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EXPERIMENTAL ASCARIDIASIS IV. VARIOUS ASPECTS OF ASCARID BEHAVIOUR WITHIN THE ORGANOPHENOTES FOLLOWING EXPERIMENTAL INFECTION OF CHICKENS

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Abstract. Various aspects of the behaviour of *A. galli* in chickens given a single dose of 200, 500 and 1000 infective eggs were studied together with the influence of infective egg dose on the rate of larval attachment in the organism of the host, the site of residence of the individual worms in the intestine, the rate of growth; in this connection the possible factors in operation were discussed. Attention was given to the ununiform development of the nematode during early phases of its ontogenesis in association with ununiformity in the time of larval emergence from the egg-shells in the organism of the host, and to the inhibiting effect of mucin and specific host reactions. The jejunum, and there particularly the section below Mecker's diverticulum, was found to be the optimal site of ascarid location. Migration of ascarids was observed in both caudal and cranial direction from this site of predilection in the early phase of nematode development. It increased in intensity as the hatching processes of the nematodes attained their peak. In chickens of our experiment, we observed two moults of *A. galli* larvae, i.e., the second and third moult both occurring in the paramucosa of the intestine. Since no larvae were found in the tissue of the intestine, it has repeatedly been emphasized that *A. galli* does not need a histotropic phase in its life cycle.

In studies on the early postinfection phase we paid attention also to several aspects of the nematode's behaviour. We were interested mainly in these problems: the intensity of larval attachment in the organism of the host, the distribution and location of the larvae in the intestine, the rate of growth of the individual worms and their moulting processes within the organophenote following a single dose of 200, 500 and 1000 infective eggs respecively.

MATERIAL AND METHODS

The material used and the techniques employed were essentially those described in an earlier paper (Birová-Volosinovičová 1973). The same applies to the terms "larvae from the lumen" and larvae from the so-called "mucosa," referring to larvae recovered from the intestine by flushing it with a strong gush of water, or collecting them from the intestine after flushing.

The four groups in the experiment consisted of 39 chickens each. For group I the dose employed was 200 infective eggs of *A. galli* per bird, for group II 500, for group III 1000. The chickens of group IV (control group) were not infected. The small intestine of each bird in the experiment was divided into three sections (according to the division suggested by Moran and Mizelle 1957); each section was separately inspected and evaluated. Section one covered the duodenum and the first 10 cm of the jejunum below the outlet of the bile duct. Section two extended past Meckel's

diverticulum to the jejunum, section three from there to the base of the caeca. In addition we inspected the caeca and the large intestine.

The results shown in the figures were evaluated mostly in intervals of five days in order to obtain a more comprehensive picture despite the fact that the chickens were examined daily from day one to day 27 postinfection.

RESULTS

a) INTENSITY OF ATTACHMENT OF *A. GALLI* LARVAE IN THE ORGANISM OF THE EXPERIMENTAL CHICKENS

The worm recovery from three groups of chickens each given a single dose of 200, 500 and 1000 infective eggs of *A. galli* is shown in Table 1. The sum of nematodes recovered from the individual experimental groups indicated that the incidence of infection was highest in group III (1000 infective eggs), less high in group II (500 eggs) and lowest in group I (200 eggs). However, the evaluation of the percentage of hatched and attached larvae in relation to the total number of eggs administered changed the situation to the advantage of group II, with group I as second in succession. The percentage of larvae fixed to the organism of the experimental chickens was 8.4 and 6.0 respectively. The lowest intensity of attachment of larvae in the organism of the host occurred in group III receiving an infective dose of 1000 eggs (4.5 %).

Table 1. Intensity of larval attachment in the organism of experimentally infected chickens during the early phase of worm ontogenesis

Days postinfection of chickens	Worm recovery after infection Egg dose:		
	200	500	1 000
1—5	30	47	185 *
6—10	129	445	630
11—15	216	928 *	784
16—20	38	159	110
21—27	44 *	24	15
Sum:	457	1,603	1,724
Per cent distribution of larvae in the intestine of the chicken	6.0	8.4	4.5

* Framed numerals indicate maximum recovery in the individual groups of days in comparison with experimental groups II and III.

The recovery of ascarids, at their individual ontogenetical stages, from the chickens in each group independent on the infective dose indicated an increase in nematode numbers from day one postinfection to a maximum on day 15 p.i. From this day onwards, the number of *A. galli* decreased rapidly in the organism of the hosts until the termination of our experiment.

An interesting feature was the shifting of the maximum number of worms from one experimental group to the other during the course of infection (see framed numerals in the table). During the initial period of infection, i.e., from day one to day 10 p.i., nematode recovery was highest in group III; from day 11 to day 20 p.i. it was highest in group II, and from day 21 to day 27 p.i., the highest number of nematodes was recovered from group I.

b) DISTRIBUTION AND LOCALISATION OF *A. GALLI* IN THE INTESTINAL SECTIONS OF THE CHICKENS

The distribution of larvae and juvenile specimens of *A. galli* in the individual sections of the intestine of the chickens each given a single dose of 200, 500 and 1000 infective eggs respectively, is shown in Fig. 1. In all experimental groups the highest number

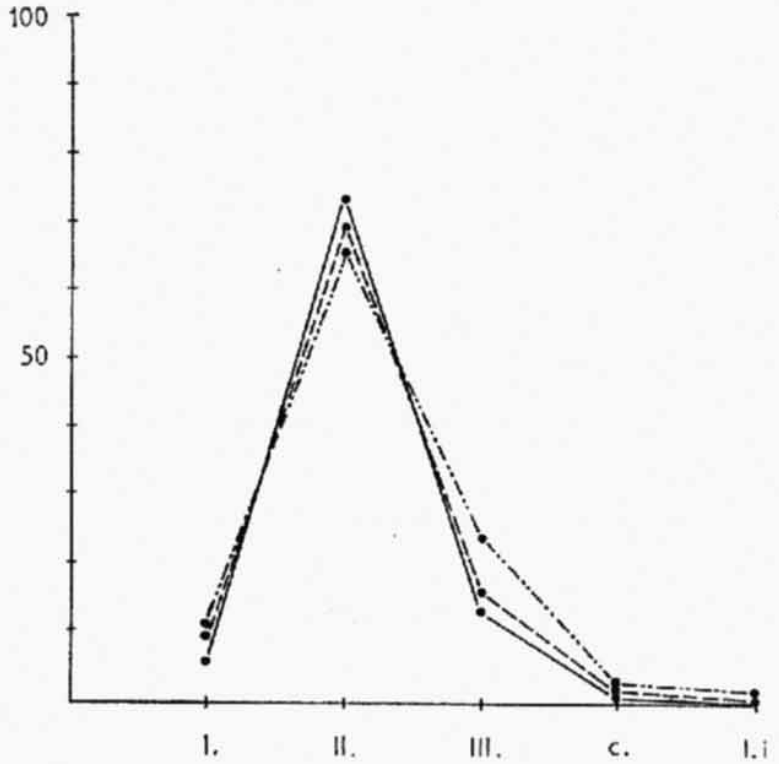

Fig. 1. Percentile incidence of *A. galli* larvae in the individual intestinal sections following experimental infection of chickens with a dose of 200 ———, 500 — — — and 1000 —·—·— infective eggs. I, II, III — sections of the small intestine, c. — caeca, l. i. — large intestine

of ascarids was recovered from intestinal section no. II, while the number of ascarids recovered from section no. III and I was lower. An occasional worm was recovered from the caeca and the large intestine. The general distribution of nematodes in the organophenote appears to be slightly influenced by the infective dose, i.e., the higher the number of eggs administered, the more worms "migrated" to section III and also to section I of the intestine. This was followed by a subsequent increase in the number of worms in the caeca and the large intestine.

Although section II of the intestine was observed to be the site of maximum ascarid location, certain "movements" of individuals were observed in the organophenote starting from day one postinfection to the termination of our experiment (Fig. 2). This ascarid "movement" was not influenced by the size of infective dose. The first

more intensive "translocation" of larvae was found to occur during the first five to eight days postinfection when an increased number of worms was recovered from section III, and later in section I. The second, more intensive translocation was recorded from day 11 to day 20 postinfection. At this time, an increased number of ascarids settled first in section I, later in section III of the intestine. Nematodes were recovered frequently also from the caeca and the large intestine.

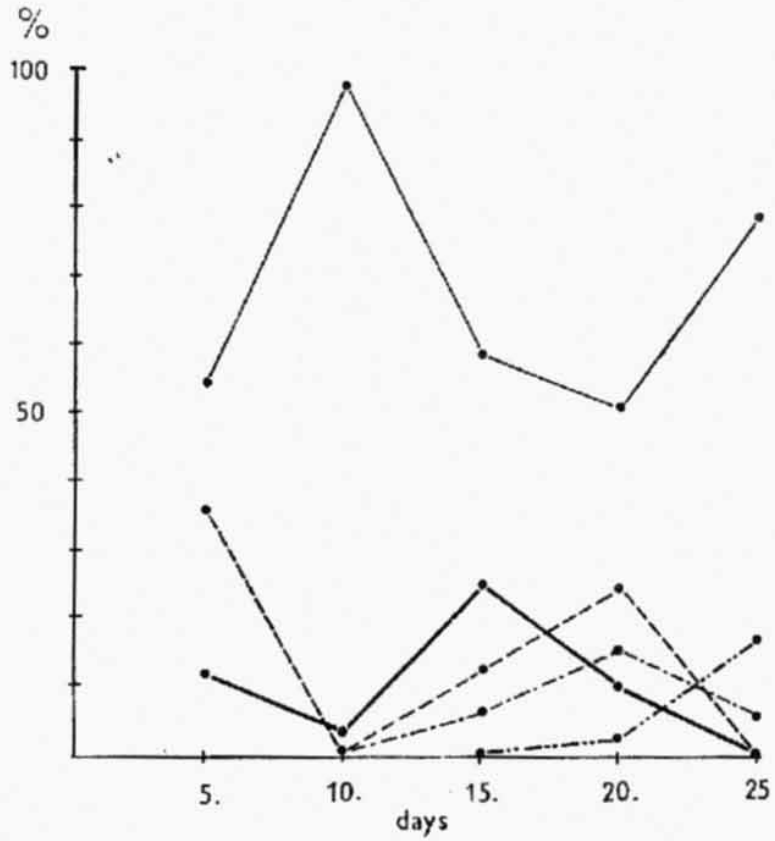

Fig. 2. Distribution of *A. galli* in the intestinal tract of experimental chickens during the early phase of nematode development. Ist intestinal section ———, IInd intestinal section — — —, IIIrd intestinal section — — —, caeca —.—.—, large intestine —..—..—..—

c) GROWTH AND DEVELOPMENT OF *A. GALLI* LARVAE

In order to determine differences in the length of larvae recovered from the lumen of the intestine and from the so-called "mucosa", and to obtain information concerning the influence of the size of infective dose on growth of the individual worms in the organophenote, the length of each ascarid was measured.

Until day 14 postinfection growth was almost uniform in *A. galli* larvae from both the intestinal lumen and the so-called "mucosa" (with a tendency for increased growth in larvae from the lumen), and not influenced by the size of infective dose. From this day onwards, growth of larvae from the lumen was more rapid than that of larvae from the so-called "mucosa" which persisted at the same growth level.

A comparison of trends in the growth of ascarids recovered from the individual experimental groups of chickens (Fig. 3) indicated that the length of the individual larvae from both the lumen and the so-called "mucosa" was generally bigger in birds given a dose of 200 infective eggs (group no. I) than in those of group no. II (500 eggs) and particularly in those of group no. III (1000 eggs). The differences in the length of worms were particularly marked in the later postinfection period.

A remarkable feature was the decline in the curve of growth on day 13 and 14 postinfection, and an immediate ascent of this curve in the following days recorded for all larvae from all experimental groups of chickens. It appears that during this period, but mainly at the age of 18—20 days, ascarid growth was intensive in chickens of all three experimental groups.

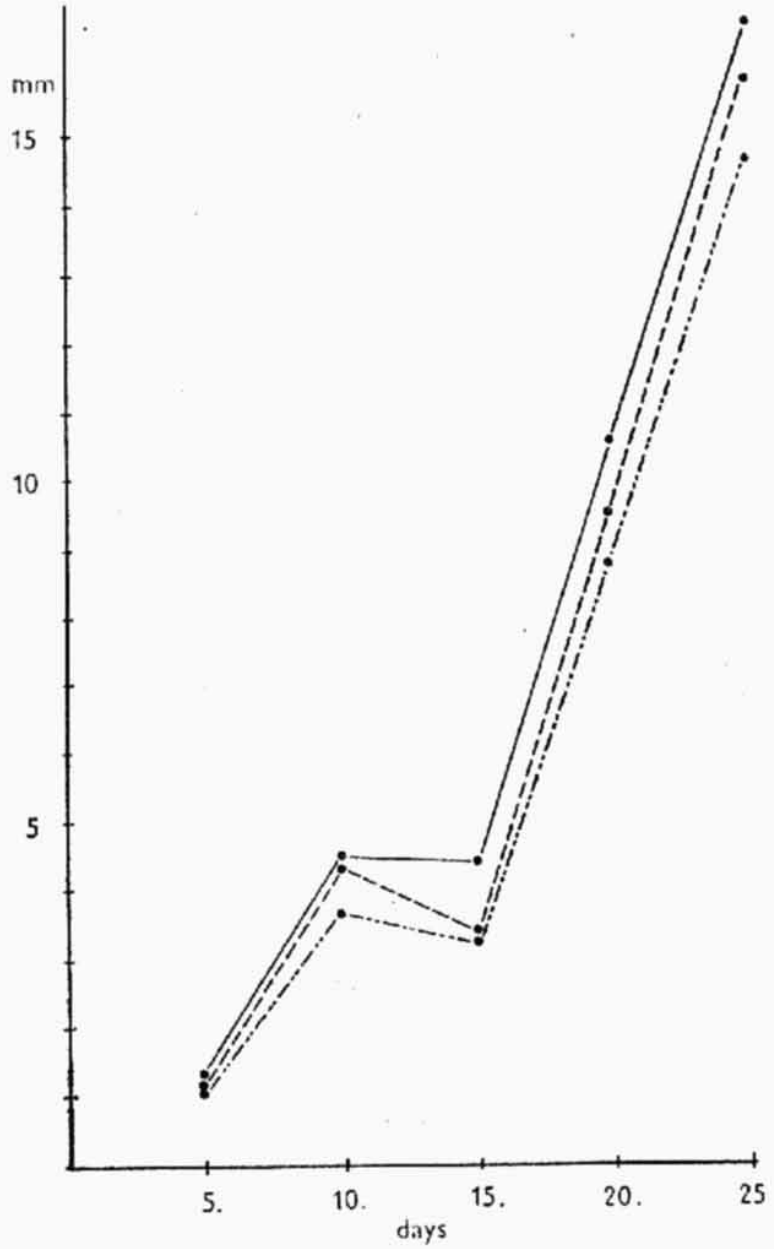

Fig. 3. Average length of *A. galli* in chickens infected with 200 ———, 500 — — — and 1000 infective eggs —. .—. .—

An interesting view on the development of *A. galli* during early developmental phases was offered by an analysis of their morphology. We evaluated simultaneously the larval stage attained and the moulting processes occurring at the individual days of our observation. A percentile representation of the individual forms is given in Fig. 4, separately for larvae recovered from the so-called mucosa and for larvae from the lumen of the intestine.

Fig. 4 shows that the metamorphosis of *A. galli* larvae was not uniform in the organophenote; this made it impossible to define exactly the time of changing of the individual

developmental forms. During the first days of infection, only second stage larvae, i.e., those which had completed their first moult inside the egg, were present in the intestinal spaces. These were found until day 10 postinfection, and again sparsely on day 14 p.i. The maximum number of these larvae was recovered from the so-called "mucosa" on day 6 p. i.

The incidence of second stage larvae was accompanied by the incidence of larvae displaying signs of their second moult. The maximum number of larvae completed their second moult on day 8 and again on day 14 and 18.

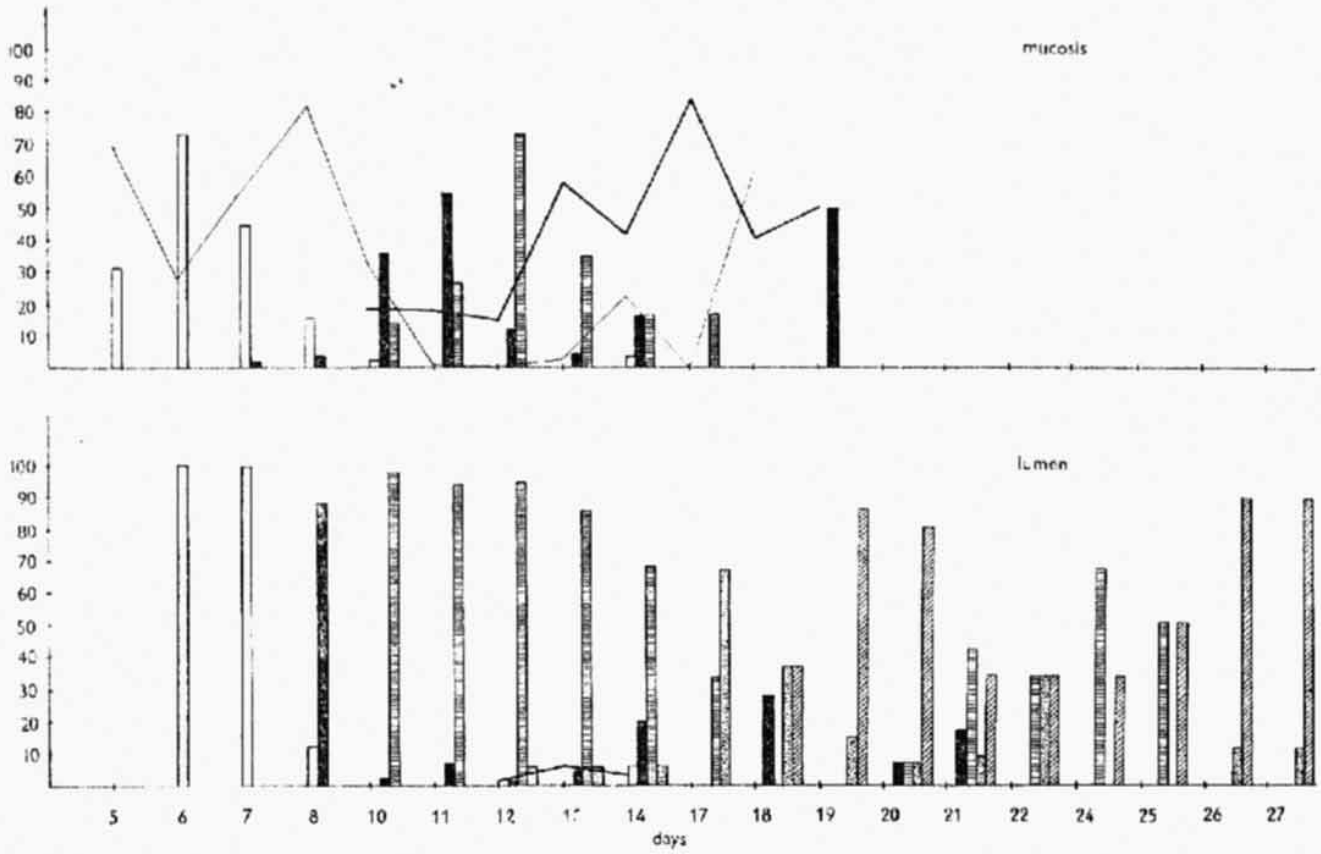

Fig. 4. Percentile representation of the individual developmental stages in chickens with experimental infection. Larvae completing their second moult ———, larvae completing their third moult ———, open columns — second stage larva, solid columns — third stage larva (early forms), hatched columns — third stage larva (late forms), dotted columns — fourth stage larva, cross-hatched columns — juvenile forms of the worm.

An occasional, early, third stage larva was recovered from the so-called "mucosa" on day 7 already, but most of these were found on day 11 p. i. Late forms of third stage larvae appeared in both the lumen and the so-called "mucosa" on day 10; they were recovered from the latter till day 17 postinfection, from the former till day 25 p.i.

The third larval moult started in the so-called "mucosa" on day 10 postinfection, the maximum number of larvae completed this moult on day 17 p.i. A small percentage of larvae completed their third moult in the lumen from day 12—14 of infection.

Fourth stage larvae and juvenile worms were recovered from the lumen only on day 12 and again on day 18. The maximum number of fourth stage larvae was found on day 17 of chicken infection.

One of the interesting features of these experimental infections was the mixture of larval stages that was found in the intestinal spaces of the infected chickens from day 10 to day 14 postinfection.

As regards the site of moulting of *A. galli* larvae, these processes occurred mostly in the so-called "mucosa" and not in the lumen of the intestine as shown in Fig. 4. The larvae completed their second moult exclusively in the so-called „mucosa", and only a small per cent of larvae at the stage of the third larval moult were located in the lumen of the intestine.

DISCUSSION

An at first increasing and later decreasing tendency in the intensity of infection with *A. galli* following experimental infection of chickens during the early developmental stage of the nematode has been discussed in earlier papers (Birová-Volosinovičová 1971, 1973). This tendency is evident also in Table 1: maximum numbers of ascarids were recovered from all experimental chickens (without regard to the size of infective dose) between day 11 — day 15 postinfection. From then onwards we observed a rapid decline in the incidence of host infection, i.e., on day 21—27 p.i., we found of the total number of ascarids recovered 9.6 % only in group no. I, 1.4 % in group no. 2 and 0.8 % in group no. III. It is indicated by our results that the higher the infective dose, the lower the number of worms present in the organism of the host in the later phase of ascarid ontogenesis, and vice versa. This phenomenon has been observed in experimental ascaridiasis by a number of authors (Dorman 1928 — cit. ex Ackert et al. 1936; Ackert et al. 1930; Shults and Daugalieva 1967; Dimitrov 1971; Birová-Volosinovičová 1973, and others) and has been considered by most of them as a sign of immunological reaction of the organism or, at least, of increased reactivity of the host to the infective agent. Shults and Daugalieva (1967) reported also a local reaction of intestinal tissue to the presence of the larvae followed mostly by worm elimination due to inflammatory processes and an increased peristalsis of the intestine. It is still in question whether this problem should not be studied also from the side of the parasite. In this connection, an interesting observation was made by several authors (Li 1933; Ackert and Whitlock 1941 — cit. ex Baron et al. 1960; Baron et al. 1960; Johnson 1970). While examining the alimentary system of both ascarids and chickens these authors found that early developmental stages of ascarids (up to an age of 50 days) fed mainly on the contents of the intestine and, hence, also on mucosa, desquamated cells, blood elements that are free in the lumen of the intestine, and on the intestinal bacterial flora. Other authors (Todd 1951; Johnson 1970; Johnson and Reid 1973) inferred that ascarids require the presence of bacteria in order to complete successfully their development. If chickens were given antibiotics the rate of larval attachment in the host was considerably reduced and larval growth was slower than that of the control group. May not a disturbance of the equilibrium of the intestinal microflora by a single large dose of infective eggs and, thereby, an impediment of further worm development in the organism of its host at a later ontogenetical phase be one of the reasons for a speedier elimination of larvae at higher infective egg doses? Although this hypothesis may be daring and lacks as yet experimental confirmation the fact that, in spite of a low worm burden in chickens infected with a high dose of eggs, the growth of the ascarids in our experiments was retarded, is in favour of this assumption. Also Todd (1951) claimed that an environment devoid of bacteria had an unfavourable effect on the growth of the worms. Dick (1971 — cit. ex Johnson and Reid 1973) observed in his study on the growth of nematodes in vitro that larval growth could be accelerated by adding bacteria from the duodenum of the chicken to the culture medium. Johnson and Reid (1973) observed in chickens differences in the rate of growth of ascarids in vivo on day 14, and mainly on day 21 postinfection, i.e., the

average body length of nematodes recovered from bacteriologically sterile hosts was 4.2 and 12.7 mm respectively, that of the controls was 4.6 and 15.9 mm respectively.

In our experiments, the rate of ascarid growth was at first almost uniform in all chickens of the three experimental groups; growth of the individual larvae was extremely variable from day 10 to day 15 postinfection. Later, however, it became differentiated in accord with the individual experimental groups. Thus on day 25 p.i., juvenile worms recovered from chickens of group I (infected with 200 eggs) measured 16.6 mm in length, those of group II (500 infective eggs) measured 16.0 mm, and those of group III (1000 eggs) measured 14.6 mm. In this case it would be difficult to talk about the influence of the "density" of the worm community on the length of the individual worms because in chickens in which this effect was to be anticipated, the worm burden was lowest and, at the same time, the length of their nematodes shortest.

Another interesting phenomenon was observed during the early developmental stages of *A. galli*. From day 13—14 p.i. a decline occurred in the growth curve for all three experimental groups of chickens (Fig. 3) coinciding with the most varied mixture of different developmental stages of ascarids. The birds still harboured second stage larvae, but also fourth stage larvae were present. The same applied to the occurrence of intensive moulting processes. While most larvae had completed their third larval moult, a small percentage completed their second moult (Fig. 4). Also Tongson and Craw (1967) observed extreme differences in the growth of larvae on day 12 of infection recovering a considerable number of second stage larvae. They considered these larvae with delayed development to be static, dormant larvae recovered even at 77 days in those birds dosed with 5000 infective eggs of *A. galli*. In our experimental chicken material, the recovery of second stage larvae was infrequent, more frequent were early and late forms of third stage larvae. On the basis of the results obtained from our earlier experiments (Birová-Volosinovičová 1970 a, b, 1971) we tried to explain the mixture of morphologically different ascarid larvae by an ununiform time of emergence of the larvae from the egg-shells after infection of the hosts. It may, however, be possible that other factors such as the delaying effect of mucin (Frick and Ackert 1948) and specific host reactions may exert their influence on larval development. There remains, however, the fact that the individual specimens of an *A. galli* community do not develop uniformly and, therefore, it is impossible to define exactly the time of origin of the individual developmental stages. The same applies also to the exact determination of the time of occurrence of the individual moulting processes. As shown in Fig. 4, these processes occurred continuously in the larvae until day 19 postinfection of the host. It is possible to talk even of a period of maximum incidence of moulting larvae occurring for the second moult from day 5—9, for the third moult from day 13—18. Of importance is the fact that apart from a small percentage of larvae, all larvae hatched in the so-called "mucosa", i. e., actually, in the paramucosa of the intestine as this has been pointed out in earlier papers (Birová-Volosinovičová 1970 a, b, 1971). We have never been able (not even histologically) to demonstrate their presence in the mucosa of the intestine.

The distribution of nematodes in the intestine of the host after experimental infection has received little attention. It is not understood whether the parasite chooses actively its site of location in the intestine, or whether this site selection is determined by the host. Most authors (Sommerville 1963; Ulmer 1971 — cit. ex Holmes 1973; Holmes 1973) are in favour of the first alternative, i.e., active site selection by the parasite himself, which may be influenced by the presence of another parasite species. In other instances the host is considered to regulate the choice of the worm's location in the intestine (Tetley 1935, 1937 — cit. ex Holmes 1973; Rogers 1957, 1960 — cit. ex Holmes 1973, Sommerville 1957; Heath 1971). The distribution of *A. galli* larvae

in chickens with experimental infection has been studied by Moran and Mizelle (1956, 1957) and Tongson and McCraw (1967). Although most of the larvae of *A. galli* resided in the area close to Meckel's diverticulum (second intestinal section) a certain part of the organophenote moved in caudal and cranial direction during the early developmental phase. Moran and Mizelle (1956, 1957) associated this translocation of the larval community with the time at which tissue penetration was observed in their experiment (day 8—16).

In our experiments the largest number of *Ascaridia* was found in the second section of the intestine (middle jejunum) independent on the infective dose and duration of infection. We observed also larval migration throughout the intestine; this was most intensive within the first 5—8 days and proceeded first mainly in caudal and then in cranial direction, but from day 11—20 postinfection, the movements were more intensive in cranial than in caudal direction. Of special interest is the fact that intensive migrations of larvae coincided with the time around which moulting processes attained their peak, i.e., at the time of the second or third moult. Similar to the experiments of Tongson and McCraw (1967), we observed a large number of moulting larvae on day 9 postinfection, i.e., at the time at which these authors recording translocations in the *Ascaridia* community. It is possible that the process of moulting is one of the reasons accounting for increased larval activity and, later, also for their elimination from the *Ascaridia* community as this has been suggested also by Tongson and McCraw (1967). This explanation of the phenomenon might be more credible than the assumption by Moran and Mizelle (1956, 1957), because host tissue penetration by the larvae from day 8 — day 16 p.i. seems unlikely in view of the natural morphological barrier, i.e., the large size of the larvae in comparison with the size of the intestinal villi. In addition, it has been suggested by our numerous observations on the location of *Ascaridia* in the intestinal spaces of the chicken that a histotropic phase does not occur during the life cycle of *A. galli* (Birová-Volosinovičová 1970 a, b, 1971).

ЭКСПЕРИМЕНТАЛЬНЫЙ АСКАРИДИОЗ IV. РАЗНЫЕ ПРОЯВЛЕНИЯ В ПОВЕДЕНИИ АСКАРИД В РАМКАХ ОРГАНОФЕНОТ ПОСЛЕ ЭКСПЕРИМЕНТАЛЬНОГО ЗАРАЖЕНИЯ ЦЫПЛЯТ

В. Бирова-Волосиновичова

Резюме. Мы изучали некоторые проявления в поведении *A. galli* после однократного заражения цыплят 200, 500 и 1000 инвазионными яичками. Мы установили влияние инвазионной дозы на степень прикрепления личинок в организме хозяина, на локализацию аскарид в кишечнике и их рост и обсудили некоторые факторы, обуславливающие эти зависимости. Внимание уделяли неравномерному развитию нематоды в течение ранней стадии онтогенеза в связи с неравномерным вылупливанием личинок из яиц в организме хозяина и с тормозящим воздействием муцина с специфическими реакциями хозяина. Тощая кишка, особенно участок ниже Меккелева дивертикула, оказалась оптимальным местом локализации аскарид. Мы наблюдали передвижения аскарид в кишечнике во время ранней стадии их развития в каудальном и краниальном направлениях от оптимального места локализации; эти передвижения стали более интенсивными во время линьки. Установлено, что личинки *A. galli* проделывают у цыплят две линьки, а именно вторую и третью линьку в парамукозной среде кишечника. Так как личинок никогда не находили в кишечной ткани, повторно можно утверждать, что аскариде *A. galli* для развития не нужна гистотропная стадия.

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