FOLIA PARASITOLOGICA (PRAHA) 24: 135—139, 1977.

DYNAMICS OF CIRCULATING ANTIBODIES AGAINST TRICHINELLA SPIRALIS AFTER APPLICATION OF ANTHELMINTICS

J. ČORBA, J. ČERMAN and R. ŠPALDONOVÁ

Institute of Helminthology, Slovak Academy of Sciences, Košice

Abstract. Formation and dynamics of circulating antibodies were studied in mice experimentally infected with *T. spiralis* and treated with mebendazole. Latex-fixation tube test was used in the experiment. In the control group of untreated mice the antibodies were detected on the 21st day after infection. The antibody level reached the maximum on day 76 and low titres were found still on day 207 after infection. In mice treated with mebendazole in the intestinal phase of trichinellosis, the antibodies were detected 10 or 7 days earlier than in the control group. At this time the antibody level reached the maximum and then it decreased gradually until no antibodies were detected on days 66—76. This phenomenon correlated with postmortem examination and suggested that the formation and dynamics of circulating antibodies against *T. spiralis* are directly dependent on the effectiveness of the treatment.

Humoral antibodies play an important role in the complex of anthelmintic immunity which was described with numerous helminthiases, among others also with trichinellosis. Our previous experiments (Čorba and Špaldonová 1974 a, b, c, Špaldonová and Čorba 1973) revealed that after the application of effective anthelmintics the mice developed an immunity manifested by a significant reduction (up to 96 %) of *T. spiralis* larvae originating from reinfection. In order to elucidate the mechanism of this immunity, a series of experiments were carried out to study the dynamics of circulating antibodies after the treatment of more or less effective anthelmintics. We followed the relationship between the antibody titre and the effect of treatment and the period of persistance of circulating antibodies in the blood.

MATERIAL AND METHODS

The experiments were carried out on 150 mice weighing 20—22 g which were infected per os with 300 *T. spiralis* larvae each. The animals were divided into 10 groups and were administered either mebendazole (Janssen Pharmaceutica, Belgium) in the dose of 100 mg/kg or tetramisol (ICI, Great Britain) in the dose of 40 mg/kg per os at intervals given in Table 1. Five days before infection and then at weekly intervals after infection blood samples were taken from the retrorbital plexus of both treated and control animals using the method of Nöller (in: Cors 1958) till day 210 after infection when the experiment was terminated. The animals were then killed and the number of muscular *Trichinella* larvae was assessed by the method of Špaldonová et al. (1965).

The antibodies were detected by latex tube test after the original method of Fischman (1960), who first used this reaction in the diagnostics of echinococcosis. The antigen applied for the reaction was prepared from *T. spiralis* larvae after their ultrasonic disintegration. The basic latex suspension of polystyrene particles measuring 0.81 μm in diameter (Imuna, n. p. Šarišské Michalany) was used as adsorbent of the antigen. It was diluted with distilled water and sensibilized latex suspension was prepared from the mixture of 10 ml of saline, 0.3 ml of diluted latex and 0.5 ml of *Trichinella* antigen.

For the reaction tests, 0.5 ml of sensibilized latex suspension was added to the same volume of diluted sera, the suspension was thoroughly mixed and incubated first in water at 37 °C for 90 min

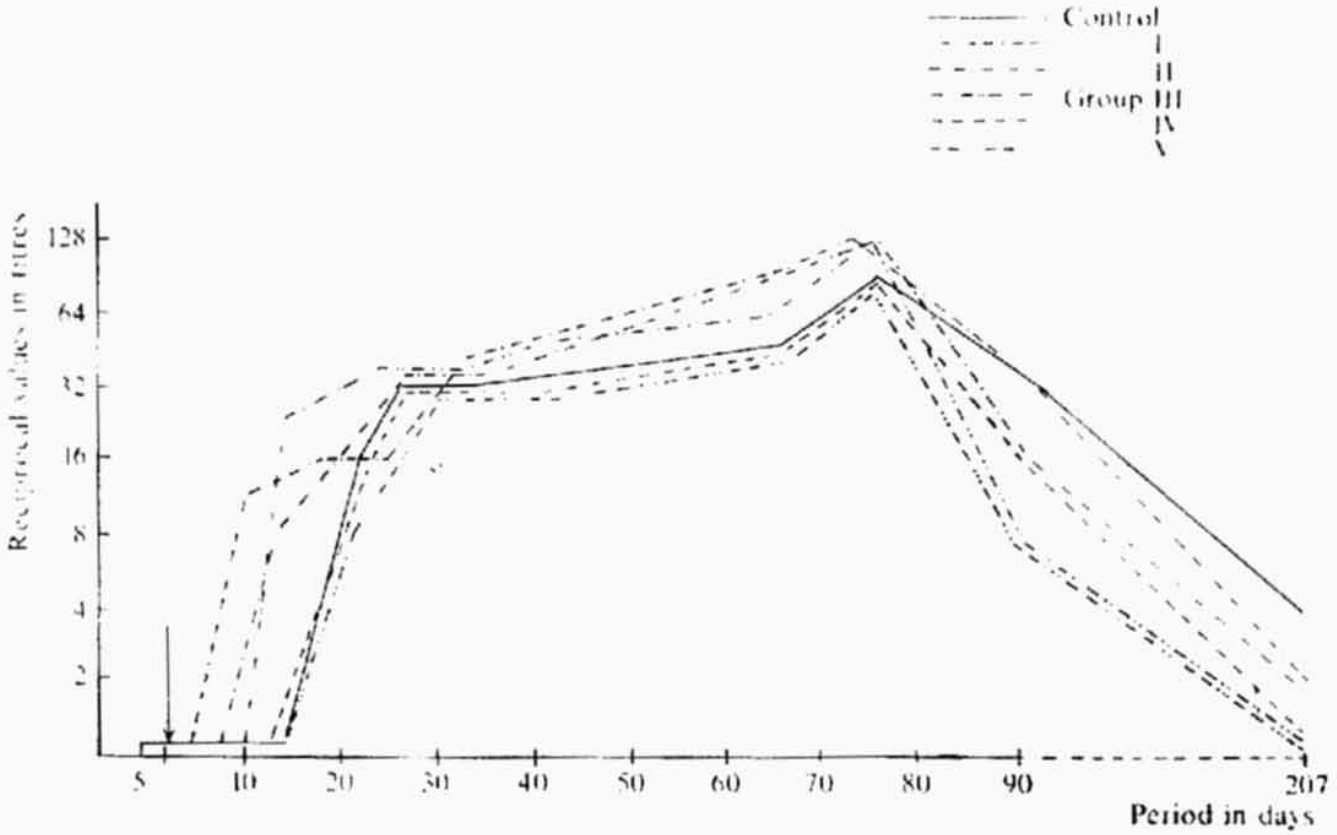

Fig. 1. Dynamics of latex-fixating antibodies in mice experimentally infected with 300 *T. spiralis* larvae and treated with tetramisol in the dose of 40 mg/kg during the intestinal phase of trichinellosis.

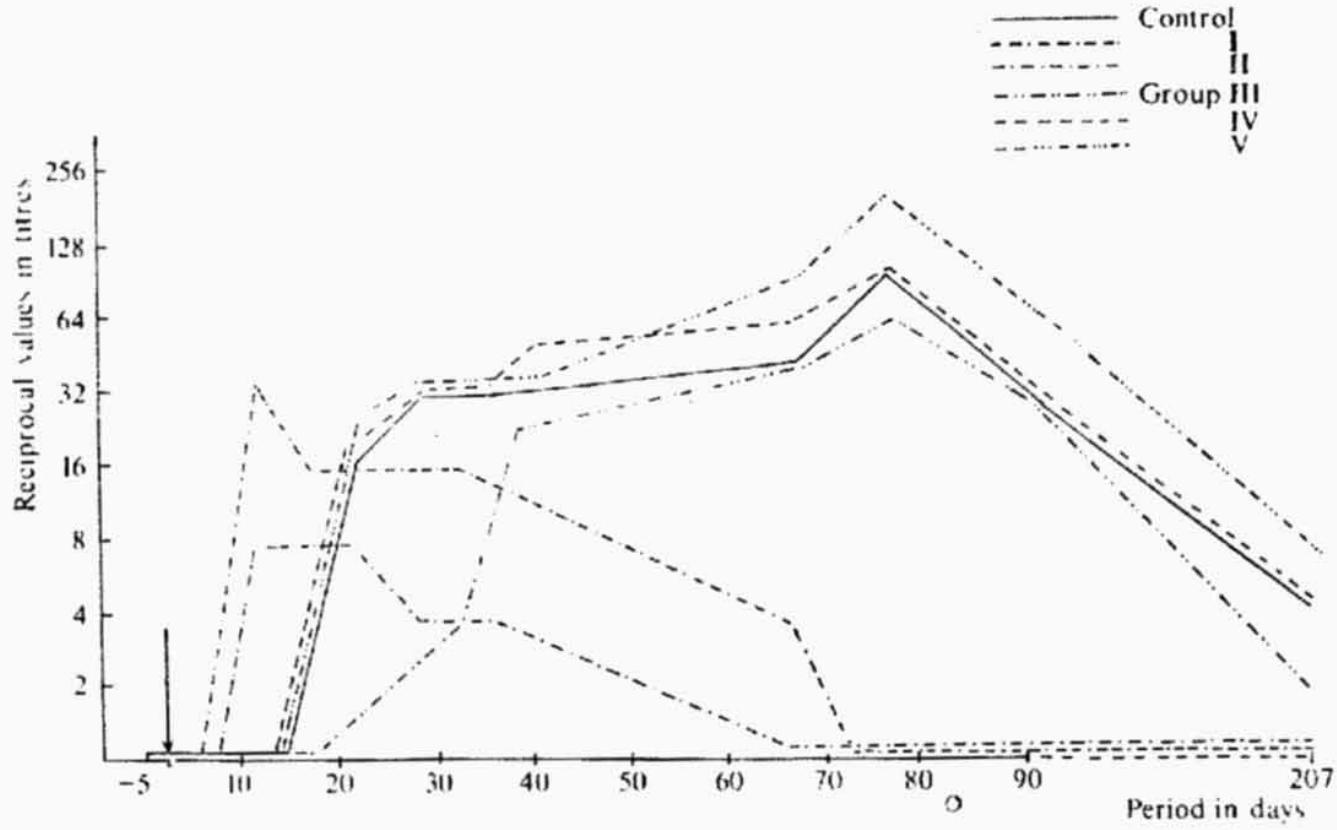

Fig. 2. Dynamics of latex-fixating antibodies in mice experimentally infected with 300 *T. spiralis* larvae and treated with mebendazole in the dose of 100 mg/kg during the intestinal phase of trichinellosis.

and then in the refrigerator at 4 °C overnight. At each examination of the sera, positive and negative controls of the sera and control of the antigen were made. On the following day the tubes were centrifuged at 2,500 r. p. m. for 3 min and the reaction was recorded.

Reactions producing opaque appearance without sediment or with a small sediment readily dispersing at slight knocking on the bottom of the tube were taken for negative. The positive reactions were evaluated according to clearing of the supernatant, the appearance of the sediment and the size of resuspended flakes and they were marked with one, two, three or four crosses. Its highest solution giving positive reaction and marked with two crosses (++) was taken as serum titre.

RESULTS

In the control group of untreated mice, the antibodies were detected on the 21st day after infection. Maximum values were obtained on day 76, but the antibodies persisted at low titres still to the 207th day.

In mice treated with tetramisole at intestinal and early migratory phase of trichinellosis, the antibodies were first detected 10 (group I), 7 (group II) and 4 (group III) days earlier than in the control animals. In the group of mice treated with tetramisol at the migratory phase of trichinellosis (groups IV and V), the therapy influenced neither the beginning of the formation nor the dynamics of antibodies.

In mice treated with mebendazole, latex-fixing antibodies were found also 10 (group I) or 7 (group II) days earlier than in the controls. At this time the antibody titres in groups I and III were maximum and decreased gradually until days 66—76, when no antibodies were detected in these groups. The therapy had a 100 % effect in these two groups, because no *T. spiralis* larvae were found (Table 1).

Table 1. Number of muscular *Trichinella* larvae in white mice 210 days after infection with 300 *T. spiralis* larvae and treatment with mebendazole in the dose of 100 mg/kg or with tetramisol in the dose of 40 mg/kg during the intestinal phase of trichinellosis.

Group	Period of application in days	Mebendazol 100 mg/kg	Tetramisol 40 mg/kg	Untreated control
I	1—2	0	22.325 ± 1826	
II	3—5	0	34.608 ± 879	
III	6—8	20.300 ± 1236	41.033 ± 1916	36.781 ± 2738
IV	9—11	35.687 ± 673	35.933 ± 2136	
V	12—14	31.233 ± 897	49.695 ± 1329	

A delated onset of antibody formation and slower increase to maximum levels were observed also in group III treated with mebendazole. The dynamics of antibodies in mice of groups IV and V was analogous to that of the control group and the differences did not exceed the value of one dilution of the geometric series. Maximum values of antibody titres in groups III, IV and V and in the control group were found on day 76 after infection, i.e. at the time when antibodies had already disappeared from the blood of mice of groups I and II. The numbers of muscular larvae in groups IV and V did not significantly differ from that in the control group.

DISCUSSION

The application of effective anthelmintics to an infected host affects the parasite-host relationship. The host organism responds to the presence of helminths with a whole complex of defensive reactions, among which an important role is played by the im-

munological mechanism. The interruption of infection by anthelminthic treatment and destruction of helminths in the host organism result in a sudden release of a large amount of antigen, which is followed by a strong immune response.

Elucidation of the role of circulating antibodies in the defence mechanism of the host against the helminths is very complicated due to the large number and variety of antigens against which the antibodies are formed. Parasitic worms contain a number of somatic, secretory and excretory antigens and therefore also the antibody response of the host is variable both in quality and quantity. The results obtained by many other authors show that humoral antibodies play an important role in the complex of anthelmintic immunity, because the resistance to many helminths was successfully transferred via the serum. Therefore in our experiments we studied the dynamics of circulating antibodies against *T. spiralis* during different developmental stages of the parasite. As it follows from the literature available to us, the dynamics of circulating antibodies after a therapy of trichinellosis have not hitherto been studied. The results of our experiments reveal that an effective therapy with mebendazole provokes a sudden increase of latex-fixating antibodies shortly after the application of the anthelmintic. We assume that this phenomenon is directly associated with the release of a large amount of antigen from the disintegrated *Trichinella* larvae. A similar effect was observed also by Ambroise-Thomas et al. (1970) in mice infected with *Schistosoma mansoni* and by Dodin et al. (1966) in patients with schistosomiasis. In contrast to our results, however, the therapy at early stage of schistosomiasis did not provoke any serological response in the organism of the treated host.

On the basis of our experiments it may be stated that the formation and dynamics of circulating antibodies against *T. spiralis* directly depend on the effect of the treatment. This phenomenon was observed during mebendazole treatment of the intestinal phase of trichinellosis, where the anthelmintic caused maximum rate of death of *Trichinella* larvae. This speeded up the formation of serologically detectable antibodies till maximum titres were attained. The correlation between latex-fixating antibodies and effective treatment may be applied in determination of the efficacy of the anthelmintic in early phase of trichinellosis.

ДИНАМИКА ЦИРКУЛИРУЮЩИХ АНТИТЕЛ ПРОТИВ *TRICHINELLA SPIRALIS* ПОСЛЕ ПРИМЕНЕНИЯ АНТГЕЛЬМИНТИКОВ

Й. Чорба, Й. Черман и Р. Шпалдонова

Резюме. Образование и динамику циркулирующих антител изучали на мышах после экспериментального заражения личинками *T. spiralis* и лечения мебендазолом. Опыты проводили при помощи латекс-фиксирующего теста. В контрольной группе нелеченных мышей антитела обнаружили на 21-й день после заражения. Максимальный уровень антител был достигнут на 76-й день и невысокие титры были найдены еще на 207-й день после заражения. У мышей леченых мебендазолом в течение кишечной фазы трихинеллеза антитела были найдены на 10 или 7 дней раньше, чем у контрольной группы. В то время уровень антител достиг максимума и после того постепенно понижался до 66—76-го дня, когда антитела уже исчезли. Это явление соответствует вскрытию и показывает, что образование и динамика циркулирующих антител против *T. spiralis* прямо зависимы от эффективности лечения.

REFERENCES

AMBROISE—THOMAS D., SARASIN G., KREN TRUONG T., Comparative evolution of the fluorescent antibodies directed against the larval stages and adults of *Schistosoma mansoni*. II. Experimental schistosomiasis in mice after treatment with niridazole. Ann. Trop. Med. Parasitol. 64: 93—99, 1970.

COHRS P., JAFFE R., MEESSEN H., Blutentnahme aus dem retroorbitalen Veneplexus. Pathologie der Laboratoriumstiere, Springer Verlag I/188, 1958.

ČORBA J., ŠPALDONOVÁ R., Immunity in mice against *Trichinella spiralis* after administration of benzimidazoles: development of immunity during the intestinal and early migration stages of infection. In: CH. W. Kim: Trichinellosis, New York, 213—219, 1974 a.

—, —, Das Studium der Wirtsimmunität gegen *Trichinella spiralis* nach Applikation von Benzimidazolen. II. Immunitätsentwicklung während der Muskelphase-Invasion. Biologia (Bratislava) 29: 213—218, 1974 b.

—, —, Development of immunity against reinfection of *T. spiralis* and *F. hepatica* after application of antihelmintics. Proceedings of III. ICOPA, Munich, 2: 666, 1974 c.

DODIN A., RATOVONDRAHETY M. I. P., MOREAU J. P., RICHAUD J., Etude immuno logique de bilharziens traités par le CIBA 32644 Ba. Acta trop. Suppl. 9: 35—44, 196

FISCHMAN A., Floculation tests in hydatid disease. J. Clin. Pathol. 13: 72:75, 1960.

ŠPALDONOVÁ R., ČORBA J., Studium der Immunität des Wirtes gegen *Trichinella spiralis* nach der Applikation von Benzimidazolen I. Entwicklung der Immunität während der Darm- und Frühmigrationsphase der Invasion. Biologia (Bratislava) 28: 411—416, 1973.

Received 3 February 1976.

J. Č. Helmintologický ústav SAV,
ul. Dukelských hrdinov 11,
040 00 Košice, ČSSR

FOLIA PARASITOLOGICA (PRAHA) 24: 139—140, 1977.

CYSTICERCOIDS OF THE CESTODE APLOPARAKSIS DIMINUENS (LINSTOW, 1905) — (CESTOIDEA: HYMENOLEPIDIDAE)

During the helminthological expedition organized by the Helminthological Laboratory of the USSR Academy of Sciences, Moscow, on the River Ob (Tyumen Region, District Salekhard) oligochaetes were collected and examined for helminths. The material was collected in tundra on the left bank of the River Ob, about 200 km north of polar circle. Three specimens of Tubificidae gen. sp. were found to contain cysticercoids of cestodes of the genus *Aploparaksis* Clere, 1903 in body cavities.

The cysticercoids are typical tailed diplocysts (according to the classification by Bondarenko and Kontrimavichus (Folia parasit. (Praha) 23: 39—44, 1976). The outer envelope of cysticercoids, surrounding the cyst proper, is elongated either on one or on both poles. Bondarenko and Kontrimavichus assume, in agreement with Mrázek (Zool. Jahrb. Abt. Anatom. 39: 515—584, 1916), that this envelope is formed by a widened tail (cercomer). The anterior part of this envelope bears a feebly discernible canal opening into indistinct pore. The size of the envelope is 0.410—0.480 × 0.128—0.144 mm. The cyst proper is of oval shape and measures 0.160—0.172 × 0.106 to 0.113 mm. The outer thin hyaline layer of the cyst is 0.008 mm thick, the inner layer has transverse striations and is thicker, 0.008 to 0.012 mm. The scolex measures 0.086—0.094 mm in diameter. The rostellum is cylindrical and measures 0.050—0.054 × 0.022—0.024 mm. It has one row of hooks of aploparaksoid type, measuring 0.014 mm in length. Oval suckers measure 0.040—0.048 × 0.022—0.026 mm. Calcareous bodies, rounded or oval, measure 0.004—0.006 mm in diameter and are concentrated mainly in the anterior part of the cysticercoid. Embryonal hooks were not found.

A characteristic feature of the cysticercoid recovered are small rostellar hooks measuring only 0.014 mm. Of the known species of the genus *Aploparaksis*, only the species *A. brachyphallos* (Krabbe, 1869), *A. leonovi* Spassky, 1961, *A. galli* (Rausch, 1951), *A. sanjuanensis* Tubangui et Massilungan, 1937 and *A. skrjabinissima* Spasskaja, 1950 have been reported to possess hooks shorter than 0.020 mm. However, their hooks differ from those of our cysticercoids in the shape. The shape and size of rostellar hooks