DUODENAL GOBLET CELLS AND AGE RESISTANCE TO PARASITISM

by

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TABLE OF CONTENTS

	Page
INTRODUCTION	3
MATERIAL AND METHODS	6
DATA	9
Lengths of Duodenal Villi	10
pH of the Duodenal Worm Habitat	12
Counts of Goblet Cells in Chickens	13
Counts of Goblet Cells in Rats	18
DISCUSSION	23
SUMMARY	26
ACKNOWLEDGMENTS	28
LITERATURE CITED	29

INTRODUCTION

There has long been empirical evidence of greater resistance in older individuals, than in younger ones, to helminthiasis and numerous other diseases. However, experimental evidence was probably not available before 1911 when Looss in administering ancylostoma larvae to dogs observed that in young animals some of the larvae were able to reach maturity. Previous investigators had been unable to obtain such results in adult dogs. Ransom and Foster (1920), working with ascaris of pigs, confirmed Looss' findings. In 1921, Ransom detected definite age resistance of chickens to the gapeworm Syngamus trachea. At about the same time Ackert and Herrick found that chickens three months of age were much more resistant to the fowl nematode Ascaridia lineata (Schneider) than were younger birds. This work was not published, however, until 1928. Herrick (1926) found a gradual increase in the resistance of chickens to the growth of this nematode up to 103 days after which no further increase was found.

Further demonstrations of age resistance of animals to helminths have been made by other workers, including Herrick (1928), Scott (1928), and Sarles (1929) working with Ancylostoma braziliense in cats and dogs; Africa (1931), Chandler (1932), and Graham (1933), with Nippostrongylus muris in rats; Winfield (1933) with Heterakis spumosa in rats; and Ackert, Porter, and Beach (1935) with Ascaridia lineata in

chickens. In this last paper the results of nine experiments on 385 chickens showed marked differences in age. Starting with chickens 45 days of age and working up to chickens 93 days of age, it was noted that the length of the nematodes decreased as the age of the chicken increased. However, at 93 days a maximum age resistance seemed to have been reached. They attributed this to the development of more potent growth--inhibition factors which react against the development of nematodes.

Much information upon resistance of chickens to the large nematode Ascaridia lineata was obtained in a series of experiments on vitamins A, B (complex), and D, as factors in the fowls' resistance to these nematodes and the effects caused by them. A review of these studies, the first of which opened a new field of investigation, was published recently by Ackert (1935).

To study further the problem of age or natural resistance, it seemed necessary to ascertain more nearly the nature of the food of the worms in the duodenum of their host. To make this study, Ackert and Whitlock (1935) and Ackert and Freeman (1936) parasitized young chickens from the same hatch with the same number of embryonated eggs of A. lineata. After one week, to allow the young worms to become established in the hosts, the chickens were divided into two groups: one to be nourished only by water and intramuscular injections of glucose, and the other to be fed water and the usual ration by mouth. When an injected chick died its control was killed and the worms from each bird isolated,

counted, and measured. The results of tests on 96 chickens showed marked differences in the growth of the worms under these contrasted conditions. The worms from glucose injected chickens were able to grow but little, whereas the Ascaridia lineata from regularly fed chicks grew normally. For example, during a period of experimentation covering 20 days, the worms from the injected birds ranged from 3 to 5.6 mm. in length, while those from the normally fed chickens ranged from 5.3 to 40.1 mm. In fact, in the absence of food in the fowl intestine, the worms made almost no growth. The results of these tests indicate that the growing Ascaridia lineata normally take food from the intestinal lumen rather than from the epithelium (Ackert and Whitlock, 1935) (Ackert and Freeman, 1936).

In many of the studies on age resistance of chickens to the Ascaridia, the fowls were secured from the same flocks. The birds of the different ages were fed from the same mixtures of feed which was a cereal basal ration supplemented with suitable protein, vitamins, and minerals as recommended by the Poultry Department of the Kansas Agricultural Experiment Station. When chickens of different ages were given 50 ± 5 embryonated eggs from the same culture and all chickens under comparison killed at the end of three weeks of parasitism, it was found repeatedly that the worms from older chickens were smaller and less numerous than those in the younger chickens (Ackert and Herrick, 1928; Ackert, Porter, and Beach, 1935; Ackert, Eisenbrandt, Wilmoth, Glading, and Pratt, 1935). These results indicated that there must be some dif-

ference between the duodenal worm habitats of the older and of the younger chickens.

In view of this condition and in the absence of any known morphological differences between the intestines of older and younger chickens, it seemed desirable to make a detailed histological study of the duodenal habitat of A. lineata in chickens of various ages.

MATERIAL AND METHODS

The chickens used for these studies were single comb white leghorns of different ages that were treated similarly. That is, they were caged the night before killing, decapitated one by one, their small intestines being removed at once and stripped of their mesenteries. Small pieces of the duodenum a few centimeters posterior to the entrance of the bile and pancreatic ducts were then removed. These distances from the bile ducts, of the pieces taken, were in proportion to the ages of the chickens. Immediately upon removal from each bird, the pieces were placed in individual vials containing the fixing reagents.

Three groups of chickens were killed. Group I consisted of chickens 2, 5, 51, 124, and 313 days of age. Group II was of chickens 12, 58, 131, and 320 days of age, while Group III contained chickens of the ages 26, 40, 55, 71, and 176 days.

Several fixatives were tried as follows: Picro-formalacetic (Bouin's fluid), Gilson's mercuro-nitric solution, saturated aqueous solution of mercuric chloride (corrosive sublimate), aqueous sublimate-

acetic acid (acetic corrosive sublimate), and sublimate-trichloracetic-formalin (Heidenhain's "susa").

Portions of the intestines of the chickens of Group I were fixed in Gilson's solution. Those of Group II in Gilson's, acetic corrosive sublimate, Bouin's and corrosive sublimate, and the tissues of Group III were fixed in "susa" and corrosive sublimate. The details of the technique were as follows:

Tissues fixed in Gilson's:

Fixative, 6-8 hours
50% (ethyl) alcohol, 2 or 3 changes at one hour intervals
70% alcohol, 3 hours
90% alcohol, and treated with iodine in 90% alcohol until all of the mercuric chloride was removed
Fresh 80% alcohol, 2 hours
90% alcohol, 3 hours
Absolute alcohol, 2 changes during 6-12 hours
1/4 toluol, 3/4 absolute alcohol, 1 hour
1/2 toluol, 1/2 absolute alcohol, 1 hour
3/4 toluol, 1/4 absolute alcohol, 1 hour
Pure toluol, 5 or 6 hours, or until the pieces of tissue appear to be clear (2 changes during the period)
Fresh toluol with paraffin shavings (all that will dissolve) left over night, or 6-10 hours
Slush poured off, freshly filtered paraffin added, put in the oven for 4-6 hours, making 3 changes of pure paraffin at one hour intervals
Tissues imbedded, mounted on blocks, and sectioned.

Those tissues fixed in corrosive sublimate and acetic corrosive sublimate were treated in the same manner as those fixed in Gilson's.

Tissues fixed in Bouin's:

Fixative, 10-12 hours
Washed in 70% alcohol and remained there until most of the excess picric acid was removed, changes of 70% alcohol being made
80% alcohol, 3 hours

90% alcohol, 3 hours
Absolute alcohol, 6-12 hours (two changes)
Remainder of technique essentially the same as that for those pieces fixed in Gilson's.

Tissues in "Susa's" fixative:

Fixative, 4-8 hours (depending upon the age of the chicken)
1/2 dioxane and 1/2 distilled water, over night
Pure dioxane (in a stoppered bottle), 3 or more hours
Dioxane (fresh) to which was added enough iodine in dioxane to make the solution straw colored. Iodine was added from time to time until the solution no longer became light or until all $HgCl_2$ was removed.
Fresh dioxane until excess iodine had been removed from tissue
1/3 dioxane, 2/3 melted paraffin placed in oven over night, or 6-12 hours
Fresh paraffin (stoppers removed) 3 or 4 changes at one hour intervals (a piece of gauze was put in the bottom of each vial to hold the tissue in suspension so that any remaining dioxane might pass to the bottom of the vial
Tissues imbedded and sectioned when convenient.

In later work on rat intestines, the "Susa"-dioxane-paraffin technique was used. Tissues fixed in it were less hardened, and could be processed with much more rapidity. Most of the tissues were sectioned 10 microns thick and mounted on slides by means of Mayer's albumin fixative. Some of the tissues, however, were cut only 6 and 7 microns thick.

Several stains were used, also several combinations of these stains. They were: alum-haematoxylin, Mallory's triple stain, orange g, triosin, eosin, and thionin. Of these, alum-haematoxylin, for a nuclear stain, and triosin as a counter, cytoplasmic stain were found to give the best results. The progressive method of staining with

alum-haematoxylin-triosin, as described by Galigher in 1934, was used largely. The alum-haematoxylin is a modification of Harris's haematoxylin and the triosin is a cytoplasmic stain which is produced exclusively by Galigher.

DATA

The purpose of this problem was to make a critical histological study of the duodenal walls of chickens of various ages to determine, if possible, any structural differences which might aid in explaining age resistance of fowls to the nematode Ascaridia lineata. To facilitate making unbiased comparisons of the intestines of the various aged chickens, the slides were catalogued by number. Measurements and average dimensions were determined for the length of villi, length of columnar epithelial cells, thickness of the cuticle, numbers of crypts, thickness of axes of villi, thickness of muscularis mucosae, and the number of duodenal goblet cells.

It was observed, in accordance with Calhoun (1933) and others, that no Brunner's glands were present as Kaupp (1918) reported. The structure of the intestine, in cross section, was found to agree with the descriptions of Calhoun (1933). In general, the small intestine, including the duodenum, is similar throughout. The duodenum is lined with simple columnar epithelium with many goblet cells. On the surface are villi between which the crypts of Lieberkühn open. The villi contain lacteals, blood vessels, muscle fibers, and lymphoid tissue, the

latter varying with the age of the chicken.

The muscularis mucosae is comprised of an outer circular and an inner longitudinal layer, the latter sending fibers into the villi. In places the outer circular layer appears to fuse with the circular layer of the lamina muscularis. The submucosa, apparent in only a few places, is a very thin layer. A few blood, lymph vessels, and nerves were visible. The lamina muscularis consisted of an inner circular and an outer longitudinal muscular layer with connective tissue layers on each side, containing plexuses of nerves, blood vessels, and lymph vessels. The sub-serous layer was thin and of white and yellow elastic fibers. Blood vessels, lymph vessels, and nerves were in the meshes. The serous or visceral peritoneum was on the outside. There was a diffuse lymphoid infiltration of the tunica propria and a few small lymph nodules in five month fowls. The villi of the duodenum were the longest found in the digestive tract (Calhoun, 1933).

Lengths of Duodenal Villi

As the villi are prominent structures in the duodenal worm habitats, a study was made of their sizes in chickens of various ages. From the mounted sections it was possible to select villi that could be measured from base to apex, or tip. To give an idea of the variability in heights of duodenal villi, ten readings are recorded for each chicken. The data are presented in Table 1. Chicks 2 days of age had duodenal villi averaging 328 μ in length; the 5-day chicks, 511.7 μ; 12-day

Table 1. Average lengths of villi from base to apex. Counts refer to divisions of the ocular scale and must be multiplied by 16.4 to obtain the lengths in microns. Low power compound microscope used.

Age of chickens	Ten counts of lengths of villi	Aver. no. ocular div.	Aver. length villi (microns)
2 days	20, 19, 14, 22, 24, 24, 25, 15, 17, 20.	20 (16.4)	328.0
5 days	32, 30, 25, 32, 34, 32, 36, 30, 30, 31.	31.2 (16.4)	511.7
12 days	32, 36, 39, 37, 31, 36, 39, 42, 40, 37.	36.7 (16.4)	601.9
26 days	43, 44, 34, 50, 40, 41, 42, 38, 40, 38.	41.0 (16.4)	672.4
40 days	47, 53, 51, 45, 40, 60, 51, 47, 41, 60.	49.5 (16.4)	811.8
51 days	54, 52, 42, 52, 45, 56, 45, 50, 50, 53.	49.9 (16.4)	818.4
58 days	65, 50, 61, 65, 50, 47, 57, 62, 53, 40.	54.8 (16.4)	898.7
124 days	52, 48, 52, 50, 53, 45, 46, 50, 49, 54.	49.9 (16.4)	818.4

chicks, 601.9 μ; 26-day birds, 672.4 μ; 40-day fowl, 811.8 μ; 51-day chicken, 818.4 μ; the 58-day bird 898.7 μ; and the 124-day fowl, 818.4 μ.

In this study the heights or lengths of the duodenal villi increased with the ages of the chickens up to 58 days. The 124-day chickens, however, showed villi no larger (longer) than were those of the 51-day bird.

pH of the Duodenal Worm Habitat

In searching for possible differences between the duodena of the older and younger chickens, the pH of the worm habitats of the chickens of Group III was taken when the birds were killed. The Quin-Hydrone method was used. The results are given in Table 2. Of the 10 chickens

Table 2. Potential hydrogen of the duodenal habitat of *A. lineata* in chickens of various ages. Parasitized birds were infected with *A. lineata*.

Chicken Number	Condition of chickens	Age of chickens	pH of habitat
A541	Normal	26 days	6.71
A545	Normal	26 days	6.89
A524	Normal	40 days	6.84
A531	Normal	40 days	6.99
A519	Parasitized	55 days	6.71
A508	Parasitized	55 days	7.15
A502	Parasitized	71 days	5.56
A411	Normal	176 days	6.70
A410	Parasitized	176 days	6.16
A412	Parasitized	176 days	6.11

examined five were without nematodes and five were infected with A. lineata. No constant differences in pH occurred between the duodena of birds infected with worms and those uninfected (normal). These findings are in accord with those of Ackert (1931) who found that among 56 chickens there was wide variation in pH in both infected and uninfected fowls, the range having been 5.7 to 7.5. While there is a slight tendency for the older birds (Table 2) to have a lower pH range, the highest pH concentration (7.15) was in the duodenum of a 55-day bird. Whether or not the pH is a factor in age resistance to duodenal parasitism can only be determined definitely after more intensive study.

Counts of Goblet Cells in Chickens

On examining the duodenal epithelia on several slides, it was noted that goblet cells were more numerous in some than in others. On checking it was found that the slides with the larger numbers of goblet cells were from the older birds. This seemed rather striking since a function of goblet cells is to secrete mucus, a lubricant of the intestine. A greater amount of mucus in older chickens would make it more difficult for the A. lineata to remain in their hosts and thus account for the smaller numbers of these worms in the older chickens. This variance in the numbers of goblet cells was apparently a new observation and warranted accurate counting of duodenal goblet cells in chickens of various ages.

The first count of goblet cells in the preliminary study was made

on one side of a whole villus from crypt to apex, and this number doubled to represent the number for both sides of the villus. Villi had been picked at random and only those counted which were sectioned longitudinally through the center of the villar axis. (See Plate I)

As seen in Table 3, the average number of goblet cells per longitudinal section (10 μ thick) of a villus was 14.3 for the 2-day chick, 26.0 for the 5-day chick, 41.0 for the 26-day chick, 48.5 for the 40-day chick, 60.6 for the 51-day chick, 90.2 for the 58-day chick, and 93.5 for the 124-day bird. This showed a gradual increase in the number of goblet cells along the sides of the villi as the age of the chicken increased.

Table 3. Counts (10 to 18) of goblet cells in median longitudinal sections (10 μ thick) through villi. All chickens normal (uninfected).

Age of chickens	Counts of villar goblet cells	Total	Average goblet cells per villus
2 days	7,19,17,13,15,17,9,13, 14,17,16,18.	172	14.3
5 days	22,22,18,36,36,24,18,18, 32,20,34,28,24.	338	26.0
26 days	46,50,48,28,46,40,30,52, 62,50,44,44,36,46,40,24, 22,34,38.	780	41.0
40 days	48,38,58,46,42,44,64,50, 34,64,46.	534	48.5
51 days	76,74,72,58,61,78,44,62, 52,54,58,68.	727	60.6
58 days	110,66,102,64,90,108,98, 80,92,90,92.	992	90.2
124 days	78,98,90,110,84,96,105, 98,90,118,62.	1029	93.5

The tips (apices) of the duodenal villi were next studied for goblet cells. The counts were upon areas of the epithelia 122 μ long by 10 μ thick near the tips of villi. Ten counts were made for each bird and the average determined. The results of the counts, as given in Table 4, show that there was a gradual increase in the number of goblet cells per given area as the age of the chicken increased, up to 124 days. For example, in the 5-day chicken the average number of goblet

Table 4. Counts of duodenal goblet cells of chickens. Each count was made in a length of 122 μ along one side of the apex of a villus.

Age of chickens	Counts of goblet cells	Total	Average per chicken
2 days	5,5,2,1,1,4,3,1,3,4	29	2.9
5 days	5,4,2,1,5,5,4,1,5,5	37	3.7
12 days	5,3,4,3,5,7,7,5,7,6	50	5.0
26 days	8,9,3,6,8,8,8,9,7,7	73	7.3
40 days	6,7,11,10,8,8,8,5,7,7	77	7.7
51 days	9,6,5,7,9,6,10,7,6,8	73	7.3
58 days	7,7,9,10,11,8,10,8,9,8	87	8.7
71 days	11,9,7,6,11,10,10,11,11,7	93	9.3
124 days	10,13,10,11,11,10,13,9,11,9	107	10.7
131 days	8,7,9,10,11,10,12,8,9,8	92	9.2
313 days	12,8,5,6,7,5,8,9,8,5	73	7.3
320 days	7,7,6,6,10,8,11,8,16,11	90	9.0

cells per area was 3.7, for 26-day chickens 7.3, for 58-day 8.7, for 71-day 9.3, for 124-day 10.7, and for 320-day 9.0. There was a drop in the average number of goblet cells per area in the 313-day bird. However, the slides for this bird were not very good, and the counts were based on a single chicken. Nevertheless, there was a definite trend toward increased numbers of duodenal goblet cells with increased age up to 124 days.

The arrangement of goblet cells in the duodenal epithelia of chickens is shown in the line drawings of Plate I. While the goblet cells are larger in the older chickens, the most evident difference is the greater number of goblet cells per area in the duodenal epithelia of older chickens than in younger ones. Actual pictures of the goblet cells as they occurred in chickens of these ages are shown in the photomicrographs of Plate II, Plate IIa.

In the preliminary observations, it was noticed that the goblet cells in the younger birds seemed to be more numerous towards the middle than at the tips of the villi, and so counts were made of areas 122 μ by 10 μ at the middle of the duodenal villi of chickens of five different ages.

The data from these counts as given in Table 5 show that the average number of goblet cells per middle villar area of the 124-day group was 11.3 goblet cells as compared with 6.0 goblet cells for the 5-day group; almost twice as many per area in the older birds. The average

Table 5. Counts of goblet cells in areas 122 μ by 10 μ on one side (middle region) of the villi.

Age of chickens	Counts of goblet cells	Total	Average per chicken
5 days	6,9,7,3,5,4,5,6,9,6	60	6.0
26 days	10,4,8,7,8,8,9,5,9,9	77	7.7
51 days	8,9,8,6,11,7,8,9,9,9	84	8.4
124 days	10,12,15,13,16,11,8, 7,9,12	113	11.3
313 days	9,6,9,8,12,6,10,11,6,7	84	8.4

numbers of goblet cells were slightly larger than those near the tip of the villi. While considerable variability occurred in the 10 counts for each bird in this study, the differences were quite evenly distributed. Differences of from 5 to 8 goblet cells per area occurred in the counts of all 5 chickens, so that increases in average numbers of goblet cells with age up to 124 days were not due to marked variation.

Herrick (1926), studying the growth of young A. lineata during 10 days of parasitism in chickens of various ages, found that as the age of the fowls increased their resistance to infection with the worms increased. The peak of resistance in his studies was reached at 103 days (Table 6). This increase of resistance with age found by Herrick is in close agreement with the increase in numbers of duodenal goblet cells

Table 6. Evidence of age resistance of chickens to the nematode Ascaridia lineata during the first 10 days of parasitism (from Herrick, 1926).

Age of chickens when fed Ascaridia eggs	Mean lengths of worms (mm.)
5 days	5.6
10 days	5.4
15 days	5.3
27 days	2.2
30 days	3.9
45 days	3.4
60 days	2.9
80 days	2.1
90 days	0.7
103 days	0.4
140 days	0.4
180 days	0.4
240 days	0.4 (.38)

which reach their peak in chickens somewhat over 100 days old.

That a relation may exist between the mucus secreted by the goblet cells and the ability of the A. lineata to remain in the chickens is possible. It has been observed repeatedly on opening the duodena that mucus was more abundant in the older than in the younger chickens. This would aid the chicken in eliminating the weaker worms and thus possibly act as a factor in age resistance to the numbers of A. lineata.

Counts of Goblet Cells in Rats

To ascertain whether or not the phenomenon of increased numbers of duodenal goblet cells found in older fowls was confined to chickens alone or characteristic of other animals as well, histological studies were made of the duodenal epithelia of a number of laboratory rats* of different ages. Rats were obtained and killed, and portions of the intestines, a few centimeters posterior to the entrance of the bile and pancreatic ducts, removed. All tissues of the rat were fixed in "susa" fixative.** Sections of tissues were cut 10 μ and 6 μ thick and stained with alum-haematoxylin-triosin. Counts were made of those sec-

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*The writer is indebted to Miss Olga Saffrey of the Department of Food Economics and Nutrition for furnishing the rats for these studies.

**The reason for using "susa" was that in the preparation of the slides of chicken material it was found that "susa" as a fixative produced less distortion of tissue and no separation of the axial villar cells from the epithelium which occurred with Gilson's fixative. In using dioxane as a dehydrating agent it was found that the tissues were never too hard to section as was sometimes the case with tissues treated by the alcohol-toluol method; also the dioxane-paraffin process was more rapid and simple.

tions which had been cut 10 μ thick, and other counts were made of those sectioned 6 μ thick.

The results of the counts of duodenal goblet cells of rats made at the tips of villi are given in Table 7. The 6- and 7-day rats had no goblet cells. Those 22 days old averaged 1.2 goblet cells per area; 30-day, 1.5; 43-day, 2.4; 66-day, 3.3; 97-day, 3.0; 123-day, 3.4; 245-day, 3.6; 555-day, 3.7; and the rats 646 days averaged 3.2 goblet cells per area. These results likewise show a gradual increase in the number of duodenal goblet cells as the rats grew older.

Table 7. Counts of goblet cells in areas of 122 μ by 10 μ at tips of villi of rat duodena.

Age of rats	Counts of goblet cells	Total	Average
6 days	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	0
7 days	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	0
22 days	2, 0, 1, 0, 2, 1, 1, 4, 0, 1	12	1.2
30 days	2, 2, 2, 1, 1, 1, 3, 1, 1, 1	15	1.5
43 days	3, 2, 2, 3, 2, 1, 3, 3, 0, 5	24	2.4
66 days	2, 4, 2, 3, 2, 3, 5, 5, 3, 4	33	3.3
97 days	3, 3, 3, 4, 3, 3, 1, 5, 2, 3	30	3.0
123 days	3, 4, 4, 2, 2, 4, 3, 4, 5, 4	34	3.4
245 days	3, 3, 2, 2, 6, 5, 3, 6, 2, 4	36	3.6
555 days	4, 2, 3, 6, 4, 4, 4, 3, 4, 3	37	3.7
646 days	2, 2, 3, 4, 3, 3, 4, 4, 3, 4	32	3.2

Counts of duodenal goblet cells were next made along the middle (one side) of the rat villi. The data from the counts are given in Table 8.

Table 8. Counts of goblet cells in areas of 122 μ by 10 μ at the middle of rat villi.

Age of rats	Counts of goblet cells	Total	Average
6 days	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	0
7 days	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	0
22 days	2, 0, 1, 3, 2, 1, 1, 2, 3, 2	17	1.7
30 days	2, 0, 3, 4, 2, 4, 1, 2, 4, 1	23	2.3
43 days	1, 4, 3, 3, 3, 3, 2, 3, 2, 3	27	2.7
66 days	7, 2, 3, 3, 3, 3, 2, 4, 3, 3	33	3.3
97 days	5, 6, 7, 7, 7, 3, 4, 3, 2, 3	46	4.6
123 days	3, 5, 6, 2, 5, 4, 6, 4, 5, 4	44	4.4
245 days	3, 4, 5, 3, 6, 5, 3, 3, 4, 3	39	3.9
555 days	6, 5, 3, 6, 5, 8, 4, 5, 5, 5	52	5.2
646 days	7, 7, 9, 5, 5, 7, 5, 9, 6, 7	67	6.7

The results of the counts given in Table 8 show that the 6- and 7-day rats had no goblet cells along the middle of the villi. The 22-day rats averaged 1.7 goblet cells per area; 30-day, 2.3; 43-day, 2.7; 66-day, 3.3; 97-day, 4.6; 123-day, 4.4; 245-day, 3.9; 555-day, 5.2; and 646-day, 6.7 goblet cells per area. This table also shows a gradual increase in the number of goblet cells per area of 122 μ by 10 μ as the age of the rat increased. When compared, the goblet cells were somewhat

more numerous at the middle portions of the villi than at the tips, especially in rats 97 or more days of age.

Table 9. Counts of goblet cells in areas 122 μ by 6 μ at the tips of rat villi.

Age of rats	Counts of goblet cells	Total	Average
6 days	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	0
7 days	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	0
22 days	0, 1, 3, 0, 2, 0, 0, 1, 1, 0	8	0.8
43 days	3, 1, 1, 2, 1, 1, 4, 3, 2, 3	21	2.1
62 days	1, 1, 2, 1, 3, 3, 1, 2, 5, 1	20	2.0
66 days	2, 2, 5, 3, 3, 0, 3, 3, 4, 1	26	2.6
125 days	4, 2, 4, 3, 2, 0, 4, 4, 4, 3	30	3.0
245 days	5, 4, 5, 3, 5, 4, 2, 2, 4, 3	37	3.7
646 days	4, 3, 3, 3, 2, 4, 3, 3, 3, 2	30	3.0

To obtain clearer definition of the goblet cells, some of the rat intestine was cut 6 u thick and studied. The results of the counts, which are given in Table 9, show that there was a gradual increase in the average number of goblet cells per area at the tips of the villi up to 245 days of age. However, as would be expected, the numbers were not as large as those of the sections 10 μ thick.

Counts were next made of 122 by 6 micron sections of areas at the middle of the rat villi.

Again, as shown by Table 10, there was a rather gradual increase

of goblet cells with the increase in age of rats up to 646 days.

Table 10. Counts of goblet cells in areas 122 u by 6 u at the middle of rat villi.

Age of rats	Counts of goblet cells	Total	Average
6 days	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	0
7 days	0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0	0
22 days	0, 1, 3, 2, 4, 1, 1, 5, 1, 0	18	1.8
43 days	3, 2, 3, 2, 5, 1, 2, 1, 2, 1	22	2.2
62 days	3, 3, 2, 4, 3, 2, 3, 2, 4, 3	29	2.9
66 days	2, 4, 3, 4, 4, 3, 4, 3, 3, 3	33	3.3
97 days	2, 3, 2, 2, 5, 2, 3, 3, 4, 2	28	2.8
123 days	4, 2, 2, 3, 5, 4, 4, 3, 5, 5	37	3.7
245 days	2, 5, 4, 2, 4, 4, 3, 6, 6, 2	38	3.8
646 days	6, 8, 5, 4, 4, 10, 3, 4, 8, 6	58	5.8

To give a more accurate average of the numbers of duodenal goblet cells per area in the rats of various ages, the data from Tables 7 to 10 were combined. The resulting averages are shown in Table 11.

From this study and from Plates III, IV, IVa, it is seen that the phenomenon of increased numbers of duodenal goblet cells is characteristic of a mammal as well as a fowl. The studies of Africa (1931), Chandler (1932), Graham (1932), Sheldon (1937), and others show that rats may develop an age resistance to their intestinal nematodes.

Table 11. Averages of combined data on counts of duodenal goblet cells at tips and middle of rat villi (lengths of area studied in each case, 122 μ).

Age of rats	Sections 6 μ thick: Aver. Tip + Aver. Middle = Aver. for area 244 by 6μ	Sections 10 μ thick: Aver. Tip + Aver. Middle = Aver. for area 244 by 10μ
6 days	0 + 0 = 0	0 + 0 = 0
7 days	0 + 0 = 0	0 + 0 = 0
22 days	0.8 + 1.8 = 2.6	1.2 + 1.7 = 2.9
43 days	2.1 + 2.2 = 4.3	2.4 + 2.7 = 5.1
62 days	2.0 + 2.9 = 4.9	
66 days	2.6 + 3.3 = 5.9	3.3 + 3.3 = 6.6
97 days		3.0 + 4.6 = 7.6
123 days	3.0 + 3.7 = 6.7	3.4 + 4.4 = 7.8
245 days	3.7 + 3.8 = 7.4	3.6 + 3.9 = 7.5
555 days		3.7 + 5.2 = 8.9
646 days	3.0 + 5.8 = 8.8	3.2 + 6.7 = 9.9

DISCUSSION

These results upon increased numbers of duodenal goblet cells in older animals, when compared with the gradual increase in the resistance of chickens to the growth of this nematode, as shown by several investigators cited, seem to indicate that goblet cells may be a possible factor in age resistance of chickens to A. lineata. Comparison of

Figures 3 and 4 shows further the possibility of a relationship between the two phenomena. The age when the peak in numbers of duodenal goblet cells in chickens is reached occurs close to the age of greatest resistance of the chickens to the nematodes, namely at three to four months of age.

A somewhat similar comparison is available between the duodenal goblet cells of rats, which increase gradually in these animals up to 646 days of age, and increased resistance of rats to their intestinal nematodes as cited earlier in this paper.

Concerning the duodenal goblet cells as possible factors in resistance of chickens to the nematode A. lineata, Ackert, Edgar, and Frick (1939) obtained evidence which indicated that mucus from duodenal goblet cells contains a factor that may inhibit the growth of the nematodes. These authors, by introducing mucus from the goblet cells into artificial culture media in which the A. lineata will grow (Ackert, Todd, and Tanner, 1938), obtained a retardation in the growth of the worms (Table 12).

Three kinds of culture media were used: (1) an isotonic-salt solution which contained no nutriment; (2) an isotonic-salt-dextrose solution which contained regular nutrient media and which served as a control and (3) an isotonic-salt-dextrose solution into which duodenal mucus (autoclaved) was introduced. Worms (A. lineata) of the same age were removed from chicken hosts, measured and placed in dishes containing one of the three media. At the end of three days all worms were

Table 12. Results of tests for an inhibitory nematode growth factor in duodenal mucus from chickens three months old.

Culture media	Larvae in culture days	Gain in length average (mm.)	Percent gain
Experiment I			
1. Isotonic-salt-dextrose solution plus mucus	3	2.2	4.0
2. Isotonic-salt-dextrose solution (control)	2.5	11.8	18.5
3. Isotonic-salt solution (no nutriment)	2	2.5 (loss)	0.0
Experiment II			
1. Isotonic-salt-dextrose solution plus mucus.	3	8.5	12.8
2. Isotonic-salt-dextrose solution (control)	3	27.0	36.9
3. Isotonic-salt solution (no nutriment)	3	.2 (loss)	0.0
Experiment III			
1. Isotonic-salt-dextrose solution plus mucus	3	2.1	2.9
2. Isotonic-salt-dextrose solution (control)	3	14.3	19.8
3. Isotonic-salt solution (no nutriment	4	11.0 (loss)	0.0

measured. The results of Experiment I showed that in isotonic-salt solution the worms lost an average of 2.5 mm. Worms in the nutrient solution grew 11.8 mm. (an increase of 18.5 per cent) whereas the worms in the nutrient solution that contained mucus grew only 2.2 mm. or an increase of 4 per cent. The results of Experiments II and III were of a similar nature. These results indicate that duodenal mucus from chickens three months of age contains a substance or factor that retards or hinders the growth of the fowl nematode Ascaridia lineata.

In view of the presence of an inhibitory growth factor in the mucus, it seems probable that a relationship exists between the increased numbers of goblet cells in older animals and age resistance of such animals to intestinal nematodes.

SUMMARY

A brief review of literature on age resistance of animals to parasitism is presented as part of a study on a possible factor in age resistance.

Fewer and shorter nematodes (Ascaridia lineata) from older than from younger chickens in various experiments led to a histological search for structural differences in the intestines of chickens of various ages.

Three groups of chickens were used in a histological study of the duodenal habitat of Ascaridia lineata. The chickens were single comb white leghorns.

Portions of the duodena from chickens ranging in age from 2 days

to 320 days were fixed, imbedded, sectioned, mounted, and stained. The chief fixatives were Gilson's fluid, corrosive sublimate, and Heidenhain's "susa." The chief staining combination was alum-haematoxylin-triosin.

Cells, fibers, muscle layers, glands, etc., were found to vary in size or number with age.

The most striking difference observed was the larger numbers of goblet cells in the duodenal epithelia of older chickens than of younger ones. For example, chickens 124 days of age had an average of 10.7 goblet cells per villar area of 122 by 10 microns as compared with 3.7 for the 5-day chick. These observations of larger numbers of duodenal goblet cells in older than in younger chickens appear to have been new to science. The results of numerous counts of goblet cells show a gradual increase in number from chicks 2 days of age to fowls 124 days of age, after which no further increases occurred.

These increases in numbers of duodenal goblet cells parallel rather closely the increases in age resistance of chickens to the large nematode Ascaridia lineata. The peaks, both in numbers of goblet cells and resistance to the growth of the nematodes, appear to occur in chickens 3 to 4 months of age.

The results of similar studies upon laboratory rats showed that older rats likewise have larger numbers of duodenal goblet cells than do young rats. For example, rats 646 days of age had an average of 9.9 goblet cells per area of 244 u by 10 u as compared with 2.9 goblet cells

in rats 22 days old. Goblet cells appeared to be lacking in rats 6 or 7 days of age. As in the case of chickens, there was a gradual increase in the numbers of duodenal goblet cells of the rats as they grew older.

From these results and from those of a related study in which mucus from the goblet cells was found to retard the growth of Ascaridia lineata in artificial media, it seems highly probable that a relationship exists between duodenal goblet cells and age resistance of animals to intestinal parasitism.

ACKNOWLEDGMENTS

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EXPLANATION OF PLATE I

Fig. 1. Section of villus of 5-day chicken showing duodenal goblet cells. gc, goblet cell.

Fig. 2. Section of villus of 124-day chicken showing duodenal goblet cells. gc, goblet cell.

Drawings with aid of a microprojector.

PLATE I

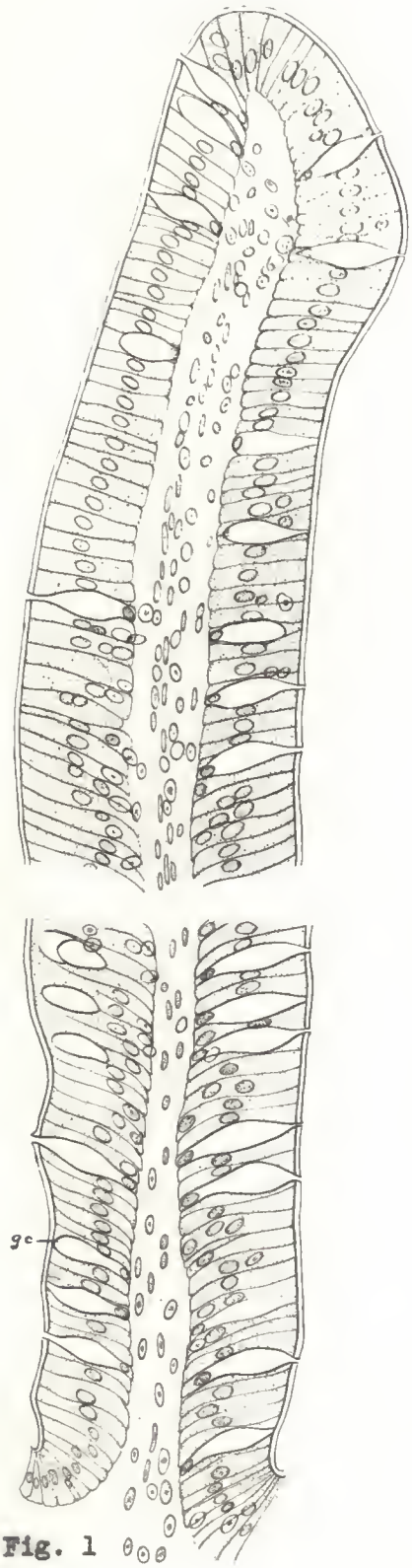


Fig. 1

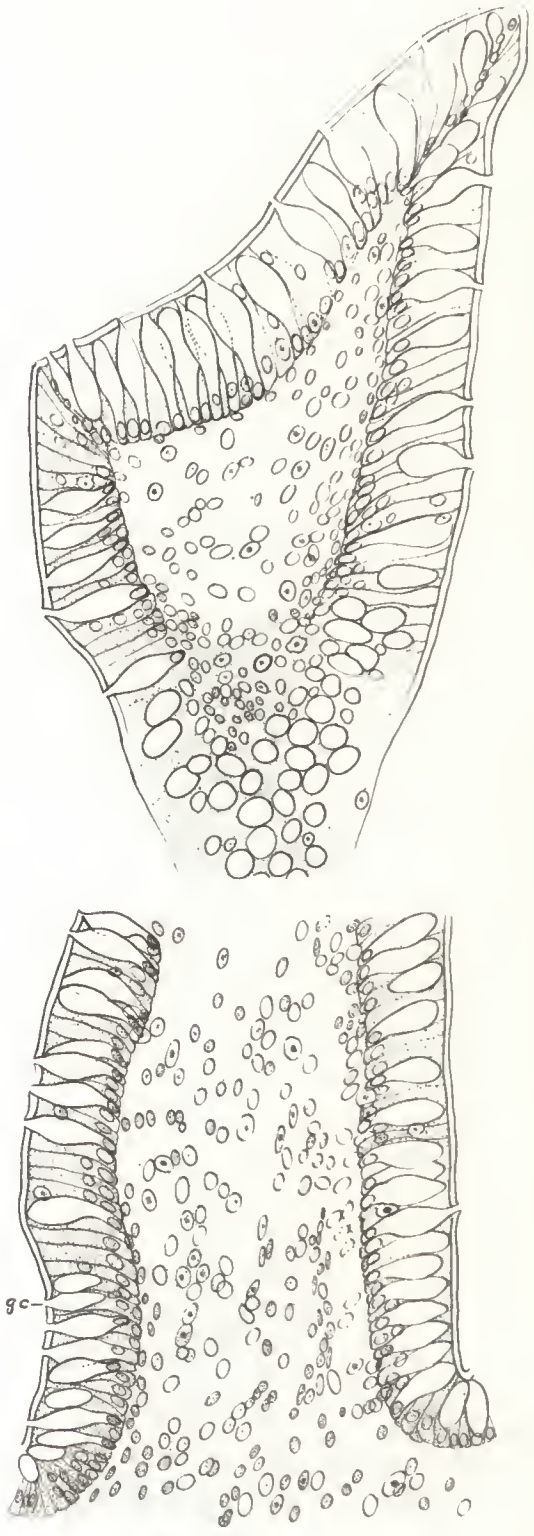


Fig. 2

EXPLANATION OF PLATE II

Photomicrograph (low power) of cross section through the duodenum of a 5-day chicken. Portion between white lines shows median longitudinal sections of villi in the epithelia of which goblet cells were counted.

PLATE II

EXPLANATION OF PLATE IIa

Photomicrograph (low power) of cross section through the duodenum of a 124-day chicken. Central portions to right of black line show median longitudinal sections of villi. Numerous goblet cells seen in epithelia.

PLATE IIa

EXPLANATION OF PLATE III

Fig. 1. Section of villus of 7-day rat. No goblet cells found.

Fig. 2. Section of villus of 646-day rat, showing duodenal goblet cells. gc, goblet cell.

Drawings with aid of a microprojector.

PLATE III

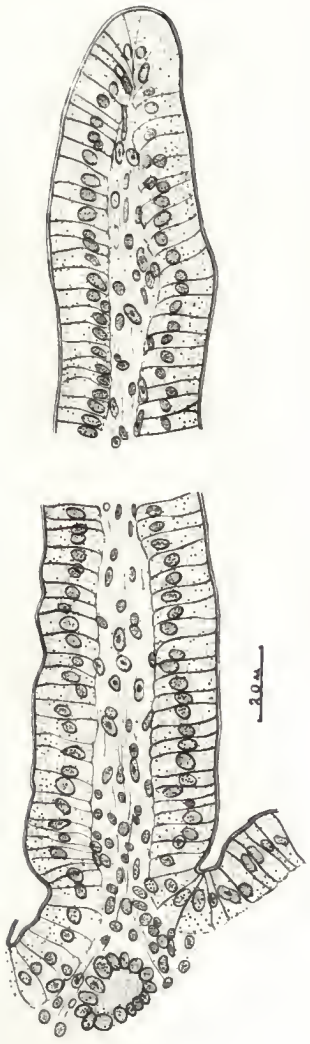


Fig. 1

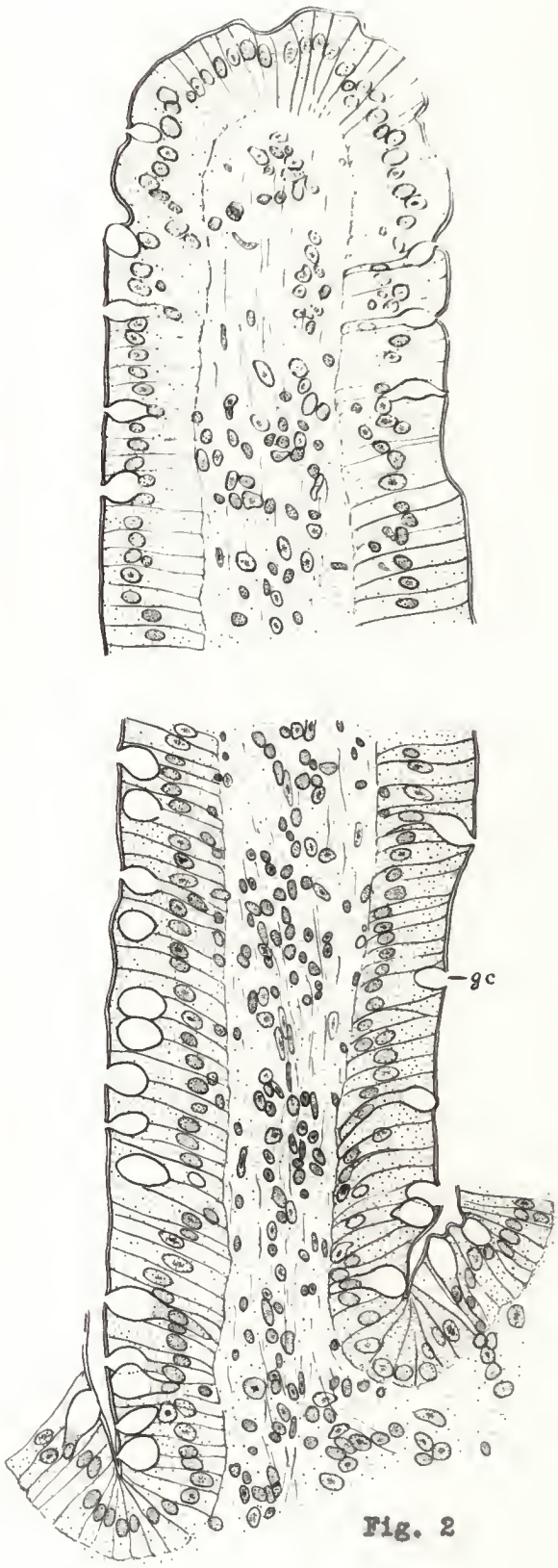
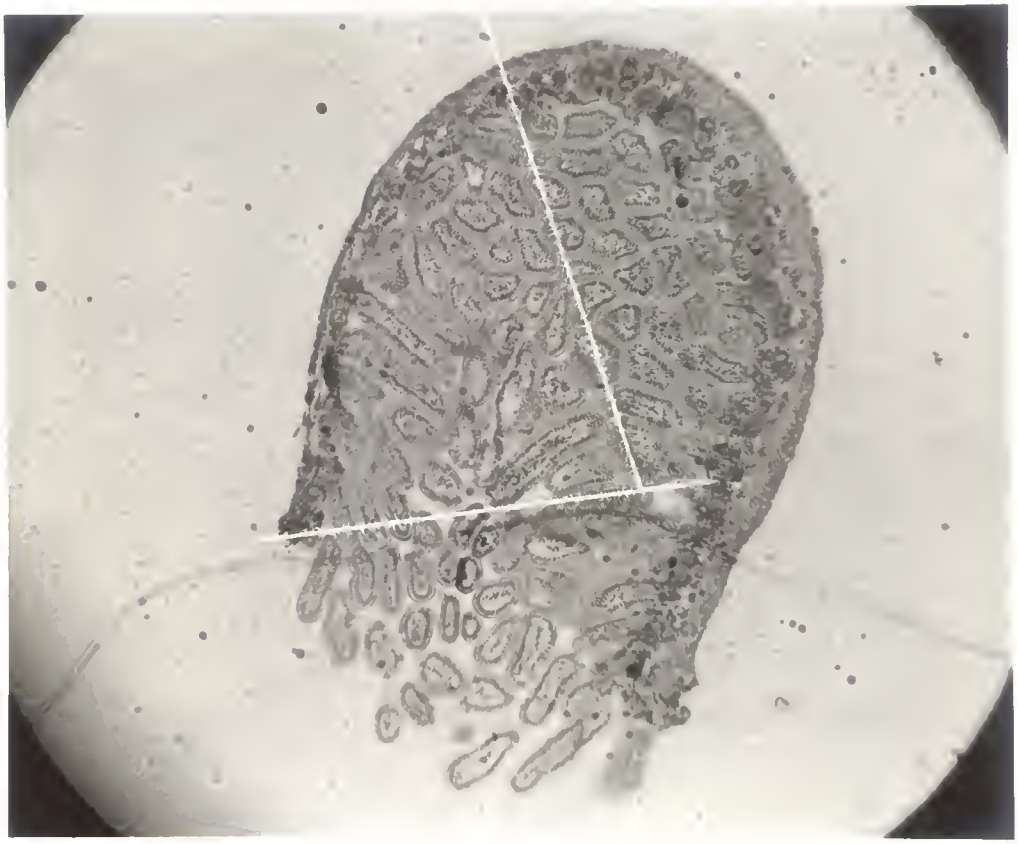


Fig. 2

EXPLANATION OF PLATE IV

Photomicrograph (low power) of cross section through duodenum of a 7-day rat. Portion in angle shows two villi in median longitudinal section. No goblet cells found.

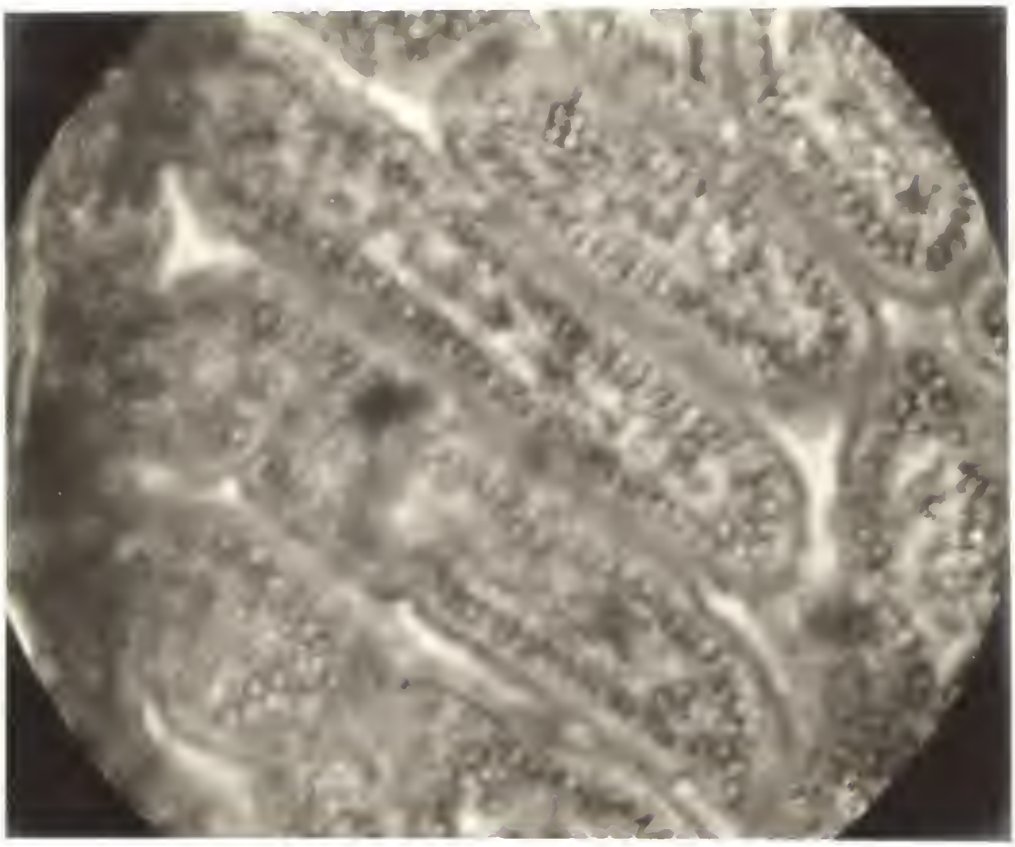
PLATE IV



EXPLANATION OF PLATE IVa

Photomicrograph (high power) of cross section through the duodenum of a 646-day rat, showing numerous goblet cells.

PLATE IVa



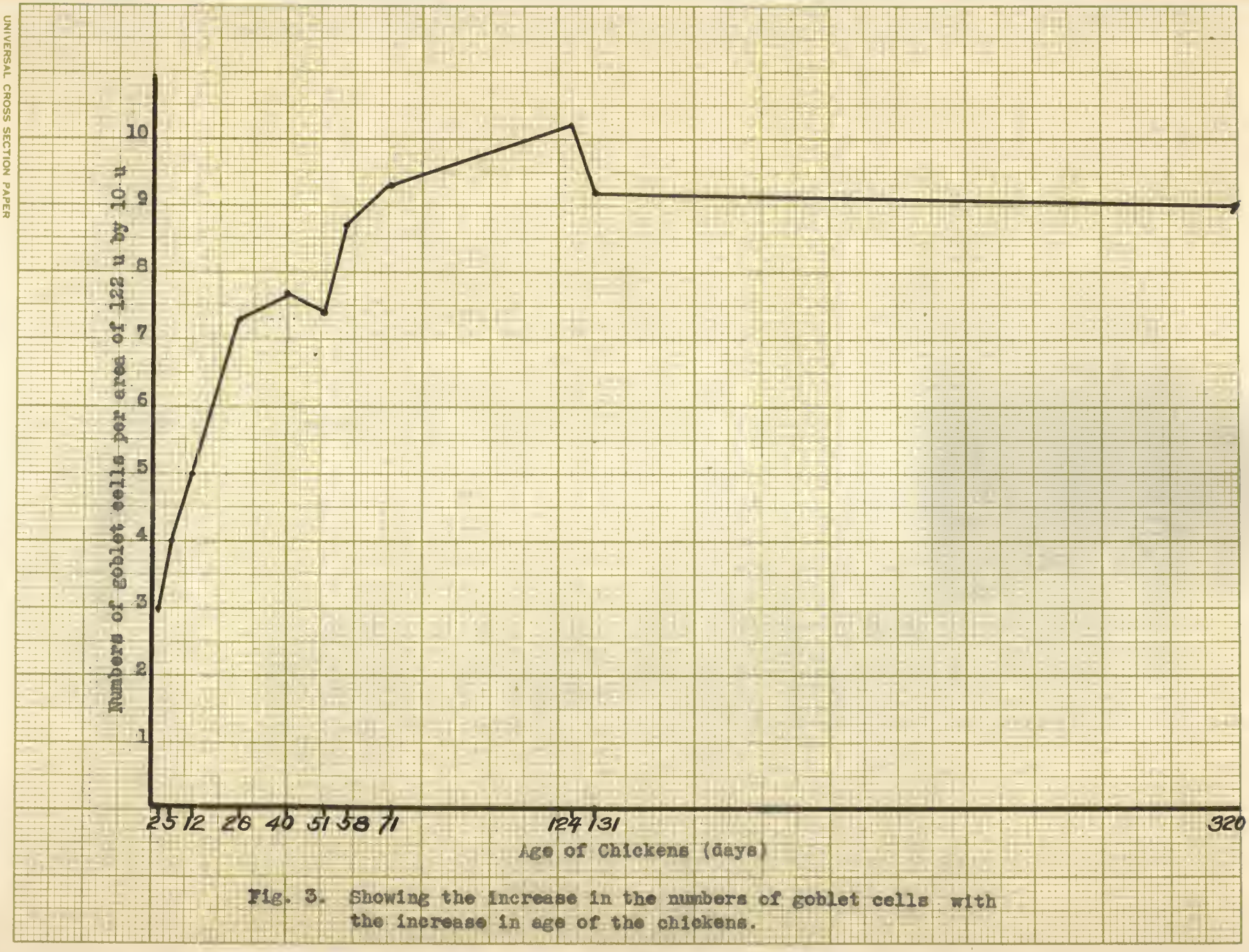


Fig. 3. Showing the increase in the numbers of goblet cells with the increase in age of the chickens.

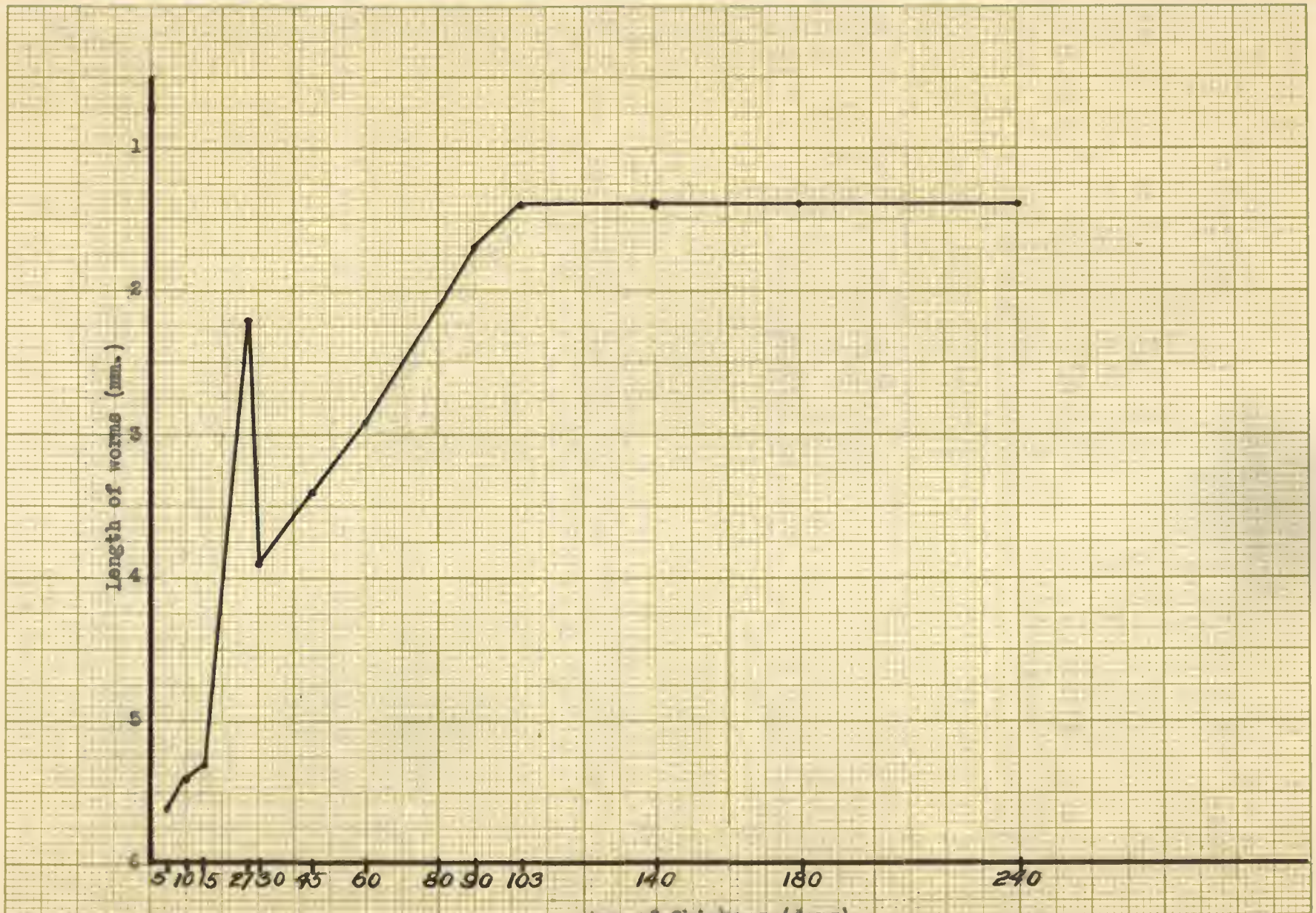


Fig. 4. Showing increase of age resistance of chickens to the A. lineata as determined by the lengths of the worms from chickens of different ages (data from Herrick, 1926).