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THE INTESTINAL PHASE OF DEVELOPMENT OF DIFFERENT *TRICHINELLA* SPECIES IN WHITE LABORATORY MICE

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Many authors [1, 6, 7] studying the localization of mature trichinellae in white laboratory mice, reported that adult trichinellae primary localize in the anterior half of the small intestine, though Berezantsev [4] described localization of trichinellae in the middle part of the small intestine of the white mice. The expulsion of nematodes from the intestine of white mice takes place on 15-30 days post infection and depends on the degree of adaptation of parasite to host.

The earlier investigations were carried out mainly with *T. spiralis* Owen, 1835, and nonidentified trichinellae of other species. Thus, we found it to be expedient studying of intestine phase of development of all four *Trichinella* species, which were maintained in white mice as standard strains for many years in our laboratory.

Material and methods

In our experiments we used 328 white laboratory mice, mostly males weighing 20-23 g. The mice were inoculated with muscle larvae of standard strains of trichinellae: *T. spiralis*: *T. nativa* Britov et Boev, 1972; *T. nelsoni* Britov et Boev, 1972; *T. pseudospiralis* Garkavi, 1972; isolated by digestion of the infected mice muscles in the artificial gastric juice. In the first series of the experiments we used a infection dose of 10 larvae per 1 g of a mouse weight, in the second 20 larvae per 1 g were administered.

The larvae were counted using the binocular microscope in 0.16% solution of agar in saline at 36°C.

The autopsy was held daily from 1 to 10 days post infection (d.p.i.) and on 13, 15, 20, 23, 25 and 30 d.p.i. Small intestine, coecum and large intestine were examined during dissection. The small intestine was divided into four equal parts, from the posterior end of each part samples

of 0.5 cm were separated for histological examinations. Each sample was placed into Petri dish or Baermann apparatus with warm saline, dissected longitudinally and placed into thermostat at the temperature of 38°C for 2-3 hours and examined using a binocular microscope. The trichinellae were collected, counted and fixated in the liquid of Barbagallo. Intestine samples, designed for histological examinations, were fixated in 10% neutral formalin and imbedded in paraffin according to generally accepted methods. Five μm sections were dyed with hemotoxilin-eosin.

Results

Mice infected with 10 larvae/g of body weight developed a mild trichinellosis. Death of mice was not observed. Mice infected with 20 larvae per g of body weight showed more severe trichinellosis and only 50% of the laboratory animals survived a month post infection.

Trichinellae localized in the mucose membrane of small intestine penetrating stroma of fibres, haemorrhages were not found. Capillaries of connective tissue stroma of fibres and blood vessels of submucouse

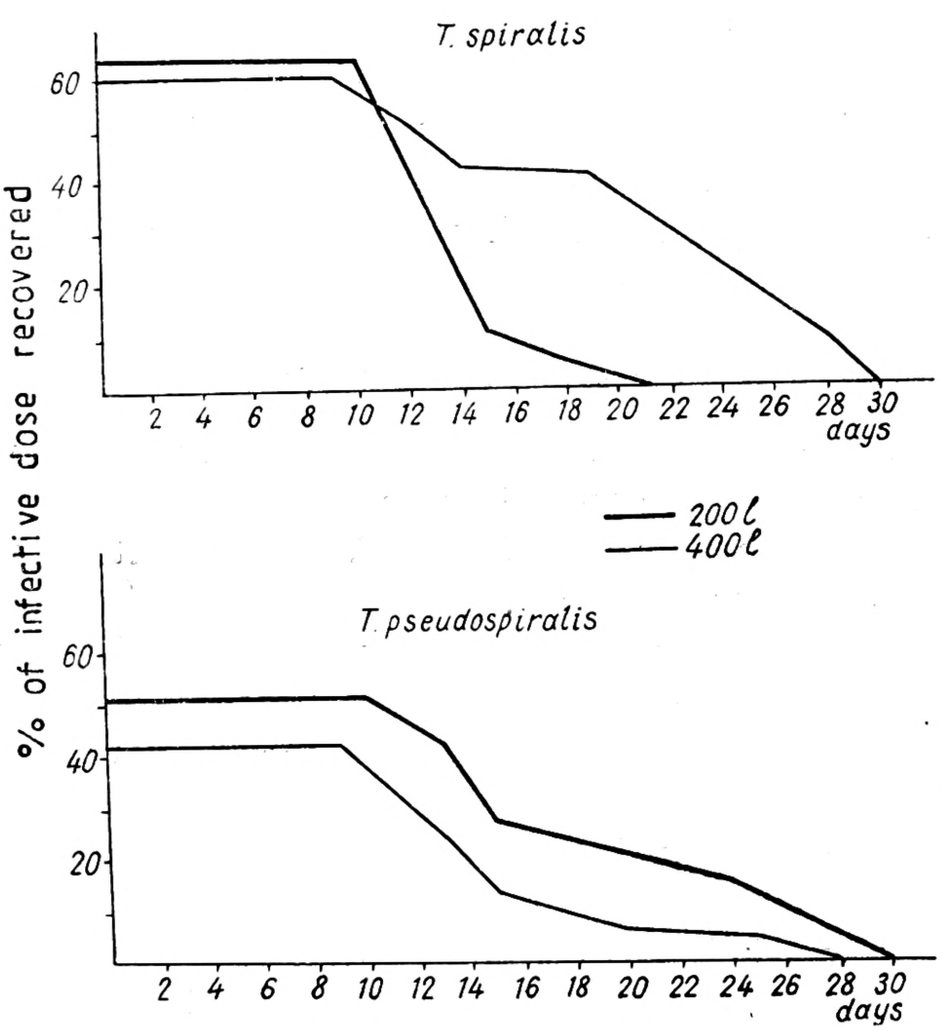

Fig. 1. Recovery of *T. spiralis* and *T. pseudospiralis* from intestine of mice infected with 200 and 400 larvae respectively

layer were congested. The connective tissue stroma of fibres was infiltrated with polymorphonuclear leucocytes.

Low infection (10 larvae/g) an average number of the established *T. spiralis* specimens constituted 64% of infective dose. The number of adult trichinellae remained constant during 10 d.p.i. and reduction of parasites burdens to 54% was observed thereafter (Fig. 1).

During the first series of the experiments (low infection) 72-86% from total number of established parasites was found in second and third parts of small intestine. On 7-9 the number of trichinellae in the third part increased, and on day 10 post infection trichinellae were distributed equally along 2nd, 3nd and 4th parts of the intestine. A considerable number of trichinellae appears in the coecum and large intestine is followed by the reduction of worm burdens. In the second series of the experiments the greatest number of trichinellae was discovered in the first half of the small intestine during the first week post infection,

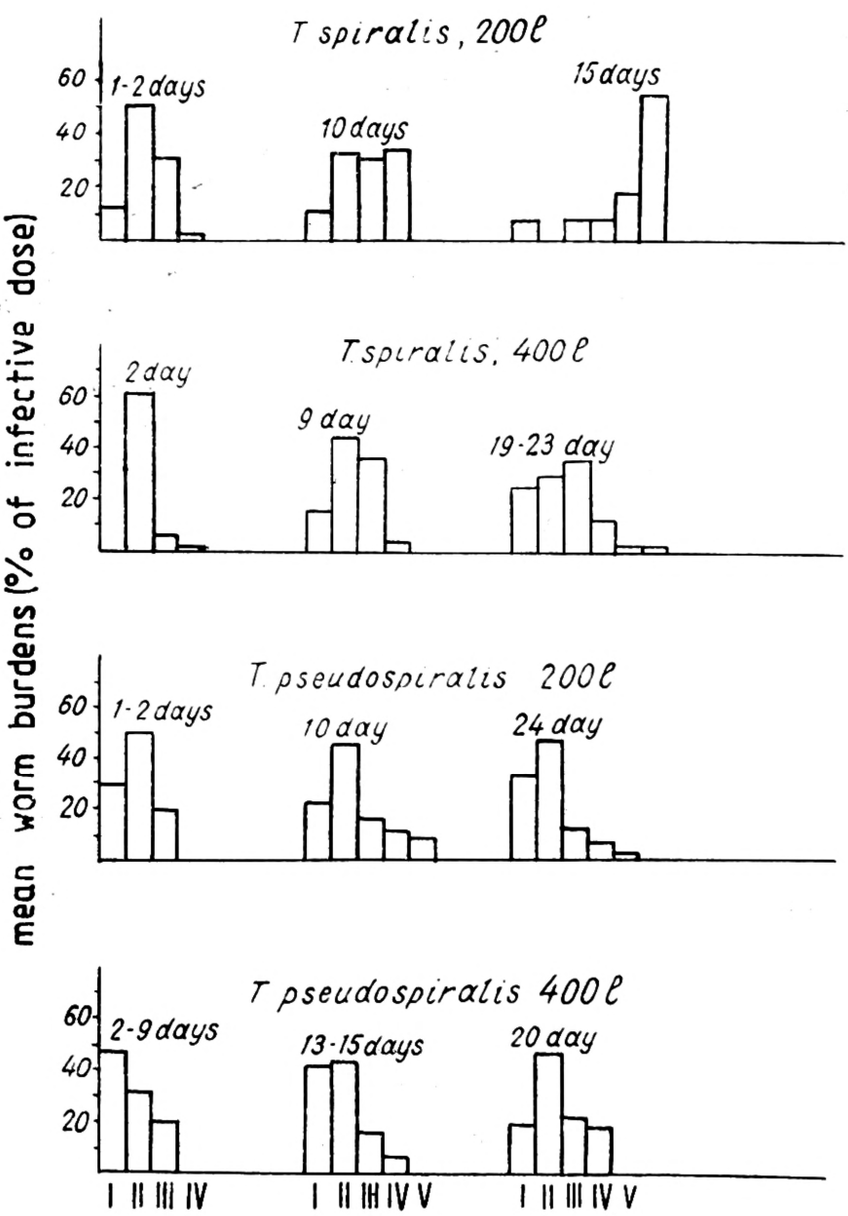

Fig. 2. Distribution of adult trichinellae in the intestine of white mice in different periods of the intestinal phase. I-IV — segments of small intestine, V — coecum, VI — large intestine

then in the middle part, and from 14 to 19 d.p.i. in the last three fourth parts of the small intestine (Fig. 2).

Establishing of mature *T. pseudospiralis* was somewhat lower than of *T. spiralis* in the first series of the experiments and reached 51% of the number of inoculated larvae. These worm burdens remainded constant till day 10 post infection then significant reduction of infection intensity (to 27% on 15 d.p.i.) occurred. Next the gradual decrease of parasite number was observed untill all the nematodes were expelled on 30 d.p.i. With the increase of the infection dose, the infection intensity reduces to 42% and the plateau phase limited to 9 days. The pattern of reduction of the infection intensity during moderate infection in general resembles the pattern of low infection. The duration of the intestine infection does not exceed 28 days.

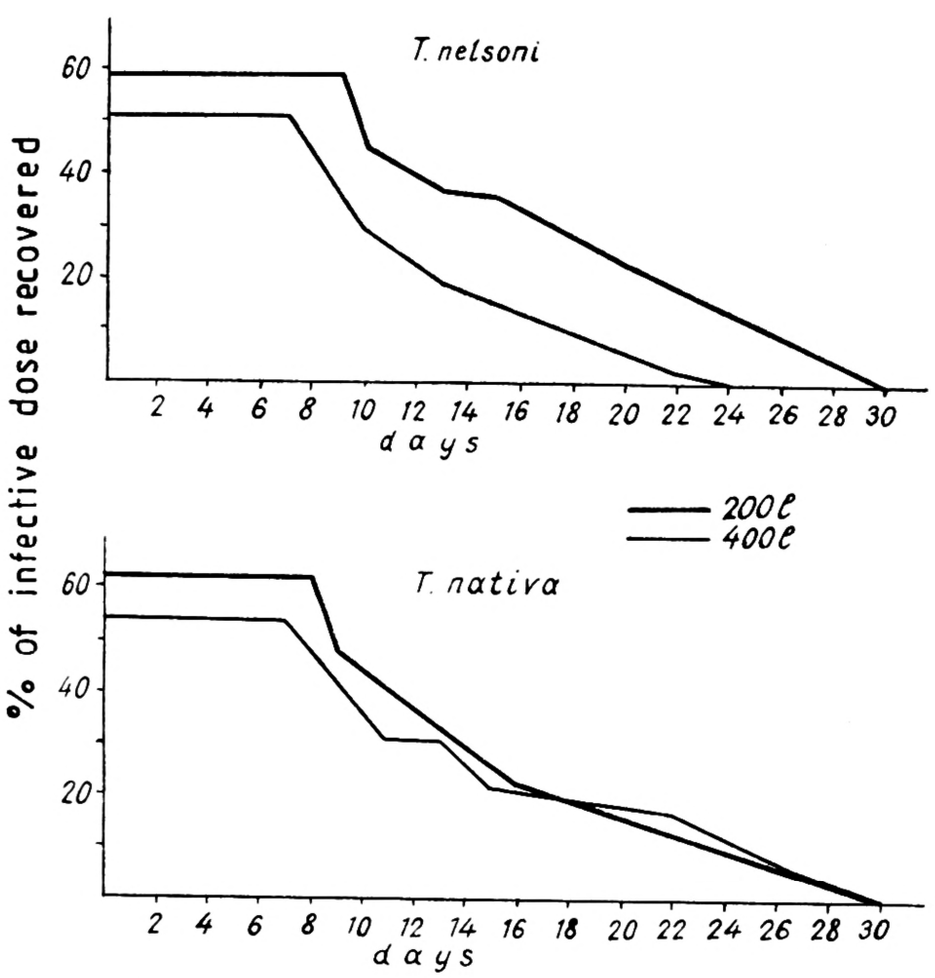

Fig. 3. Recovery of *T. nelsoni* and *T. nativa* from intestine of mice infected with 200 and 400 larvae respectively

In low infection the mature specimens of *T. pseudospiralis* primary localized in the first half of the small intestine, while the greatest number of trichinellae were discovered in the second part of the small intestine, 50-55% on day 1-6 p.i. and about 45% on days 10-24 p.i. Only on the third day p.i. trichinellae were equally distributed along the first three parts of the small intestine. In moderate infection the majority of trichinellae also localize along the first half of the small intestine. On

all days post infection singular parasites were found in the most dystal (fourth) part of the small intestine and only on day 20 p.i. their number increased.

During the first series of the experiments with *T. nativa* infections the rate of the adult nematode establishing was reasonably quite high, 62% of the infection dose and remain constant during 8 d.p.i. then significantly reduces to 48% on day 9 p.i. In the second series of the experiments the rate of the established larvae is quite lower, 54% and the constant period of the intestine phase is shorter (7 days). The diagram is a stepped curve with significant reduction of infection to 30.5% on day 11 d.p.i. The duration of the intestinal phase of development also made 30 days (Fig. 3).

During the first week of low infection the greatest number of trichinellae were found in the first half of small intestine, from 65 to 88% on day 3 p.i. and on the following days in the middle part of the small intestine. In the second series of the experiments during the first days

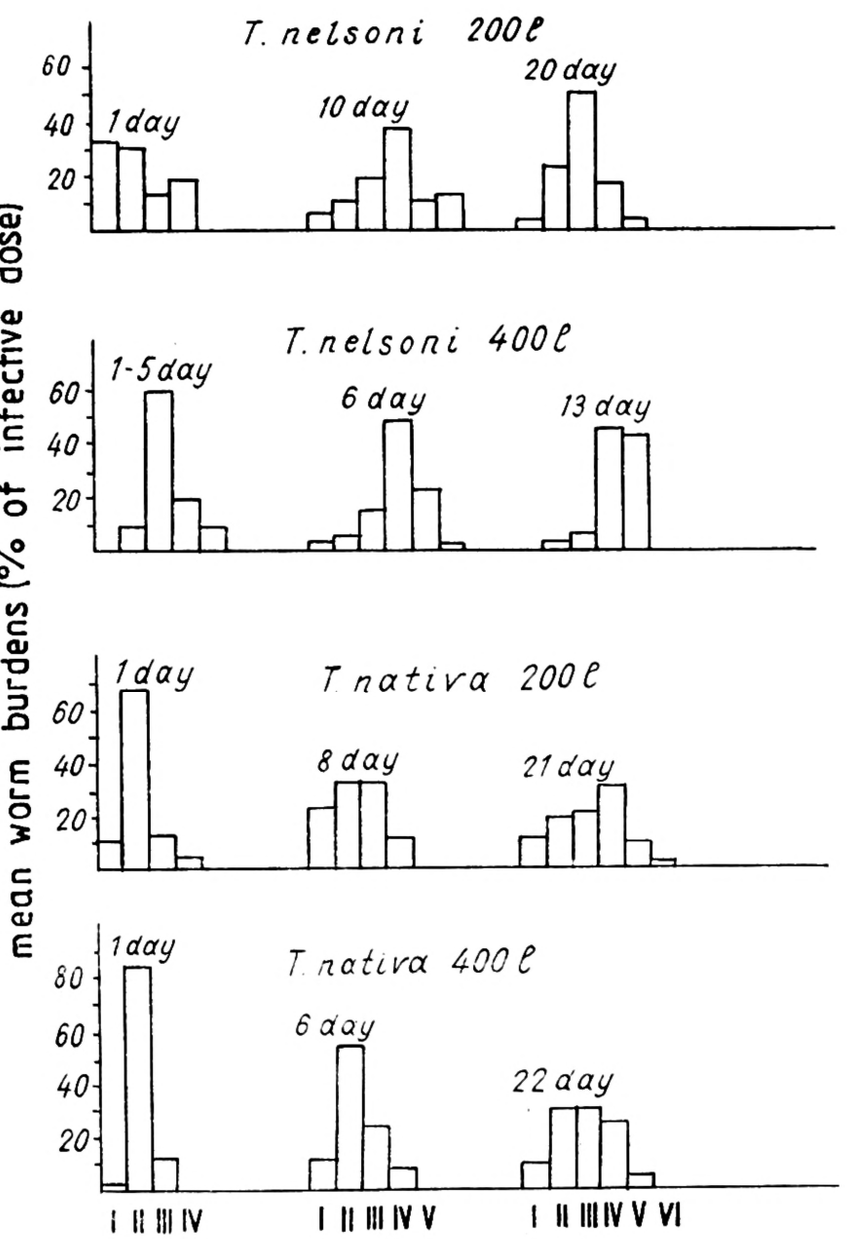

Fig. 4. Expl. cf. Fig. 2

of the infection the were second and third parts of the small intestine main localization sites (up to 88% of trichinellae), on the following days of the infection the main sites were third and fourth parts.

The average number of the intestinal nematodes of *T. nelsoni* in the first series of the experiments reached 59% from the primary dose, the worm burdens remained constant during nine days and reduced to 45% on day 10 p.i., next constantly decreased untill all the helminths were expelled by day 30 p.i. In the moderate infection the intensity of intestine infection was lower, the plateau phase was shorter, after which the intensity of infection reduced to 29% on day 10 p.i. On the following days of the infection the number of the nematodes decreased gradually and on day 24 we didn't observe parasites in the intestine (Fig. 2).

In the low infection 76 to 90% of established trichinellae localized in the first half of the intestine during the first days of infection, and on days 3-7 p.i. in the last three fourth parts of the small intestine. In the moderate infection the greatest number of trichinellae were found in the third part of the small intestine, and 6 days p.i. in the fourth part (Fig. 4).

As a result of our investigations we found that moderate and low infection there did not cause any significant pathological changes in the mucous membrane which corroborated previous reports [4, 5, 6, 7]. We didn't find trichinellae in the lumen of the intestine.

The main site of adult trichinellae localization was the small intestine, where 90 to 100% of the recovered trichinellae were found. In coecum and large intestine usually single trichinellae were observed, the incre-

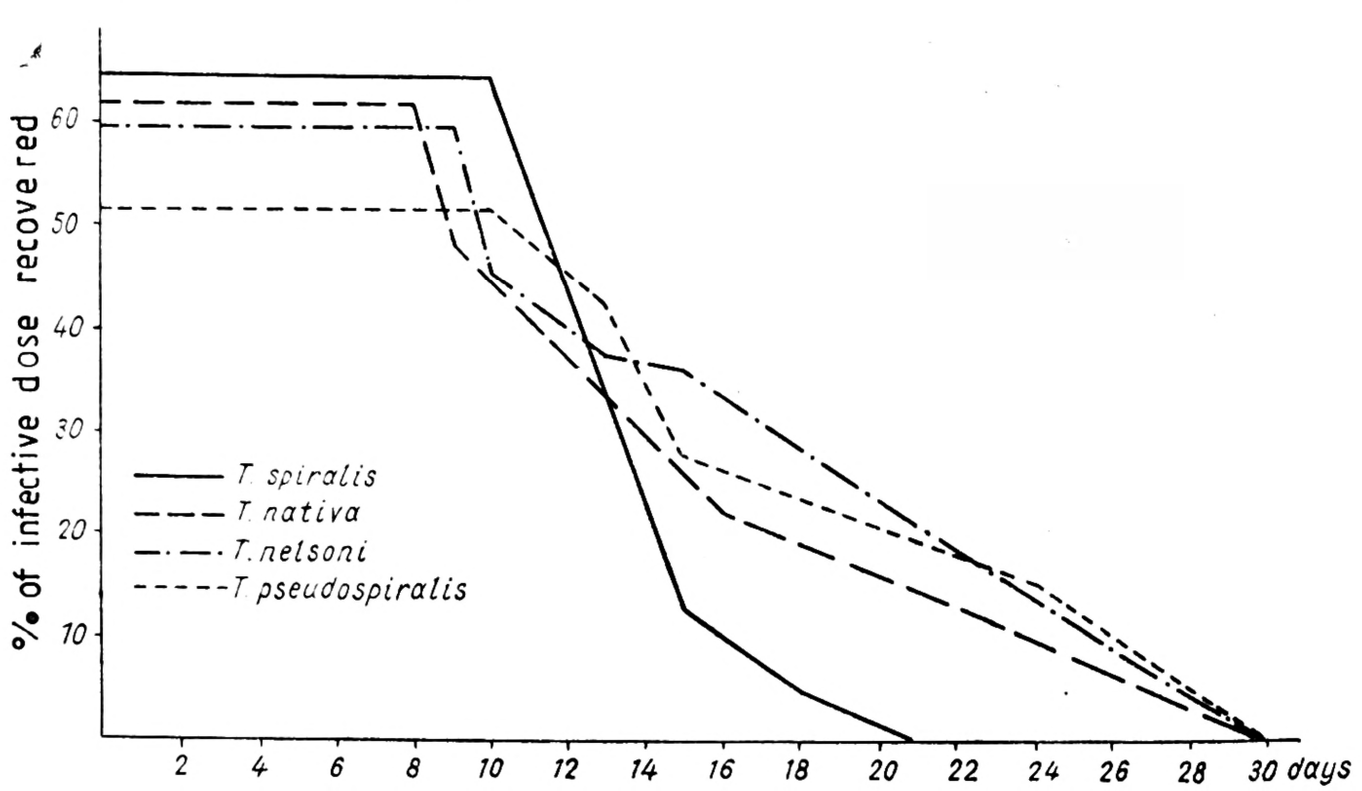

Fig. 6. Recovery of adult trichinellae from the intestine of mice in moderate infection

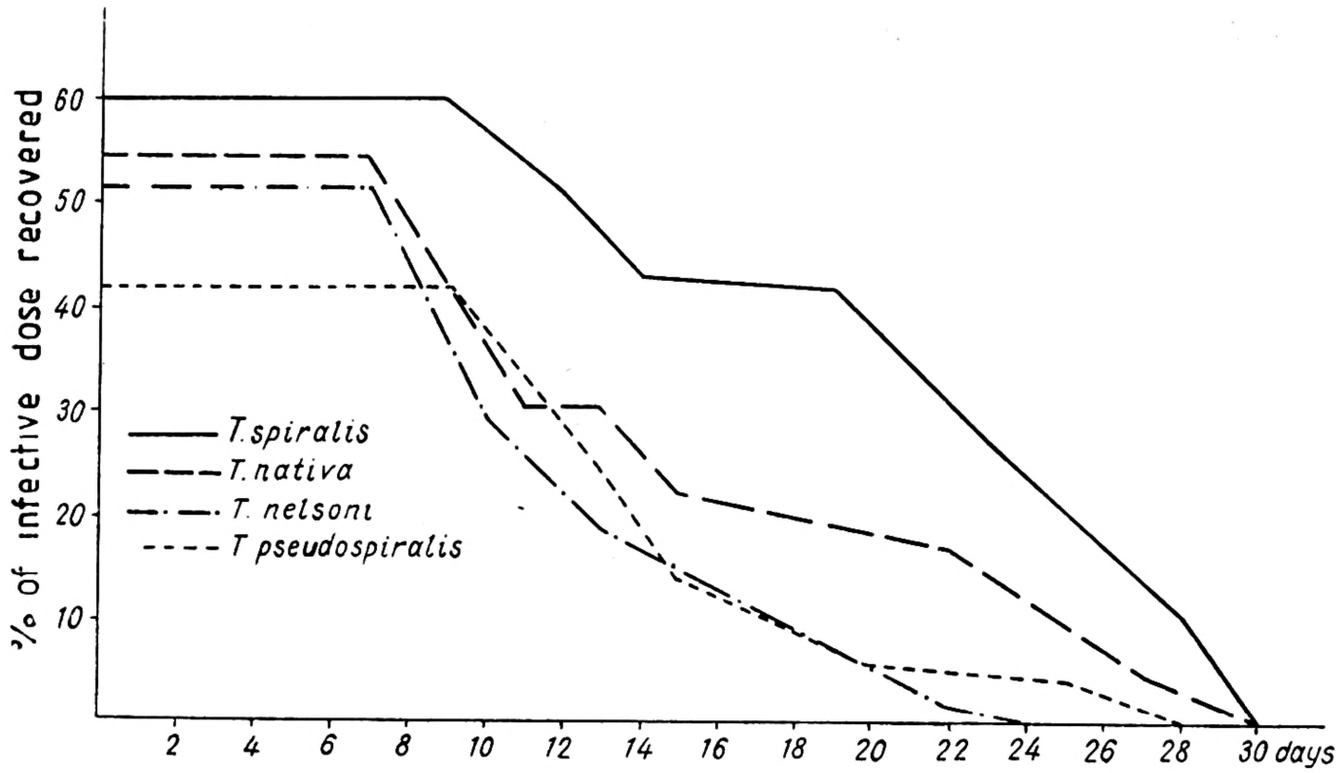

Fig. 5. Recovery of adult trichinellae from the intestine of mice in low infection

ase of the nematods in coecum and large intestine was followed by the reduction of the intestinal infection. That fact was considered to be a sign of worm expulsion [4, 8, 10].

The intensity of intestine infection of *T. spiralis* was higher than in the other species, the number of the recovered trichinellae reached 64% of the primary dose. The number of mature *T. pseudospiralis* was lower, 51% (Fig. 5). After twofold increase of the infection dose the intensity of intestine infection reduced in *T. spiralis* by 4%, in *T. pseudospiralis* by 9%, in *T. nativa* and *T. nelsoni* by 8% (Fig.6).

Discussion

According to the results of Belosevic and Dick [2] after the moderate infection the establishing of *T. spiralis* reached 50%, according to Pereverzeva [9] after the low infection it was higher (57%). After the second passage of arctic isolate of trichinellae, which we considered to be *T. nativa*, Pereverzeva [9] observed the increase of infection intensity to 44-66% in early stages, and Belosevic and Dick [2] to 43%. Aracava and Todd [1] report that the number of species of arctic isolate is quite lower in comparison to isolates of the moderate zone. In our results these meanings differ slightly, what we consider to be a result of different trichinellae species adaptation to white mice.

The shortest intestinal phase (21 days) we observed in low infection with *T. spiralis*. Similar results were reported by Campbell [7]. After

a moderate infection the nematodes persisted in the intestine till day, 30 p.i. however Belosevic and Dick [2] observed intestinal phase till day 20 p.i. Pereverzeva et al. [9] in low infection found 4-5% of trichinellae till 28-30 d.p.i. With the increase of the infection dose, the course of the intestine infection of *T. spiralis* also changes: in low infection after the 10 day plateau followed a reduction of the parasites number in the intestine, in the moderate infection plateau phase persisted until 14-17 d.p.i.

During moderate *T. nelsoni* infection the expulsion of the mature nematodes from the intestine occurs a little earlier, and the reduction of the nematode worms number in the intestine proceeds faster. The duration of intestinal phase of *T. pseudospiralis* infection after the increase of the infection dose is reduced only by two days. In the infection with *T. nativa* the duration of the intestinal phase does not depend on the infection dose, but the decrease of worms number in low infection after the switch reduction on day 9 p.i. takes place gradually, and in the moderate the diagramme is a stepped curve.

The greatest number of the mature *T. pseudospiralis* localize in the anterior half of the small intestine. In the first series of the experiments maximally 50% of parasites were found in the second part of the small intestine, and in the second series the parasites were found in the first part of the intestine.

During the first days of parasitizing a preferable site of mature *T. nelsoni* localization is the first half of the small intestine with the following displacement to the posterior half. In the moderate infection the parasites localize mainly in the posterior half of the intestine.

The greatest number of parasites in the infection with *T. nativa* were found at the beginning of the infection in the second part of the small intestine, on the following days the parasites were found in the middle part.

For low *T. spiralis* infection typical localization of the greatest of trichinellae was the middle part of the small intestine which corresponds to the data of Berezantsev [4], Podhajecky [10] and Campbell [7], who used the same dose of infection and found the greatest number of trichinellae in the anterior half of the small intestine. After a low infection dose the greatest number of trichinellae on the early days of the infection localize initially in the anterior half of the small intestine; from 90% on 1-3 d.p.i. to 85% on day 5 p.i. According to the data of Belosevic and Dick [2] these numbers on day 5 p.i. are little higher: 88.9 and 94%.

Intestinal infection with *T. spiralis* and *T. pseudospiralis* showed a similar plateau periods; 10 days in low infection and 9 days in moderate infection. For *T. nativa* and *T. nelsoni* in both cases this period was a little shorter.

Thus the duration and intensity of the intestine infection depend on the dose of infection and species of *Trichinella*.

The pattern of distribution of trichinellae in the intestine of white laboratory mice depends on the species of trichinellae and time post infection.

Otrzymano: 16 IX 1985

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