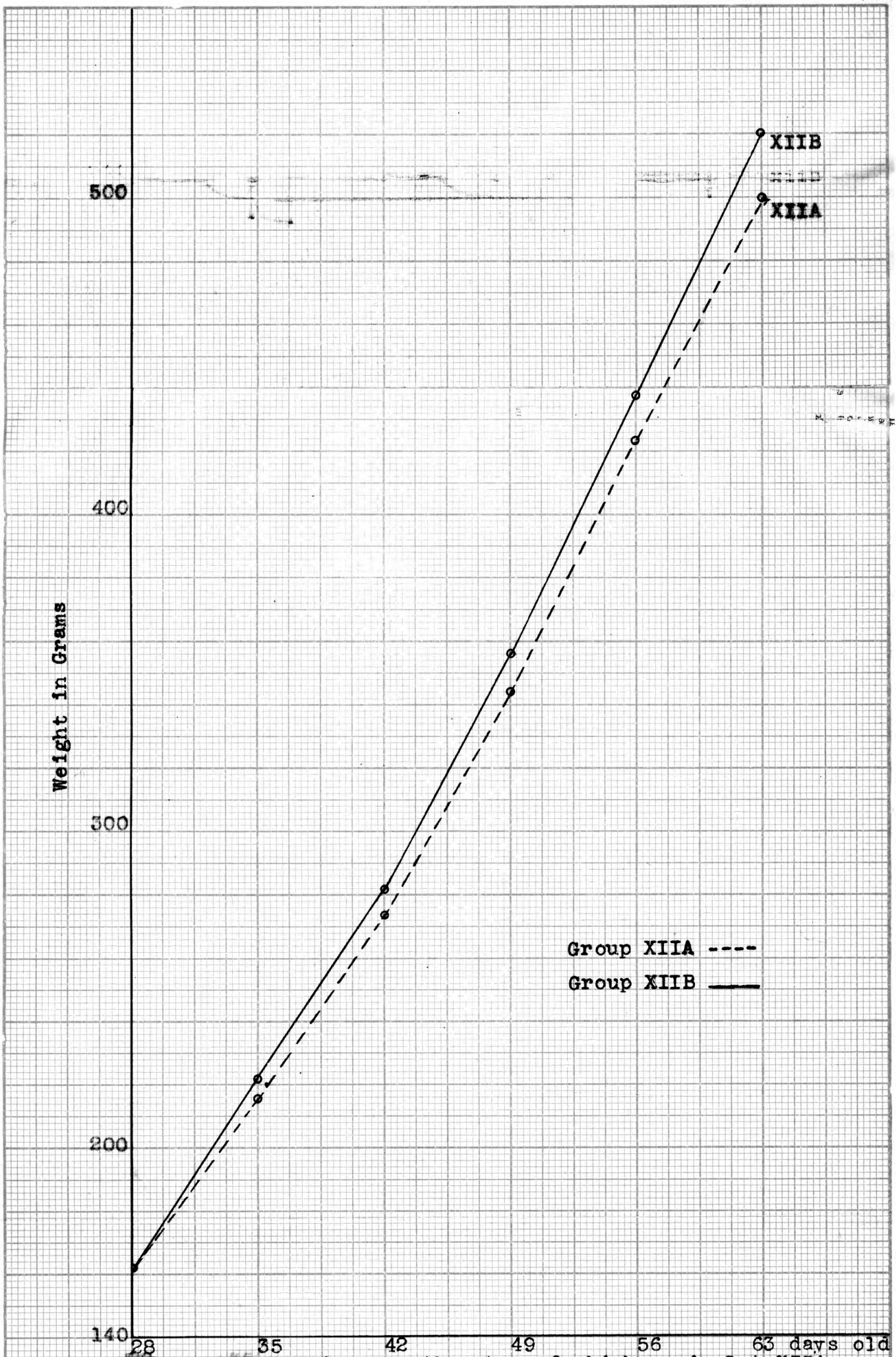


Fig.12. Showing comparative growth rates of chickens in Lot XIIA (bled weekly) and Lot XIIB (not bled). Parasitized (300 A. lineata eggs) when 42 days old.

difference in means was within the limits of experimental error.

In Lot XIIA 79 worms were taken from 15 chicks or an average of 5.26 worms per chicken. These worms ranged from 2.7 to 26.6 mm. in length and averaged 15.53 mm. per worm. However, in Lot XIIB which had 14 chicks 82 worms were recovered or an average of 5.85 worms per bird. This is a larger mean number than found in Lot XIIA but the difference is not significant (Table III). The worms in Lot XIIB ranged in length from 2.0 to 26.2 mm. Their mean length was 16.12 mm. Comparing the mean lengths of the worms in Lot XIIA with those in Lot XIIB it was found that the mean length of worms in Lot XIIB was 2.58 mm. longer than those in Lot XIIA, which is 3.59 times its probable error and is considered significant (Table III). The presence of a slightly larger mean length of worms in Lot XIA indicates that the resistance of the birds in this lot was not appreciably lowered by bleeding. That the resistance of the chicks in Lot XIIA was not lowered is demonstrated by the presence of significantly longer worms in Lot XIIB (control). Also the fact that more worms are found in the B lots in both groups does not demonstrate lowered resistance in the A lots. The reason that no effects of bleeding upon resistance was noted in this experiment may have been due to the fact that more

vigorous chicks were used here than in Experiment II or III.

When Lot XIB (control) fed 500 eggs was compared biometrically with Lot XIIB (control) fed 300 eggs it was demonstrated that there were more worms in Lot XIIB and that these worms had a mean length of 5.90 mm. larger than that in Lot XIB. This difference is considered significant. These results check with the corresponding lots compared in Experiment I and Experiment II except that in Experiment I the worms are longest in Lot IB fed 500 eggs when compared with Lot IIB fed 300 eggs (Table V).

A comparison of Lot XIA and XIIA showed that there were more worms in Lot XIIA fed 300 eggs than in Lot XIA fed 500 eggs. The worms in the lot fed 300 eggs were also longer. This result confirmed the results of similar comparisons of lots in Experiments I and II.

When 500 eggs were fed to Lots XIA and XIB the percentages of survival were 1.1 and 1.1, respectively; and when 300 eggs were administered to Lots XIIA and XIIB the percentages were 1.8 and 1.9 showing the corresponding increase in percentage of survival of larvae that was noticed in the other experiments when the size of the egg dose was decreased.

## DISCUSSION

From the evidence in Experiments I and II it is obvious that the resistance of the chickens in most of the lots was lowered by bleeding. This is demonstrated by a larger number of longer worms occurring in a majority of the A lots in both experiments. No significant difference occurred in numbers of worms but significantly longer worms were found in the lots bled in the cases of IA and IB, and VIA and VIB (fed 500 eggs) and IIA and IIB (fed 300 eggs). The cause of the lowered resistance in chickens that were bled is uncertain. It probably is due, however, to a physiological reaction in which the growth inhibiting mechanism is affected.

Doses of 50 or 25 eggs are more conducive to the growth of the worms than are doses of 500 or 300 eggs. Further evidence on this point is given by Ackert, McIlvaine and Crawford (1931) and by Ackert, Graham, Nolf and Porter (1931). It seems probable that the growth of the worms is enhanced when small numbers of nematodes are present because the growth inhibiting substance in the albumin-globulin fraction of the blood has been diluted. With large infestations this resisting substance would be present in sufficient quantity to inhibit growth of the

young worms.

The percentage of survival was seen to increase as the egg dose decreased in size. A similar result occurred in a part of Dorman's work (1928). Dorman fed various numbers of embryonated eggs of the caecal worm, Heterakis papillosa, to chickens. As the percentage of survival and the mean length of A. lineata larvae increase as the size of the egg dose decreases, it is thought as Graham, Ackert and Jones (1932) have suggested, that it is a seriological relationship in which an inhibiting factor is produced in the chickens.

In Table V four comparisons are made in which there is a significant difference in mean numbers of larvae. In Table IV there are no comparisons in which a significant difference occurs though in both Tables IV and V there are several comparisons made in which the difference in mean numbers of larvae approach significance. The cause for this phenomenon is thought to be the variation in number of worms recovered from the various lots (Ackert, Graham, Nolf and Porter, 1931). This is probably due to the variability in hatching rate. Thus, worms from slowly hatching eggs would be unable to establish themselves in the anterior end of the small intestines before being swept out by the rapid peristalsis of the chicken's intestine. The vigor of peristal-

sis may materially affect the number of larvae present, for large numbers of worms are usually present in intestines having poor muscular tone.

## SUMMARY

1. Three experiments on a total of 360 white leghorn chickens were conducted to ascertain if the resistance of chickens to variable degrees of parasitism is affected by periodic blood losses.

2. The general method of procedure was to separate the chicks of the same hatch into equal lots according to weight, rejecting the extremely large and small birds, use one lot of each group as controls and the other as experimental animals (bled weekly), feed all lots on an adequate diet in well kept pens, make weekly weights of each chicken, parasitize all lots on the same day by feeding to each bird in each of five groups the same number of eggs, i.e., 500, 300, 100, 50, and 25 eggs, respectively, of the intestinal nematode Ascaridia lineata (Schneider), and after three weeks of parasitism, kill the chickens and collect the worms.

3. The criteria for determining whether the resistance of the chickens to the parasite was affected were the numbers (size of infestation) and the lengths of the worms

(rate of growth) that occurred in the various lots under comparison.

4. When large numbers of worm eggs (500 and 300) were fed in Experiments I and II, the resistance of the chickens was lowered by repeated loss of blood. Significantly longer worms were found in the A lots (bled) of Groups I and VI (fed 500 nematode eggs) and the A lot in Group II (fed 300 eggs). In a majority of the lots under comparison there was a larger number of longer worms in the lots that were bled. No evidence of lowered resistance was obtained in Experiment III because larger, more resistant birds were used. The lowered resistance is thought to be due to a physiological reaction in which the growth inhibiting mechanism is affected.

5. When 500, 300, or 100 nematode eggs were fed no appreciable differance in size of infestation resulted. The fluctuations in the hatching rate of the worm eggs and the vigor of peristalsis might have been the cause of this result. A marked difference however was noted between the sizes of the infestations resulting from these doses and from the doses of 50 and 25 eggs.

6. The percentages of survival of larvae from the lots under comparison increased as the size of the egg dose decreased but not in an inverse proportion. This is perhaps due to a seriological relationship in which an inhibiting factor is produced in the chicken.

7. Greater growth of worms occurred when egg doses of 50 and 25 eggs were given than when doses of 500, 300, or 100 eggs were administered. In large infestations the slower growth of the worms is thought to have been retarded by the presence in the host of an abundance of growth inhibiting substance.

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