ON THE NUTRITION OF THE CHICKEN ROUNDWORM, ASCARIDIA LINEATA (SCHNEIDER)

by

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INTRODUCTION

Relatively little work has been done on the nutrition of the Ascaridae and few attempts have been made to correlate critically the results obtained. Even the extensive reviews of nematode physiology by Winterstein (1911) and McCoy (1935) leave much to be desired in the discussions of ascarid nutrition.

Nutritive Needs of Ascaridae

Flury (1912) has shown that the nutritive needs of the Ascaridae are probably the same as those of other animals since the composition of their bodies was found to be almost the same qualitatively, although somewhat different quantitatively. Weinland's (1901) results indicated and Flury's (1912) work showed that although the ascarid glycogen was, as nearly as could be determined, chemically identical with that found in the higher animals, it constituted from one-fourth to one-third of the dry substance of the worm. The only outstanding differences in quality of composition noted by Flury in the Ascaridae were (1) a lack of uric acid and creatine and (2) the substitution of a high molecular alcohol (called by him "ascaryl alcohol") in place of glycerin in combination with the fatty acids. Ackert (1930) has shown that there is no evidence to indicate that nematodes (Ascaridia lineata) require vitamin A or vitamin B complex.

Method of Feeding

The fairly complex structure of the ascarid enteric tract, particularly the anterior portion, indicates that it is functional. Li (1933 a and b) demonstrated that various materials (charcoal, starch, and beef) fed to the hosts could be found in the digestive tract of ascarid parasites

shortly afterward and that the bacterial flora of the worm's gut was similar to that of the host's intestine.

Archer and Peterson (1930) observed that the digestive tract of Ascaris lumbricoides could be seen with a Roentgen ray machine after a barium cereal meal eaten by the host had passed completely through the jejunum. These observations indicate that the ascarids ingest food of the host. Garin (1913), on the other hand, has submitted evidence indicating that all of the Ascaridae tend to bite the mucosa, some species remaining attached throughout life.

Many of the older writers believed the cuticle and muscle layer of the nematodes to be an important factor in the absorption of food stuffs. Winterstein (1911) pointed out that in many members of the group the digestive apparatus is stunted or completely missing. The Acanthocephala, of course, are entirely dependent upon the cuticle as an organ of nutrition. Leuckart (1876) believed the peculiar structure of the "cuticular musculature" to be due to the possession of a nutritive function . Rauther (1907) on the basis of experiments with dyestuffs upon a free living nematode concluded that the cuticle is the most important organ for the absorption of water and dissolved substances, at least in that case. Schopfer (1925) thought that the molecular concentration of the fluid of the body cavity of

Ascaris is regulated by the cuticle. There is no direct evidence to refute this view. The well-known resistance of ascarids to poisons such as formaldehyde might be cited as being due to the impermiability of the cuticle. On the other hand, the protoplasm of nematodes may be resistant to such poisons. The only evidence which could be interpreted as indicating that the cuticle is impermiable is the fact that it is composed of a sulphur-rich albuminoid resembling keratin (Flury, 1912), and the results of experiments by Weinland and Ritter (1903) which showed that the worms could not absorb simple sugars through the cuticle from the solution surrounding them; thus making it necessary to inject the test solutions into the body cavity for the worms' utilization. However, these tests do not eliminate cuticle absorption of water and ionizing compounds, since if the worms depended upon their digestive tract as a source of such materials, they would have also ingested some sugar, rendering it unnecessary to inject the test solutions. It is likely that a certain amount of undissolved material must be present in a solution to stimulate the worms to ingest it.

Digestion and Metabolism

Unless their absorptive membranes are much more permiable than those of other animals, some form of digestion must occur in the Ascaridae. The nematode's position in the gut of the host, especially that of Ascaridia lineata, immediately behind the opening of the bile duct (Ackert, 1931) indicates that food stuffs broken down to their simplest, diffusible products are not available. Weinland and Ritter (1903) have shown that the ascarid glycogen is probably formed as is the glycogen in man, namely, from the simple sugars and maltose. Similarly, the other complex substances of the worm's body are probably formed in a like manner from simple diffusible products.

Schneider (1906) and Rauther (1907) have demonstrated that the digestive tract of certain nemas is acid in reaction in the midgut and possibly for the extent of the entire digestive tract. Abderhalden and Heise (1909) have demonstrated the presence of a peptolytic enzyme in the gut of Toxocara canis, albeit by rather questionable methods inasmuch as they did not control satisfactorily bacterial or autolytic enzyme action. The most conclusive experimental evidence of the existence of enzymes in the digestive tract of the nematodes comes from Garin (1913). He found

that digestion of leucocytes and tissue occurred about the heads of certain worms imbedded in the host enteric mucosae. Such material was found in the digestive tract of the parasite and seemed to serve as its source of food.

Numerous enzymes have been demonstrated as being secreted by or produced in the bodies of Ascaridae, but before discussing them the question of the type of oxidative metabolism in Ascaridae will be considered. Bunge (1884, 1888, 1890) believing that ascarids live under conditions entirely precluding the existence of free oxygen first suggested that they were anaerobes. He found that they could exist in media from which oxygen had been extracted as long as they could in oxygen-permeated media. These results were duplicated by Weinland (1901, 1902, 1903) and Weinland and Ritter (1903), who in addition found that a large amount of valerianic acid was produced and the normal carbohydrate content of the worms diminished under these conditions. An extract of the worms produced the same effect. Since he had used antiseptics (arsenic trioxide, sodium fluoride, toluol, chloroform) to control bacterial action, he believed that the fermentation of the carbohydrate to valerianic acid was the result of the mechanism possessed by the worms for leading an anaerobiotic existence. He advanced for this reaction the equation $4C_6H_{12}O_6 = 9CO_2 + 3C_5H_{10}O_2 + 9H_2$. Flury (1912)

while accepting Weinland's work believed that the reaction was more complicated than this equation indicated and that it produced butyric acid before the valerianic acid formation.

Slater (1925) has taken a diametrically opposed view. He repeated Weinland's experiments and produced the same results. However, he found that if the worms in the oxygen-free media were stimulated to move at regular intervals with an electric current they did not live as long as the controls in the oxygenated media. He also found that it was impossible to inhibit the bacteria present on the cuticle of the worm without killing the nematode. Thus, he concluded that the fermenting action observed by Weinland and others was due to bacteria. Instead of anaerobiosis the condition really existent was a type of suspended animation. Slater believed that the increase in the portal circulation when food was passing through the intestine would bring sufficient oxygen to supply the ascarids' needs. However, Campbell (1931 and 1932) has shown that during digestion the oxygen tension in the intestinal mucosa is reduced to absolute zero, that the tension only approaches that of other tissues when digestion is not in progress. It is possible that the nibbling of the intestinal mucosa by non-fixed Ascaridae reported by Garin (1913) may be the result

of the worms' seeking oxygen.

Much other indirect evidence has accumulated which points to other than an anaerobiotic existence for the Ascaridae. Keilin (1925) has found that they contain cytochrome, a respiratory pigment not found in anaerobic bacteria. Magath (1918) showed that Ascaridae contained large amounts of catalase which also is not found in anaerobes. Although their theory is not generally accepted, Burge and Burge (1915) believed that the protection of intestinal worms against enzymes was due to the presence of free oxygen in their tissues. The old evidence that similar processes occur in other lower forms of life, especially the annelids, which was advanced by Lesser (1909 a and b) and others and which gave support to the theory of anaerobiosis in Ascaridae by analogy, has not withstood modern investigation (Dolk and Van der Pasuw, 1929).

Other enzymes reported by various workers for the Ascaridae include fibrin-lysing (Kobert, 1903), protein, glycogen, starch, cane sugar, grape sugar and fat splitting enzymes, aldehyde oxidase, grape sugar invertase, peroxidase, and a blued resin ferment (Flury, 1912).

From these considerations it is inferred that the nutritive needs, alimentation and metabolism of the Ascaridae do not differ fundamentally from those of other animals.

But the fact remains that little is known of the food and feeding habits of the Ascaridae. It is for the purpose of ascertaining information on these subjects that the present work is undertaken.

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MATERIALS AND METHODS

With the exception of 11 chicks secured locally, the 141 white leghorns used in these experiments came from an accredited commercial hatchery. Immediately upon arrival they were placed in electrically heated brooders. When the chicks were ten days old, they were put in screened, steam heated, well lighted and ventilated pens described by Herrick, Ackert, and Danheim (1923) who showed that under such conditions and on a well-balanced ration chickens may be bred and raised successfully indoors.

The diet given these chickens consisted of yellow corn meal 1000 parts, alfalfa leaf meal 100 parts, meat meal 260 parts, milk powder 160 parts, ground oats 300 parts, cracked wheat 375 parts and sardine oil .11 parts. Feed sufficient for 300 chickens was mixed each week to insure the retention of an adequate amount of vitamin D.

As indicated elsewhere the parasite used in these experiments was the chicken roundworm, Ascaridia lineata (Schneider).

Eggs for parasitizing the chickens were from a "pure line" of A. lineata started September 22, 1934, with the eggs from one worm. The eggs were obtained from the uteri in the following manner: After removing the cephalic end of the worm just posterior to the esophago-intestinal junction the tail of the worm was grasped by forceps, and by constant but gentle forward pressure with a small spatula, the genital organs were forced out of the incision. The uteri were isolated and transferred to a sterile, Petri dish, where they were uncoiled and stretched out. Then, after making a number of incisions at regular intervals along the extended uteri, the eggs escaping from the openings were examined with a microscope to determine their fertility. The criterion for fertility was the presence of the "equatorial clear spot" described by Ackert (1931).

As much of the uteri as contained fertile eggs was transferred to another, sterile, Petri dish, the eggs expressed out upon its surface, and the uterus and any other tissue debris present removed. The eggs were then covered with sterile, distilled water to which a few drops of 2 per cent formalin had been added to prevent mold growth and incubated at either room temperature or at 30° C. The cultures were regularly aerated to insure the developing embryos' receiving an adequate amount of oxygen, and fresh, sterile, distilled water was added when needed.

With the exception of the chickens used in Experiment 1, all of the birds used in these experiments were parasitized at 25 days of age. The egg cultures used were at least three weeks old, which allowed sufficient time for most of the eggs to reach the infective stage.

Some of the chickens in Experiment 1 were given 50 ± 5 embryonated eggs of A. lineata, the dose shown by Ackert, Graham, Nolf, and Porter (1931) to "ordinarily produce in young chickens infestations suitable for comparing the resistance of hosts." However, since in many cases very small larvae were being sought, the chickens in the larger experiments were given 100 ± 10 eggs to facilitate finding the young worms. To parasitize the chickens a drop of water from a culture of infective eggs of

A. lineata was placed upon a slide under a microscope equipped with a mechanical stage and the eggs counted. When the desired number of eggs was found in a drop or in a series of drops, they were wiped off the slide with a piece of filter paper which was folded with the eggs on the outside and inserted into the esophagus of the chicken. The chicken was then banded and subsequently treated in accordance with the plans of the experiment.

As the method of attacking the problem of ascarid nutrition consisted essentially of providing the parasite with a normal environment free from the food of the host the chickens were given parenteral injections of various solutions in an effort to keep them alive as long as possible. The first solution tested was a suspension of egg yolk prepared under aseptic precautions in the ratio of one or two egg yolks to 100 cc. of sterile Locke's solution. This was administered hypodermically gauging each dose by the amount of absorption of the previous dose. While chickens fed by these means lived noticeably longer than completely starved chickens, absorption of the solution was very slow. Subcutaneous injections of normal chicken serum proved to be no more efficient than the injections of yolk suspension. Sterile solutions of glucose in Locke's solution were then tried. According to Milks (1930) the

usual dosage of glucose in man is 1 gm. per kilogram of body weight. While this dose was effective, double this dose was found to be more so. Using it in 25 per cent solution intramuscularly it was possible to keep 45-day-old chickens alive 18 and 19 days, respectively, while two chickens a week older lived only 9 days and 10 days, respectively, when completely starved. No attempt was made to gauge the dosage accurately. Thus chickens weighing from 125 to 225 gm. were treated as weighing 200 gm., chickens weighing from 225 to 325 gm. were treated as weighing 300 gm., etc. The dosage was gauged by the weight of the chicken at the time it was taken off feed. The glucose was administered per os in 25 per cent solutions, intravenously in 50 per cent solutions (wing veins), intramuscularly in 25 per cent solutions, and subcutaneously in 10 per cent solutions. Of all these methods intramuscular injections into the breast muscles of 25 per cent glucose in Locke's solution in the dosage of 2 grams per kilogram of body weight three times a day was the most effective.

To facilitate injecting, the breast feathers were clipped, the chicken suspended by the feet and the breast sponged with 60 per cent alcohol. A Luer-Lok syringe and 26 gauge needle which previously had been sterilized in a 10 per cent formalin solution in 70 per cent alcohol, was

washed with sterile water and filled with the requisite dose of glucose solution from a rubber-stoppered serum bottle. The solution was then injected, .1 to .08 cc. at each insertion, into the pectoral muscles. Occasionally a chicken developed a sucutaneous edema in the pectoral region. Drainage by a liberal incision was fairly effective in reducing the swelling and the accompanying distress.

In Experiment 2 the control and experimental chickens of each group were selected on the basis of estimated weight. In Experiment 3 the birds were grouped on the basis of weights at the time of parasitizing. The heaviest fowls were used at the beginning of each experiment and the lightest ones at the end to enable the latter to gain as much weight as possible and hence be more resistant to starvation.

To collect the worms, the chicken was decapitated and the gut from the gizzard to the caeca removed. This was broken into two or more pieces and the contents flushed out with a pressure apparatus such as described by Ackert and Nolf (1929). The material obtained was carefully examined with a binocular microscope; the worms, if present, were removed and placed in 4 per cent formalin. Measurements of the length of the worms were obtained by tracing

on onion skin paper the image projected upon the ground glass of a photographic bellows so adjusted as to enlarge six times. These lines were subsequently traced with a milled wheel whose divisions were six times as long as the unit of length. The length of each worm was then recorded.

The hydrogen-ion determinations made in Experiment 3 were done by the quinhydrone method. Immediately after the chicken was killed, a piece of gut 1.5 inches long was taken from immediately behind the entrance of the bile ducts. One end of this was slipped over the salt bridge electrode of the potentiometer, which had been made especially small for this purpose. The free end was smeared with quinhydrone and the other electrode applied to it. Readings were then made in the usual manner. It was manifestly impossible to determine the pH of the duodenal content of the starved chickens; so the above described method was adopted as standard. While tests of the pH of the intestinal content in the control animals, and of the intestine itself as well as determinations made before and some time after death varied from determinations made in the described manner, it was believed that this method of determination would show any significant variation between one group and the other since it was constant.

EXPERIMENTAL DATA

As mentioned elsewhere, the aim of this work was to obtain information on the food and methods of feeding of the fowl nematode, Ascaridia lineata. To ascertain whether or not the parasite feeds on the ingesta of the host, certain chickens in each group were deprived of their normal food and fed parenterally or completely starved. These are designated as experimental chickens. Other chickens fed with the usual ration, parasitized at the same time, and with the exception of the type of food supplied treated essentially the same as the experimentals are called control chickens. All chickens had constant access to water.

Experiment 1

Five groups of chickens were handled in Experiment 1. In Group 1 there were two chickens, one experimental and one control. The experimental chicken immediately after receiving 50 ± 5 embryonated eggs of A. lineata was taken off feed and kept alive for the 6 days of the experiment by injections of yolk suspension. The control chicken was taken off feed each night. Upon examination 6 small worms 2.1 mm. in length or less were recovered from the experimental bird but none from the control.

Group 2 consisted of 6 experimental chickens and 2 controls. They were parasitized when 5 days old with 100 ± 10 embryonated eggs of A. lineata. Two of the experimental chickens were fed parenterally with yolk suspension, one living 4 days, the other 6. Two other experimental birds fed with yolk suspension and normal chicken serum lived 4 days, and 2 completely starved birds likewise lived 4 days. The control chickens lived until killed on the 6th day after parasitizing. Upon examination no worms were recovered from any of the birds. This may have been due to the exceedingly small size of the larvae.

The 10 controls and 10 experimental chickens of group 3 when 18 days old were given 50 ± 5 eggs of the parasite. At this time also the parenteral feeding began. Of the experimental chicks 2 lived 6 days, 6 lived 7 days, and 2 survived 8 days. Again no A. lineata were recovered from any of the chickens.

The chickens of Group 4 were given 100 ± 10 embryonated eggs of A. lineata when 5 days old. As has been mentioned the dosage of yolk suspension was gauged by the amount of absorption of the previous dose. In this test 2 of the experimental chickens were given yolk suspension in excess and lived only 2 and 3 days, respectively. The 2 chickens which were given no more of the suspension than they could

absorb lived 4 and 5 days, respectively, while the birds injected with normal chicken serum lived 4 days, and the 2 completely starved animals lived 3 and 4 days. The control chickens were off feed each night. An average of 2.5 worms was recovered from the control and 2 worms from the experimental chickens; so the incidence of parasitism was about the same. The worms from the controls averaged 3.9 mm. in length as compared with 2.6 mm. from the experimentals.

The 7 chickens of group 5 received 50 ± 5 embryonated eggs of A. lineata when 21 days old and were placed on experiment 19 days later. The experimental chickens were injected with yolk suspension for the first 3 days of the experiment and glucose afterward. One of these died on the 5th day of the experiment and the rest on the 6th. The control chickens were sacrificed on the 6th day. An average number of 6 worms per chicken was recovered from the controls, but none from the experimentals (Table 1).

The results of Experiment 1 showed (1) that only in one case (Group 1) were worms recovered which were under 12 days old; (2) that worms 12 days old were recovered from both experimental and control chickens and that the worms were smaller in the former chickens (Group 4); and (3) that several 25-day old worms were recovered from the control chickens but none from the experimental ones.

Table 1. Giving Data From Experiment 1.

Group Number	Control Chickens							Experimental Chickens						
	Number of chickens	Age when parasitized	Duration of Experiment (days)	Age of worms at beginning (days)	Age of worms at end (days)	Average number of worms	Average length of worms (mm.)	Number of chickens	Age when parasitized	Duration of Experiment (days)	Age of worms at beginning (days)	Age of worms at end (days)	Average number of worms	Average length of worms (mm.)
1	1	28	6	0	6	0	0	1	28	6	0	6	6	2.1*
2	2	5	6	0	6	0	0	6	5	4-6	0	4-6	0	0
3	10	18	4-5	0	4-6	0	0	10	18	4-5	0	4-6	0	0
4	2	5	7	5	12	2.5	3.9	7	5	3-7	5	12	2'	2.6
5	3	21	6	19	25	6	33.4	4	21	5-6	19	24-25	0	0

*Only one worm available for measuring.

'All worms found in one chicken which lived 7 days.

Experiment 2

Since the yolk suspension and normal chicken serum were not very efficacious as a parenteral source of food, glucose was tried. Starting with the day the chickens were parasitized with 100 ± 10 eggs of the parasite, each week a new set of control and experimental chickens was placed on experiment, the grouping being made in Table 2 on the basis of the age of the worms recovered from the hosts.

The experimental chickens in Group 1 were given 1 gm. of glucose per kilogram of body weight twice a day. One died 3 days later, 3 died 4 days later, and 1 on the fifth day. No worms were recovered from either the controls or experimentals in this group.

In Group 2 the experimental chickens were given 1 gm. of glucose per kilogram of body weight. One of these was fed *per os* and the other 2 parenterally. The control chickens were off feed each night. An average of twelve 13-day old worms was recovered from the controls and 10 from the experimentals, and since the worms averaged 4.6 mm. in the former and 3.9 in the latter in length, it is evident that no marked difference occurred in incidence or in length of worms.

In Group 3 one experimental chicken was given 1 gm. of glucose per kilogram of body weight intramuscularly and the

other double the dose *per os*. The control chickens were off feed each night. An average of .5 worms averaging 2.7 mm. in length was found in the experimentals and an average of 1.5 worms 11.9 mm. long was recovered from the control birds.

The experimental bird in Group 4 was given 1 gm. of glucose per kilogram of body weight and lived 10 days under the conditions of the experiment. No worms were recovered from this chicken but the control had 3 *A. lineata* averaging 27.5 mm. in length. From the birds in Groups 5 and 6 no worms were recovered. Receiving twice the dosage of glucose that the experimental chicken of group 4 received, the experimental chickens of Groups 5 and 6 lived almost twice as long. Since this dosage of glucose was so markedly efficient, from this point on all experimental chickens were given 2 gm. of glucose per kilogram of body weight, three times a day.

The incidence of worms in the control animals for Groups 3, 4, 5, and 6 was much lower than that found by Ackert (1931). These controls were off feed for at least eight hours a day, this constituting the only difference between the treatment accorded them and that given the animals in Ackert's (1931) experiments. The lowered incidence of worms might be ascribed to a faulty worm culture

were it not for the fact that in the control chickens of Groups 7, 8, 9, 10, 11, and 12, which were parasitized with the same culture, the incidence of worms increased immediately when the chickens were kept on full feed until they were sacrificed.

No worms were obtained from the experimental chickens of Groups 7 and 8, although 4 worms were recovered from the control of the former and 6 from the control of the latter.

Out of the 3 experimental animals in Group 9 but 1 worm was recovered, and it was 10.6 mm. smaller than the average length of worms from the corresponding controls, which had an average number of 4.3 worms each (Table 2).

In Group 10 the average number of worms in the experimental birds was 1 compared with an average in the controls of 6.3. In the former chickens the average length of worms was 24.3 mm. smaller than in the former. It is interesting to note that these worms which were 35 days old were smaller than the worms obtained from the controls of Groups 7, 8, and 9, which were a week younger.

No worms were recovered from either the control or experimental chicken in Group 11, while seven 38-day old worms averaging 45.8 mm. in length were found in the control of Group 12 compared with one averaging 41 mm. in

Table 2. Giving Data From Experiment 2.

Group Number	Control Chickens				Experimental Chickens					
	Number of chickens	Age of worm at chickens death (days)	Average number of worms	Average length of worms (mm.)	Number of chickens	Age of worms at start (days)	Age of worms at finish (days)	Duration of Experiment (days)	Average number of worms	Average length of worms (mm.)
1	4	3-5	0	0	4	3-5	0	4-5	0	0
2	3	13	12	4.6	3	7	13	6	10	3.9
3	2	25	1.5	11.9	2	15	25	10	.5	2.7
4	1	26	3	27.5	1	15	26	11	0	0
5	1	33	0	0	1	15	33	18	0	0
6	1	34	0	0	1	15	34	19	0	0
7	1	26	4	38.3	1	21	26	5	0	0
8	1	27	6	30.3	1	21	27	6	0	0
9	3	28	4.3	41.0	3	21	28	7	.33	30.4
10	3	35	6.3	52.3	3	28	35	7	1	27.8
11	1	37	0	0	1	28	37	9	0	0
12	1	38	7	45.8	1	28	38	10	1	41

length from the experimental bird. This time the worm was about the same size as those obtained from the control animals a week previously.

To briefly recapitulate the results of Experiment 2, no worms were found before the 13th day, although several were recovered from both the control and experimental animals at that time. The worms were considerably smaller and fewer in the experimental birds by the 25th day. From that time on worms were found only rarely in the experimental fowls and when found, were, with only one exception, smaller than those recovered from controls a week earlier.

The irregular feeding of the controls in Groups 3, 4, 5, and 6 seemed to lower the incidence of worms in these animals.

Comparing the "duration of experiment" columns in Table 1 and Table 2, it is seen that the glucose was more efficient than the yolk suspension. The chickens on glucose feeding lived from 5 to 19 days compared with a length of life of 3 to 7 days with the parenteral administration of yolk suspension. This becomes even more apparent when it is noted that the experimental chickens in Groups 9 and 10 were killed after 7 days parenteral glucose feeding, and were not allowed to die of starvation as previously was the case. The experimental chickens which

lived the longest (Groups 5 and 6) were fed by intramuscular injections, proving the greater efficacy of this method over the others which were tried. It is noteworthy that chickens a week older and consequently having greater reserve lived only half as long when totally starved (Groups 11 and 12). The chickens in Group 1 were injected only twice a day and lived only 3 to 5 days. One chicken in Group 3 and the one in Group 4 were given 1 gm. of glucose per kilogram of body weight intramuscularly. (The other chicken in Group 3 was given 2 gm. of glucose per kilogram of body weight per os.) The chickens in Groups 5 and 6 were given double the dose and lived almost twice as long. Because of these results the procedure was adopted of giving each experimental chicken 2 gm. of glucose per kilogram of body weight, intramuscularly, three times a day.

Experiment 3

It has been shown by Ackert and Herrick (1928) that the incidence of A. lineata decreases markedly in regularly fed chickens from the 18th day on. The results of Experiment 2 showed that such a drop occurred in the incidence of parasites in the control chickens some time between the 13 and 18th day. However, the elimination of parasites was even more complete in the experimental chickens. To

determine just when this drop occurred the experimental chickens were handled in the following manner: starting 3 days after they had received 100 ± 10 embryonated eggs of A. lineata, each day an experimental chicken was taken off feed and fed parenterally with the standard amount of glucose. Seven days later, unless it had died in the meantime the chicken was killed and the average number and length of worms obtained compared with those from control chickens, which had been parasitized with the same amount of parasite eggs at the same time, and had been kept on full feed until sacrificed. The results of Experiment 2 also indicated that irregularly fed chickens lost more worms than did those kept on full feed. To ascertain if irregular feeding is a factor in the incidence of A. lineata a third set of chickens called semi-controls was added to the experiment. From the third day after parasitizing, each day a semi-control chicken was placed on experiment and deprived of food for at least 8 hours of each of the 7 days it was on experiment. At the end of that time it was sacrificed along with one control and one experimental chicken, and the worms from the control and semi-control compared. The results of Experiment 3 are given in Table 3.

Table 3. Giving Data from Experiment 3.

	Controls				Semi-controls			Experimentals		
Group number	Age of worms (days)	Number of chickens	Average number of worms	Average length of worms (mm.)	Number of chickens	Average number of worms	Average length of worms (mm.)	Number of chickens	Average number of worms	Average length of worms (mm.)
10	10	2	2.5	3.2	2	0.5	3.0	2*	3.0	3.1
11	11	1	3.0	3.7	1	13	3.5	1	0	0
12	12	1	4.0	3.8	1	0	0	1	1.0	4.0
13	13	1	0	0	1	2	4.3	1	0	0
15	15	1	6.0	4.1	1	3.0	4.7	1	3.0	3.1
16	16	1	1.0	10.6	1	0	0	1	1.0	4.6
17	17	1	0	0	1	6.0	3.4	1	8.0	3.3
18	18	1	4.0	11.1	1	1.0	8.5	1	7.0	2.4
19	19	2	7.5	13.1	2	1.0	19.1	2*	2.0	5.5
20	20	1	3.0	14.9	1	2.0	7.8	1	0	0
22	22	1	?	?	1	0	0	1	1.0	4.0
23	23	1	4.0	8.8	1	2.0	21.2	1	0	0
24	24	1	6.0	26.4	1	0	0	1	0	0
25	25	1	0	0	1	0	0	1	1	3.6
26	26	1	2	26.5	1	0	0	1	0	0

* One chick did not live 7 days without food; ? = worms lost

One experimental chicken in Group 10 did not live the full 7 days. However, no marked differences can be observed in size of the worms recovered from any of the three chickens in the group, although fewer were found in the semi-control.

In Group 11 no worms were recovered from the experimental bird; 3 were taken from the control; and 13 from the semi-control. The worms recovered were approximately the same size.

No worms were found in the semi-control of group 12, although 4 were found in the control and 1 in the experimental chicken, all of them about the same size.

Two worms, averaging 4.3 mm. in length were found in the semi-controls of Group 13, but none was found in the other two chickens.

On the 15th day (Group 15) 6 worms were recovered from the control, 3 from the semi-control, and 3 from the experimental bird. The parasites averaged 4.1 mm. in the first fowl mentioned, 4.7 in the second, and 3.1 mm. in the third.

Although no worms were recovered from the semi-control of Group 16, and only one was found in each of the other two chickens, again the worm in the experimental chicken was considerably smaller than in the control

(4.6 mm. compared with 10.6 mm.).

No worms were found in the control of Group 17. However, the 6 worms from the semi-control and the 8 from the experimental chicken were of almost the same size.

Four worms averaging 11.1 mm. in length were taken from the control of Group 18; one 8.5 mm. worm was found in the semi-control, and again the worms in the experimental animals were the smaller, the 7 worms found averaging 2.4 mm.

The experimental chicken scheduled to be examined on the 21st day died on the 19th; so the data dealing with the worms from it and from the other members of this group are included in Group 19. The average number of worms from the controls of this group was 7.5, and they averaged 13.1 mm. in length. The incidence of parasites is as in the previous group, lower in the semi-controls (average of 1 worm), and it is also lower in the experimentals (average of 2 worms). However, unlike the previous group, the worms are larger in the semi-controls (average 19.1 mm.), although those from the experimentals are still much smaller (average 5.5 mm.).

From the 19th day on (including Groups 20, 22, 23, 24, 25, and 26) the general results noted in Group 19 were repeated. An average number of 3.2 worms occurred in

the controls, .71 worms in the semi-controls and .57 *A. lineata* in the experimental chickens. The worms from the experimental birds were always markedly smaller (2.5 to 6.5 times) than those from the controls. What worms were found in the semi-controls did not differ markedly in length from those found in the controls since they were longer (Group 23) as often as they were shorter (Group 20).

The results of Experiment 3 have shown that the worms from the experimental chickens tend to get shorter on the 15th day and to remain consistently shorter than those from the control chickens, although no such difference was observed between the worms from the semi-controls and those from the controls. The results of Experiment 3 have also demonstrated an abrupt and sustained drop in incidence of parasitism which occurred in the experimental birds on the 18th day and in the semi-control chickens on the 17th day, although no corresponding drop was noted in the control chickens.

Combined Data of Experiments 2 and 3

In Table 4 the data from Experiments 2 and 3 are combined. The chickens are grouped according to the age of the worm at the death of the chicken. The data from Experiment 1 and from Group 1 of Experiment 2 are not included because of different procedure. With the exception

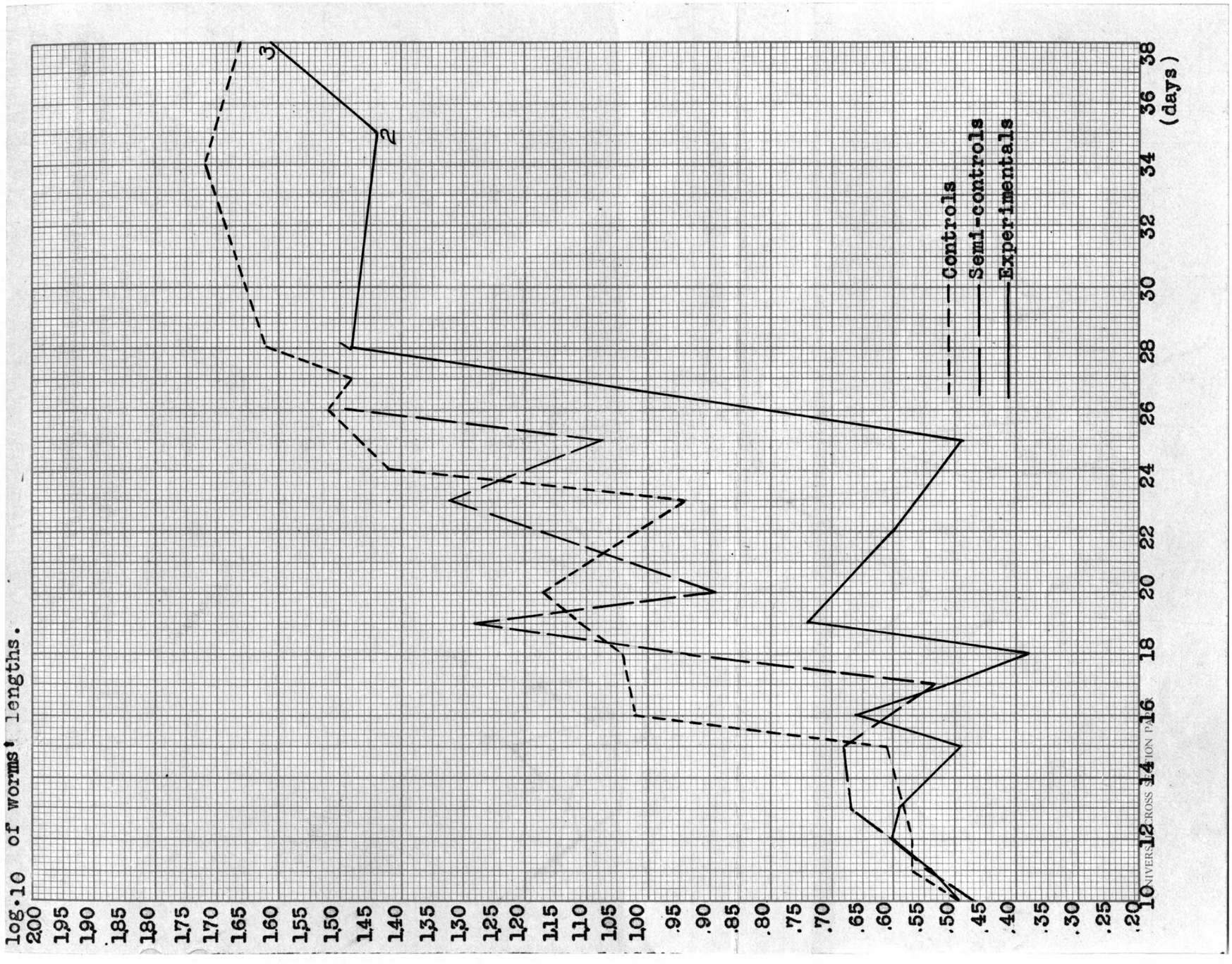

Fig. 1. Giving the comparative lengths (Briggsian logarithms) of Ascaridia lineata from the chickens in Experiments 2 and 3.

of one chicken in Group 13 and one in Group 25 which were fed glucose per os all the experimental birds were fed by parenteral injections of glucose. These exceptions should not vitiate the results because (1) sugars in such amounts would probably be absorbed in the glandular stomach and (2) Weinland and Ritter (1903) showed that Ascaridae did not use the experimental sugars in solutions about them. All the experimental chickens with the two exceptions noted on the table lived at least 6½ days under the conditions of the experiment. The semi-control group consists of the birds designated as such from Experiment 3 and the control birds of Experiment 2 from Groups 1 to 6, inclusive, since the latter were handled essentially in the same manner as the former.

In examining Table 4 it is seen that there are no striking differences in incidence of worms in Groups 10 to 18, inclusive. In averaging the controls had the lowest numbers, 2.5 per chicken. The experimental chickens were next with an average of 3.7 worms per bird, and the semi-controls highest with 4.1 parasites per fowl.

While the incidence of worms in the control birds was increased to 4.7 from the 19th day on, the numbers of worms found decreased to .83 in the semi-controls and .42 in the experimental animals in the same period. It is to be noted

that the drop in incidence occurred in the semi-controls on the 18th day and in the experimental chickens on the 19th day. Generally speaking, the incidence of parasites is over ten times larger in the control birds than in the experimentals, and four to five times larger than in the semi-controls from the 19th day of parasitism on.

To consider the differences in lengths of worms which occurred in the three sets of chickens the data on the average length of worms (Table 4) have been graphed in figure 1, the Briggsian logarithms of the lengths of the worms being plotted against their ages. Upon studying fig. 1 two things become evident: (1) from the fourteenth day on the worms in the experimental birds tend to be shorter than those in the controls, the difference in length becoming markedly evident upon the 18th day; (2) the growth curve of the worms recovered from the semi-controls follows the growth curve of worms from the controls rather closely, although there is some tendency evident for the worms to be somewhat shorter in the former group. Points 1 and 3 on the growth curve of the worms from the experimental animals, which are fairly close to corresponding points on the growth curve of worms from the controls are of doubtful signifance since they were each plotted on the basis of the length of one worm. The worms plotted at point 2 are even smaller

Table 4. Giving Data from Experiments 2 and 3, Combined.

Group number	Age of worms (days)	Controls: Number of chickens	Controls: Average number worms	Controls: Average length of worms (mm.)	Semi-Controls: Number of chickens	Semi-Controls: Average number worms	Semi-Controls: Average length of worms (mm.)	Experimentals: Number of chickens	Experimentals: Average number worms	Experimentals: Average length of worms
10	10	2	2.5	3.2	2	0.5	3.0	2	3.0	3.1
11	11	1	3.0	3.7	1	13.0	3.5	1	0	0
12	12	1	4.0	3.8	1	0	0	1	1.0	4.0
13	13	1	0	0	4	9.8	4.6	4	7.5	3.9
15	15	1	6.0	4.1	1	3.0	4.7	1	3.0	3.1
16	16	1	1.0	10.6	1	0	0	1	1.0	4.6
17	17	1	0	0	1	6.0	3.4	1	8.0	3.3
18	18	1	4.0	11.1	1	1.0	8.5	1	7.0	2.4
19	19	2	7.5	13.1	2	1.0	19.1	2*	2.0	5.5
20	20	1	3.0	14.9	1	2.0	7.8	1	0	0
22	22	1	?**	?	1	0	0	1	1.0	4.0
23	23	1	4.0	8.8	1	2.0	21.2	1	0	0
24	24	1	6.0	26.4	1	0	0	1	0	0
25	25	1	0	0	3	1.0	11.9	3	0.66	3.6
26	26	2	3.0	33.0	2	1.5	30.8	3*	0	0
27	27	1	6.0	30.3	0			1*	0	0
28	28	3	4.3	41.0	0			3	0.33	30.4
33	33	0			1	0	0	1	0	0
34	34	0			1	0	0	1	0	0
35	35	3	6.3	52.3	0			3	1.0	27.8
37	37	1	0	0	0			1	0	0
38	38	1	7	45.8	0			1	1.0	41.0

*One chicken died before being off feed six days.

**Worms lost.

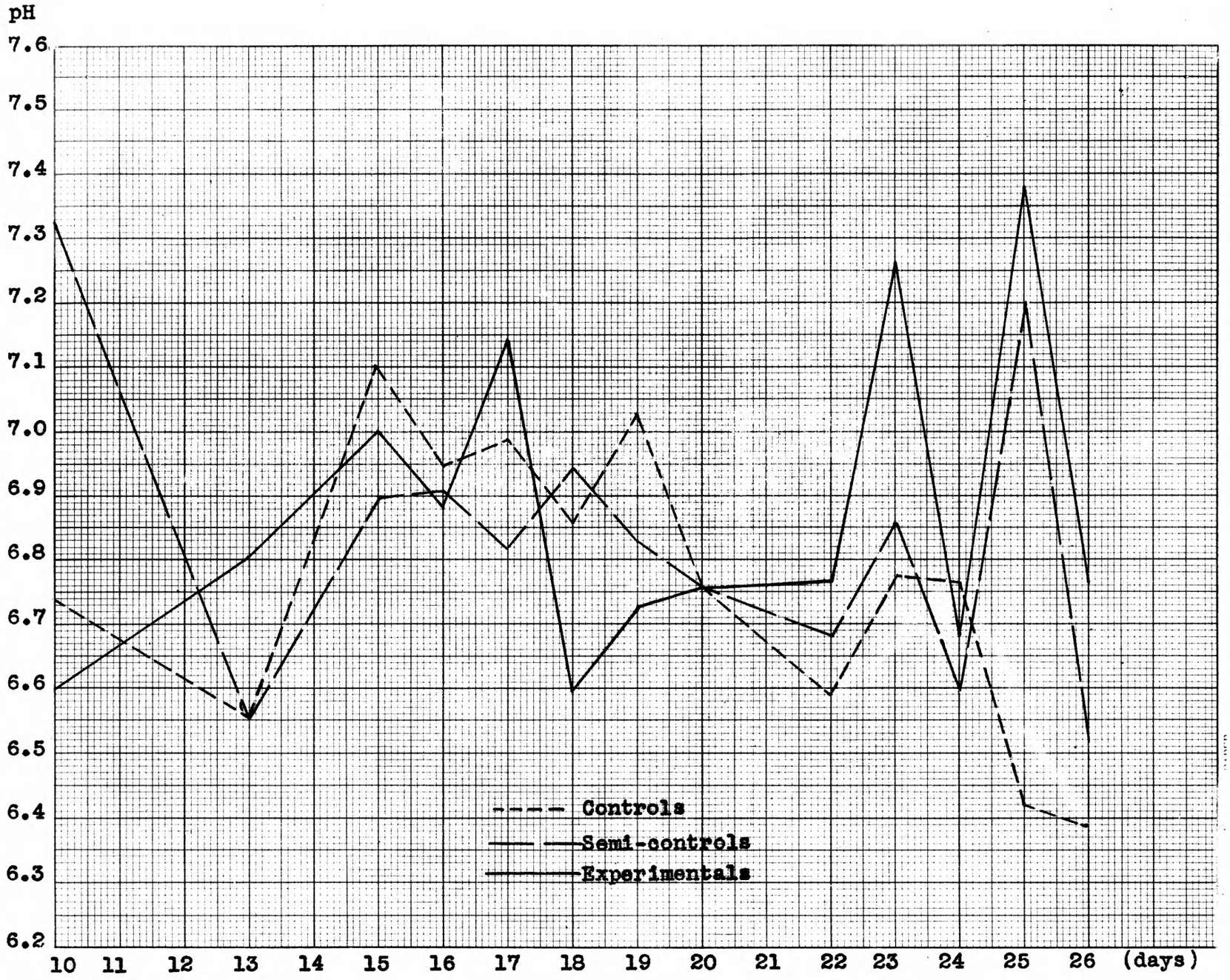

Fig. 2. Graph of range of pH values of worm habitat in the chickens of Experiment 3.

than the worms plotted from the control animals a week previous. The same is true of the worm plotted at point 3. While the present experiments give no record of a 21-day old worm, 30 mm. long, it is not inconceivable that such could occur, thus accounting for the recovery of one that size from an experimental chicken at 28 days after parasitizing and allowing no exceptions to the rule that from the 14th day until the time the normal growth curve flattens, the worms from the experimental chickens are much smaller than those from the controls. After the latter time all worms found in the experimental birds are the same size if not smaller than those obtained from controls a week previous.

DISCUSSION

The absence of food in the intestinal tract of the host might cause several factors to become operative to the detriment of the parasite and cause the low incidence and shorter length of worms from the experimental chickens. Chief among these are: (1) alterations in the pH of the worm habitat, (2) alterations in oxygen supply, (3) secretion of intestinal products poisonous to the worm, (4) alterations in peristaltic action, (5) alterations of the bacterial flora of the intestinal tract, and (6) lack of

food for the parasite.

Hydrogen-ion determinations were made on the worm habitat of the intestines of freshly killed chickens; the results of which are given in fig. 2. The pH range was 6.6 to 7.4 in the experimental chickens, 6.5 to 7.3 in the semi-controls and 6.4 to 7.1in the control birds. No marked differences were observed between the groups observed. The range of fluctuation was within the limits recorded by Ackert (1931) although not, in every case, within those set by Mayhew (1935). However, differences in the technics may account for the varying results obtained by the two workers. The results of these tests would indicate, then, that pH alterations are not a factor in the incidence and growth of A. lineata.

Likewise it is doubtful if alteration of the oxygen supply can be a factor. Campbell (1931, 1932) has shown that the oxygen tension in the intestine approaches that of other tissues except at the time that the active reduction processes of digestion are in progress. No digestion is taking place in the empty gut of the experimental animals; so there is plenty of oxygen available for the parasites if they lead, as is highly probably, an aerobic existence. If they lead an anaerobic existence, as some workers suspect, the presence of the oxygen should not be

a detriment to them since Weinland (1902) has shown that the processes which he called anaerobiotic could take place in the worms in the presence of oxygen.

Unless it is postulated that the larvae of A. lineata are more resistant to toxic products than the parasite after its third molt, it is unlikely that any toxic product of the starved intestine such as was shown by Bahrs (1931) to influence the growth-promoting power of rabbit intestine for Planarian worms can be a factor in the lowered incidence of parasites in the experimental animals. Ackert and Herrick (1928) have shown that the immature A. lineata up until the 9th day live in the lumen of the duodenum and in the intervillar spaces and that from the 10th to the 18th day they have their anterior ends buried in the mucosa. Surely these larvae so intimately associated with the mucosa would be more susceptible to a poisonous product in it than the adults living free in the lumen of the intestine. But the evidence of the foregoing experiments point out that the drop in incidence of parasitism does not occur until after the worms have left the mucosa. If the larvae before the third molt are more resistant than afterward to such a toxin, then the drop in incidence of parasitism should occupy several days, since Ackert (1931) has shown it takes as long as 4 days for all of the parasites to molt.

Ackert and Nolf (1931) have shown that the higher incidence of parasitism in chickens fed on a vitamin B deficient diet can probably be directly attributed to the partial paralysis of the intestine which accompanies this type of avitaminosis. There is no question but that an abnormally active intestine provides an adverse environment for the worms since violent cathartics have a certain amount of Ascarid anthelmintic action. Logically, a slowing of the peristalsis should be more favorable than otherwise for the parasite.

The ingestion of water by the starved chickens might conceivably stimulate the normally quiet, empty intestine to such an extent as to eliminate some worms.

During the course of the experiments it was noted that the experimental chickens took considerable quantities of water. Sometimes their crops would be full of it; fluctuations in their weights would be observed which could only be accounted for by the presence of comparatively large amounts of water in their bodies. However, this excessive water inbibition was not a regularly occurring phenomenon (either in the chickens of any group or in any one chicken). This would indicate that sudden increases of peristaltic action in the gut caused by the presence of large amounts of water could not be a constant factor in the elimination of worms

by the experimental chickens, although the possibility that it has a certain amount of anthelmintic action in the concerned cases cannot be denied. The normal, regular amounts of water inbibed by the host are probably of less importance as a factor causing the lowered incidence of worms in the experimental chickens than the foregoing primarily because the small amounts of water ingested normally at one time by the chicken should not stimulate enough peristalsis to eliminate the worms. At any rate, abnormal peristalsis could not account for the smaller size of the worms in the experimental animals.

Another factor detrimental to the parasite which the empty digestive tract of the experimental chicken could cause is an alteration of the bacterial flora of the host intestine. Li (1933a) has shown that bacteria are probably unimportant as a food source for the Ascaridae since microorganisms were found in the enteric tracts of only half the parasites examined and then in extremely limited numbers. It is conceivable that the slowed peristaltic action of the host intestine due to starvation might result in fewer bacteria than normal being eliminated. Whatever favorable influences this factor may have for the increase of bacteria population are undoubtedly limited by other influences such as the absence of food for the bacteria, since in no case

were any indications of excessive bacterial growth such as significant alterations of the intestinal pH, manifestations of excessive fermentation in the gut, or symptoms of auto-intoxication evidenced by the experimental chicken. However, these considerations do not eliminate the possibility that a bacterium having an anthelmintic action on the parasite may be present. If such were the case, the active principle responsible for this action is undoubtedly readily diffusible since Weinland (1902) has shown that in all probability the Ascaridae do not feed unless first stimulated to do so by particles resembling those of ingesta and since in the starved gut it is very improbable that particles large enough to cause this reaction do not occur. The hypothetical toxic principle in all probability, therefore, exerts its action by way of the cuticle of the parasite. If it diffuses through the cuticle of the parasite older than 18 days of age, it must have thoroughly permiated the nematode younger than 18 days both by way of its food, the host mucosa, and the cuticle. This being the case, its anthelmintic action should have occurred with equal efficacy before as well as after the 18th day. If we postulate that the larvae before the third molt, which occurs about the 18th day were more resistant than those after the third molt to the bacterial toxic substance, the drop in incidence

of parasitism should have occupied a period of four days since Ackert (1931) has shown that this is the time consumed by the parasites for this molt.

It would seem, then, that starvation of the parasite is the most plausible factor in the drop of incidence of parasitism in the experimental animals. It is impossible with the present information at hand to determine just what the larvae eat before they enter the intestinal mucosa. From that time until they leave the mucosa on about the 18th day, they probably feed on intestinal secretions, lymph, and possibly cells and tissue debris. They probably cannot use blood since Garin (1913) has shown that close relatives of theirs belonging to the genus Ascaris contain no hemolysins in their bodies, this being an almost exclusive property of the true blood-suckers. The fact that the worms from the experimental animals tend to be smaller than those from the controls after the 14th day may be due to the influence of a toxic product such as Bahrs (1931) has suggested, or it may be due to the fact that the emaciated, non-secreting, non-active intestine does not provide quite enough food for the parasites.

Garin (1913) believed that the Ascaridae which lived free in the lumen of the intestine were nourished at the expense of the intestinal mucosa of the host, and not at

the expense of the contents of the digestive tract. This is very doubtful, primarily because there are so very few reports of the more common Ascaridae being attached to the intestine. (Garin cites only three cases and no more recent references have been found.) Dr. J. E. Ackert[1] stated that in all of his experience with Ascaridia lineata and the other non-fixed Ascarids, he has never found an adult worm attached to the mucosa. Surely, if this were the common method of feeding more of the members of this family would have been found feeding in this manner. Then, too, the lesions in the intestine which Garin believed to be due to the gnawing of the worms are not commonly observed, particularly with A. lineata. If the worm could live at the expense of the mucosa, the incidence of parasitism should be the same in both experimental and control animals of the foregoing experiments after they had left the wall of the intestine. This was not the case and fails to support Garin's view. The drop in incidence of parasitism coincides exactly with the time of the worm's detachment from the mucosa determined by Ackert and Herrick (1928) to be 18 days.

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1 Personal communication.

It has already been shown (Introduction) that the digestive tract is probably the chief organ for providing A. lineata with nourishment. By a process of elimination the conclusion has been drawn that the loss of parasites in the experimental animals is probably due to an absence of food for the parasite. Since the intestinal mucosa has been eliminated as a chief source of food for A. lineata, the parasite must either use the intestinal secretions or the ingesta of the host as food. If the intestinal secretions are the chief source of food for the parasite, the ingesta present serves a two-fold purpose: it stimulates the secretion of intestinal juices, and it probably stimulates the parasite to ingest them since Weinland and Ritter (1903) have shown that Ascaridae will not ingest pure fluid material even if heavily laden with foodstuffs in solution. The non-ingestion of fluids by Ascaridae is believed to be a protective mechanism against the swallowing of water taken in by the host since such an event would probably result in immediate disintegration of the worm due to osmotic changes. The fact that the Ascaridae are so host-specific lends support to the theory that the host intestinal secretions constitute at least an important part of their diet. The theory that the ingesta of the host constitutes part of the diet of the Ascaridae needs no discussion. Suffice it

to say that the food of A. lineata (and probably of the other Ascaridae) is composed of either the intestinal secretions or the ingesta of the host or both and that the absence of this food is the chief factor responsible for the greatly lowered incidence and much shorter length of parasites in the experimental animals as compared with those from the control animals in the present experiments.

The only cases where worms were found in the experimental animals, after 18 days of parasitism, which were comparable in size to those of the control animals, occurred after the growth curve (fig. 1) of the worms in the latter animals had flattened out and the worms were reaching adulthood with its accompanying greater resistance to starvation. It is noteworthy that in these aforementioned cases the worms recovered from the experimental animals were, in all but one instance involving one worm, smaller than the worms recovered from the control animals a week previously.

Although the incidence of parasitism in the irregularly fed animals, the semi-controls, was lower than in the controls after the 18th day, the growth curves of the worms of the two groups were fairly close with a tendency toward smaller worms on the part of the semi-controls. Alterations in pH and oxygen tension can be eliminated in this case as

in the case of the experimental animals. Toxic products would have little if any chance to form in the intestine of these animals and even if formed, could be eliminated from consideration by the same plausible factors discussed in connection with the experimental animals. Lack of food for a period each day might conceivably be responsible for the tendency for shorter worms in the semi-controls but surely could not directly cause the elimination or death of the worms. It is probable, therefore, that alteration in peristalsis is responsible for the lowered incidence of parasites in the semi-control animals. Ascaridia lineata is used to an environment almost constantly filled with the ingesta of the host. The crop of the chicken keeps a supply of food almost unceasingly moving through its digestive tract. When the chicken is taken off feed and the supply of food passing downward from the crop is exhausted, intestinal peristalsis slows down. Probably the worm can accommodate itself readily to this situation. When the host is returned to feed, however, the sudden increase in peristalsis to which the parasite has not become adapted plus the appearance in the digestive trace of large quantities of food with which the chicken has gorged itself probably has an anthelmintic action forcing some of the worms from their habitat. Repeat this seven times or, as was the case in some of the controls in Experiment 2, even more often and

the lowered incidence of parasites in the semi-control animals is not inexplicable.

SUMMARY

1. Careful analysis of the work of previous writers on the subject of nematode nutrition revealed that in all probability the method of taking food and the metabolism of the Ascaridae do not differ fundamentally from those of other animals.

2. Intramuscular injections of two grams of glucose for each kilogram of body weight in 25 per cent solution in Locke's solution three times a day were found to be the most effective method of keeping young chickens alive by parenteral feeding. Two chickens, 45 days old, were kept alive 18 and 19 days, respectively, by such treatment while two, a week older, lived only nine and ten days when completely starved.

3. The results of three experiments upon 141 chickens showed that the incidence of parasitism, after the 18th day, in the control birds was ten times as large as that of the experimental animals and four to five times as much as that of the semi-control fowls. No marked differences were noted before this time.

4. From the 14th day till the time that the normal growth curve flattens the worms from the experimental

chickens are much smaller than those from the controls; after the latter time all worms found in the experimental animals at most are the same size, if not smaller, than those obtained from the controls a week earlier. Although there is some tendency in the semi-control birds for the worms to be smaller than in the controls, the growth curves follow each other fairly closely.

5. The lowered incidence and smaller size of worms from the experimental animals is apparently primarily due to starvation of the parasites, and the food of Ascaridia lineata is shown to consist of either the intestinal secretions of the host or the host ingesta or, more likely, both.

6. The lowered incidence of parasitism in the semi-controls is thought to be due mostly to the abnormal irregular peristalsis resulting from irregular feeding.

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