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EFFECT OF VITAMINS ON TRICHINELLA PSEUDOSPIRALIS GARKAVI, 1972 INFECTION IN MICE

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Abstract. The effects of vitamins A, B complex, E, and ADE on the eosinophilia, body weight, number of obtained larvae and distribution of larvae of *Trichinella pseudospiralis* were examined in mice. The increased eosinophilia started to appear in the majority of infected mice from day 7 p.i., the maximum being around day 21 p.i. The highest values of eosinophilia were observed in the mice after application of vitamin B complex. In spite of considerable individual differences, the greatest decrease in body weight occurred in mice receiving vitamins E and B complex. The greatest numbers of larvae were obtained from mice receiving vitamins E and B complex, which is proportional to the increase in eosinophilia. The numbers of muscle larvae in untreated mice and in those receiving vitamins ADE were approximately the same. The lowest numbers of larvae occurred in mice receiving vitamin A. In all mice groups the greatest numbers of larvae were localized in the left foreleg and diaphragm.

Four species of the genus *Trichinella* are known at the present time: *Trichinella spiralis* Owen, 1835, *T. nativa* Britov et Boev, 1972, *T. nelsoni* Britov et Boev, 1972, and *T. pseudospiralis* Garkavi, 1972. Their morphology is so similar that for 140 years they were described as a single species, *T. spiralis*. *T. pseudospiralis* differs from the other three species in some characters, particularly in the absence of muscle cysts and in the ability to terminate its life cycle within different bird species.

All of the four *Trichinella* species are used in the laboratories of the Institute of Parasitology. *T. pseudospiralis* was applied for the present studies of the effect of vitamins.

MATERIALS AND METHODS

The larvae of *T. pseudospiralis* were obtained from the Zoological Institute, Academy of Sciences of the Kazakh S.S.R., Alma-Ata. They were used for the infection of 25 females of SPF ICR white mice aged 3.5 months and weighing 29.9 g on the average. The mice were divided into five groups and five mice served as controls. They were fed with commercially produced pellets and water ad libitum and kept under standard conditions. A month before infection with *T. pseudospiralis* larvae, mice of four groups received vitamins in the dose of 0.1 ml per mouse administered intramuscularly into the femoral muscle at one-week intervals. The following vitamins were used: vitamin A (Axerophthol Spofa, 50,000 I.U. in 1 ml), vitamin B complex Spofa (10 mg Thiaminium dichloratum, 4 mg Natrium riboflavin-(5)-phosphoricum, 3 mg Pyridoxolium chloratum, 5 mg Calcium panthothenicum, 40 mg Nicotinamidum in 1 ml), vitamin E (Erevit — 30 mg Tocoferolum aceticum in 1 ml), and vitamins A + D_2 + E (Axetocal: A — 100,000 I.U. Retinolum aceticum, D_2 — 100,000 I.U. Ergocalciferolum, E — 30 mg Tocoferolum aceticum in 1 ml). Each mouse was infected perorally with 100 *T. pseudospiralis* larvae obtained by digestion of muscles of an infected mouse with 3 % pepsin in 1 % of hydrochloric acid at 37 °C for 14 h. The mice were then weighed at regular intervals (2, 7, 14, 16, 18, 21, 28, 35, 42, 49, and 70 days) at the same time of day and simultaneously blood was taken from their tails. The blood smears were stained after Pappenheim (1911) by May-Grünwald and Giemsa-Romanovsky stains and the changes in the blood picture, particularly eosinophilia, which is characteristic of helminthoses, were followed. On day 70 after infection the mice were killed and individual parts were examined by compression method. The larvae released from the body parts by digestion were counted and their numbers were calculated per 1 g of muscle tissue.

RESULTS

During the experiment (70 days p.i.), changes in the blood picture and body weight, total number of muscle larvae and their distribution in different body parts calculated per 1 g of muscle tissue were determined in mice infected with *T. pseudospiralis* larvae and receiving different vitamins.

Table 1 and Fig. 1 show the changes in eosinophilia which were evident from the increase in the number of eosinophilic leukocytes starting from day 7 p.i. After the application of vitamins A, B complex, and E, the number of eosinophiles increased up to the maximum on days 21—28 p.i., whereas after the application of vitamins ADE the maximum occurred already on days 14—18 p.i. The highest numbers of eosinophilic leukocytes were found after the application of vitamin B complex (26.3 %) and vitamin E (24.7 %) on day 21 p.i. The course of eosinophilia in the mice receiving vitamin A and in untreated mice was very similar, with the maximum on day 21 p.i. The course of eosinophilia after the application of vitamins ADE was most different, since the maximum values were obtained already on days 14 (14.9 %) and 18 (14.7 %) after infection. The increased eosinophilia was observed in experimental animals of all groups during the whole experiment, i.e. for 70 days.

Table 1. Number of eosinophilic leukocytes (in %) in mice infected with *T. pseudospiralis* after application of vitamins

Day p.i.	Vitamin				Untreated	Control
	A	B complex	E	ADE		
0	0.4	0.2	1.03	0.8	0.3	0.4
2	0.42	0.2	1.4	0.8	1.02	0.8
7	2.0	4.3	5.8	3.8	3.2	1.3
14	11.2	14.3	13.7	14.9	12.6	1.4
16	14.1	17.2	14.02	13.8	14.3	1.4
18	16.2	20.1	19.4	14.7	15.5	1.6
21	20.1	26.3	24.7	12.9	20.7	1.6
28	18.5	23.1	24.04	10.8	19.6	0.7
35	18.6	20.7	19.1	10.1	19.3	1.3
42	16.8	17.8	10.3	8.6	16.1	1.5
49	14.3	16.6	4.3	9.1	14.6	1.6
70	8.9	10.4	5.2	4.2	7.8	0.8

Table 2. Mean total number of muscle larvae of *T. pseudospiralis* in individual mice groups

Mice group	Mean No. of larvae
with vitamin A	1,039
with vitamin B	3,198
with vitamin E	2,731
with vitamins ADE	1,384
untreated	1,308

The changes in the mean weight of mice infected with *T. pseudospiralis* larvae are shown in Fig. 2. The weight increased evenly in the control group and in mice infected with the larvae but not receiving the vitamins. The most pronounced decrease in the

Table 3. Distribution of *T. pseudospiralis* larvae in different parts of body calculated per 1 g of muscle tissue

Mean No. of larvae/1 g	Mice groups with vitamin				Untreated mice
	A	B complex	E	ADE	
Diaphragm	140	1,383	1,129	298	318
Left hind leg	120	364	296	173	158
Right hind leg	66	424	401	81	69
Left foreleg	433	1,640	1,483	502	488
Right foreleg	380	458	417	310	351
Left chewing muscle	260	1,280	1,101	307	263
Right chewing muscle	120	1,140	993	211	211
Tongue	20	150	150	34	31
Ventral muscles	63	128	133	52	79
Intercostal muscles	46	191	244	37	74
Others	8	33	26	10	10

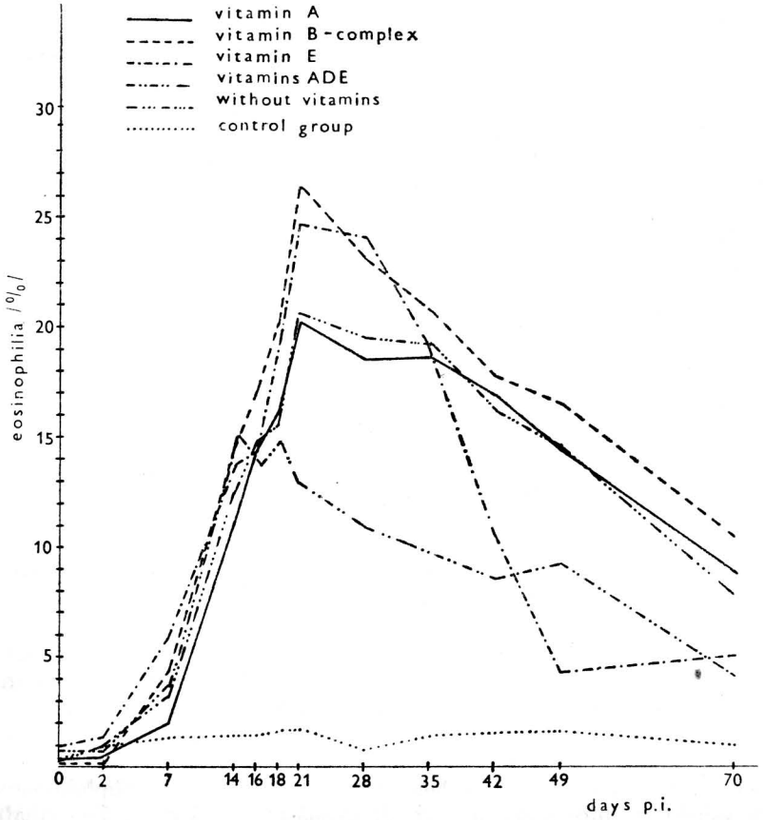

Fig. 1. Changes in the number of eosinophilic leukocytes in the blood of mice infected with *Trichinella pseudospiralis* larvae after application of different vitamins.

body weight occurred in the mice receiving vitamin E (on day 21 p.i.) and vitamin B complex (on days 14—21 p.i.).

If the mice were infected with 100 larvae of *T. pseudospiralis*, in no case their weight decreased below the initial value.

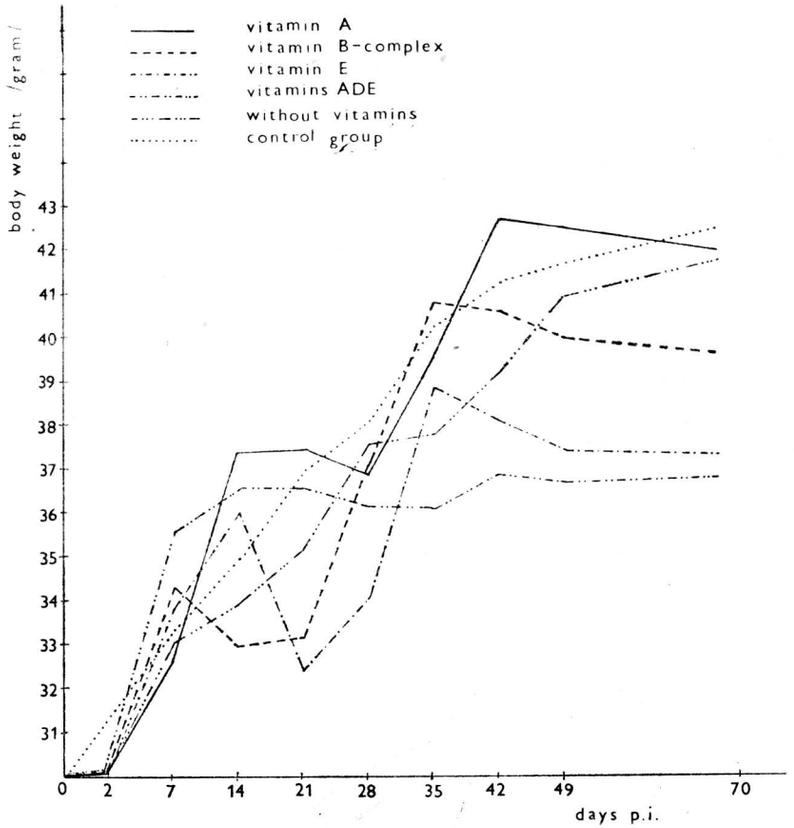

Fig. 2. Mean weight of mice infected with *Trichinella pseudospiralis* larvae after application of different vitamins.

The distribution of *T. pseudospiralis* larvae in different parts of body is shown in Table 3. The distribution of muscle larvae was uneven in all experimental mice; the greatest numbers of larvae were obtained from the left foreleg, diaphragm, masseters, and right foreleg. The greatest numbers of larvae were found in the left forelegs of mice of all experimental groups. Then followed the diaphragm in mice receiving vitamin B complex and vitamin E, and right foreleg in mice receiving vitamins A and ADE and in untreated mice. The left chewing muscle contained more larvae than the right one.

The results obtained show a correlation between the numbers of eosinophilic leukocytes and body weight in mice infected with *T. pseudospiralis* larvae. The greatest numbers of larvae occurred in the mice groups receiving vitamin B complex and vitamin E, which exhibited the greatest eosinophilia but the lowest body weight.

DISCUSSION

In the blood smears from non-infected mice bred in our laboratory the percentage of eosinophilic leukocytes was 1.2 % on the average, Schäffer (1940) reported 0—4 %, Albritton (1955) 0—5 %, and Nejedlý (1965) even 0—7 % as a normal level. Rapidly increasing eosinophilia in the blood is a very important diagnostic character of trichinosis. Its level, however, is no index of severity of the disease (Gould 1970). In our experiments, the number of eosinophilic leukocytes in the blood of mice started to increase after day 7 p.i., reaching the maximum mostly on days 21—28 p.i., and then it decreased again. An increased number of these blood elements, however, persisted during the whole experiment. There was a certain relationship between the maximum number of eosinophiles and decrease in the body weight of experimental mice. The body weight of mice infected with *T. pseudospiralis* larvae never decreased below the initial value. Similarly Penkova (1982) observed an increase in the weight and size of mice experimentally infected with 100 larvae of *T. pseudospiralis*.

Althought the mice were of the same age, sex and weight (maximum difference ± 1 g) and the infection dose was calculated for each animal, relatively great differences occurred between them. Individual differences in the susceptibility of different hosts to *Trichinella* infection were described also by Kozar and Kozar (1963), Ooi and Oku (1984), and Wojcik et al. (1973). According to Kozar (1973), the number of parasites in the muscles is not related only with the female productivity, but also with immunological factors of the host. The immunity is usually manifested by a more rapid elimination of intestinal parasites at different time after infection, sometimes by an inhibition of the growth and smaller body size of adult parasites and decrease in their reproductive ability. Shanta and Meerovitch (1967) reported that the differences in the results obtained by different authors are affected by different virulence of the genus *Trichinella*, species of the experimental animal, individual differences between the experimental animals of the same species, and techniques applied in the experiments. An uneven distribution of *Trichinella* larvae in the groups of cross-striated muscles in mice was described also by Bizyulyavichyus et al. (1976). They found the greatest number of larvae in the chewing muscles, tongue muscles and diaphragm.

A similar effect produced by different vitamins on the intensity of infection was observed also in mice infected with *T. spiralis* larvae. The application of vitamin B complex and vitamin E, due to their positive effect on the skeletal muscles, led to an increased intensity of *T. pseudospiralis* infection, in contrast to vitamin A, which caused a decrease in the intensity. Tarczyński (1963) performed similar experiments as McCoy (1934) and confirmed his conclusion that hamsters with a complete avitaminosis harbour twice as many muscle larvae of *Trichinella* sp. as the control animals fed with a full diet and infected with the same dose of larvae of the same parasite.

ВЛИЯНИЕ ВИТАМИНОВ НА ЗАРАЖЕНИЕ МЫШЕЙ ЛИЧИНКАМИ *TRICHINELLA PSEUDOSPIRALIS* GARKAVI, 1972

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Резюме. Изучали влияние витаминов А, Б комплекс, Е и ADE на эозинофилию, вес тела, количество личинок и распределение личинок *T. pseudospiralis* у мыши. У большинства зараженных мышей повышенная эозинофилия начала проявляться на 7 день после заражения и максимальную эозинофилию наблюдали около 21 дня после заражения. Самая большая эозинофилия встречалась у мышей, получивших витамин Б-комплекс. Несмотря на значительные индивидуальные различия, самое большое понижение веса тела наблюдали у мышей, получивших витамин Е и Б комплекс. Самое большое количество личинок

выделяли из мышей, получивших витамин Б комплекс и витамин Е, что прямо пропорционально повышению эозинофилии. Приблизительно одинаковое количество мышечных личинок *T. pseudospiralis* обнаружено у мышей без витаминов и после применения витаминов ADE. Меньше всего личинок обнаружили у мышей, получивших витамин А. Во всех группах мышей самое большое количество личинок было локализовано в левой передней ноге и диафрагме.

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