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INFLUENCE OF COMBINED ACTION OF HISTAMINE AND THEOPHYLLINE ON MAST CELLS, ANTIBODY-BEARING CELLS AND PARASITES IN THE COURSE OF EXPERIMENTAL TRICHINELLOSIS IN MICE*

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The mechanism of "self-cure" in intestinal helminthoses has attracted the interest of many investigators in recent year. Factors mentioned as playing an important role in this process include mast cells and their products (Jarrett et al., 1968, Murray et al., 1971a, b). In our studies over a number of years on the phenomenon of "self-cure", conducted on a model of *Trichinella spiralis* infection, we have tried to elucidate the role of mast cells by observing enhanced (Karmańska et al., 1973b) as well as inhibited (Karmańska et al., 1973a, 1974; Karmańska, Michalska, 1975; Michalska, Karmańska, 1976) degranulation of these cells. In continuation, in the present study we have investigated the course of "expulsion" of adult trichinellae under conditions of maximally reduced numbers of degranulating fast cells. To obtain maximal inhibition of degranulation, we treated mice infected with trichinellae with histamine and theophylline, the synergistic action of which raises intracellular levels of cAMP which enhances stability of the cells.

Material and methods

The study was carried out on 100 male mice of the CFW strain weighing 20 ± 0.9 g, aged 3 months, maintained under constant conditions during the experiment.

Mast cells were studied by the method of Enerböck (1966a, b),

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and paraffin sections for histopathologic study were stained with hematoxylin and eosin. The immunocytoadherence test with *T. spiralis* antigen was set up acc. Płonka (1974) and Machnicka (1976). The manner of infecting the animals and counting parasites was described previously (Karmańska et al., 1977).

Results

The mice were divided into two groups: controls (group 1) infected with 200 *T. spiralis* larvae per mouse, and an experimental group (group 2) which besides being infected as above, were injected intraperitoneally every 12 hours with histamine in doses of 30 mg/kg/day and theophylline, 24 mg/kg/day. Animals of both group were killed at 10, 16, 20, 30 and 42 days p.i. (post infection), and cells from the peritoneal exudate were used to set up the immunocytoadherence test with erythrocytes coated with trichinella antigen. Sections of the jejunum and masseter muscle were used for histopathologic studies. In addition, adult trichinellae were counted, and at 42 days p.i. also muscular larvae.

1. Jejunum.

Mast cells. In the lamina propria of the jejunal mucosa of the control mice (group 1), 10 days p.i. the mean number of mast cells was 235.0/sq mm, and on day 16 p.i. increased to 356.4/sq mm. However, at 20 days p.i. the number of mast cells dropped to 305.8/sq mm and 30 days p.i. to 166.1/sq mm. The lowest number of mast cells, 40.7/sq mm, was observed on the 42nd day p.i.

The rise in the number of mast cells was accompanied by a rise in the number of degranulated cells to 50.6/sq mm on day 10, and at 16 days p.i. to 102.6/sq mm. On the 20th day of the experiment, the number of degranulated cells dropped to 49.4/sq mm, 30th day — to 46.2/sq mm, and on day 42 p.i. to 11.0/sq mm.

In the group of mice treated with histamine and theophylline (group 2), the number of mast cells in the intestinal mucosa was lower. On day 10 p.i., 224.4 mast cells/sq mm were counted, but on day 16 p.i. the peak number of mast cells, 277.2/sq mm was reached. At the next count, on day 20 p.i., the number of mast cells dropped somewhat to 247.3/sq mm, and after 30 days to 137.5/sq mm. The lowest mean number of mast cells. 48.4/sq mm, was found on the 42nd day p.i.

Similarly, the numbers of degranulated cells in the intestinal mucosa of mice treated with histamine and theophylline was lower than in the control group of mice. On day 10 p.i., 48.3 degranulated cells/sq

mm were found, on day 16 p.i. 53.9/sq mm, and on day 20 p.i. 48.4/sq mm. At 30 and 42 days p.i. the number of degranulated cells underwent futher reduction, to 14.3 respectively 7.7/sq mm (Fig. 1).

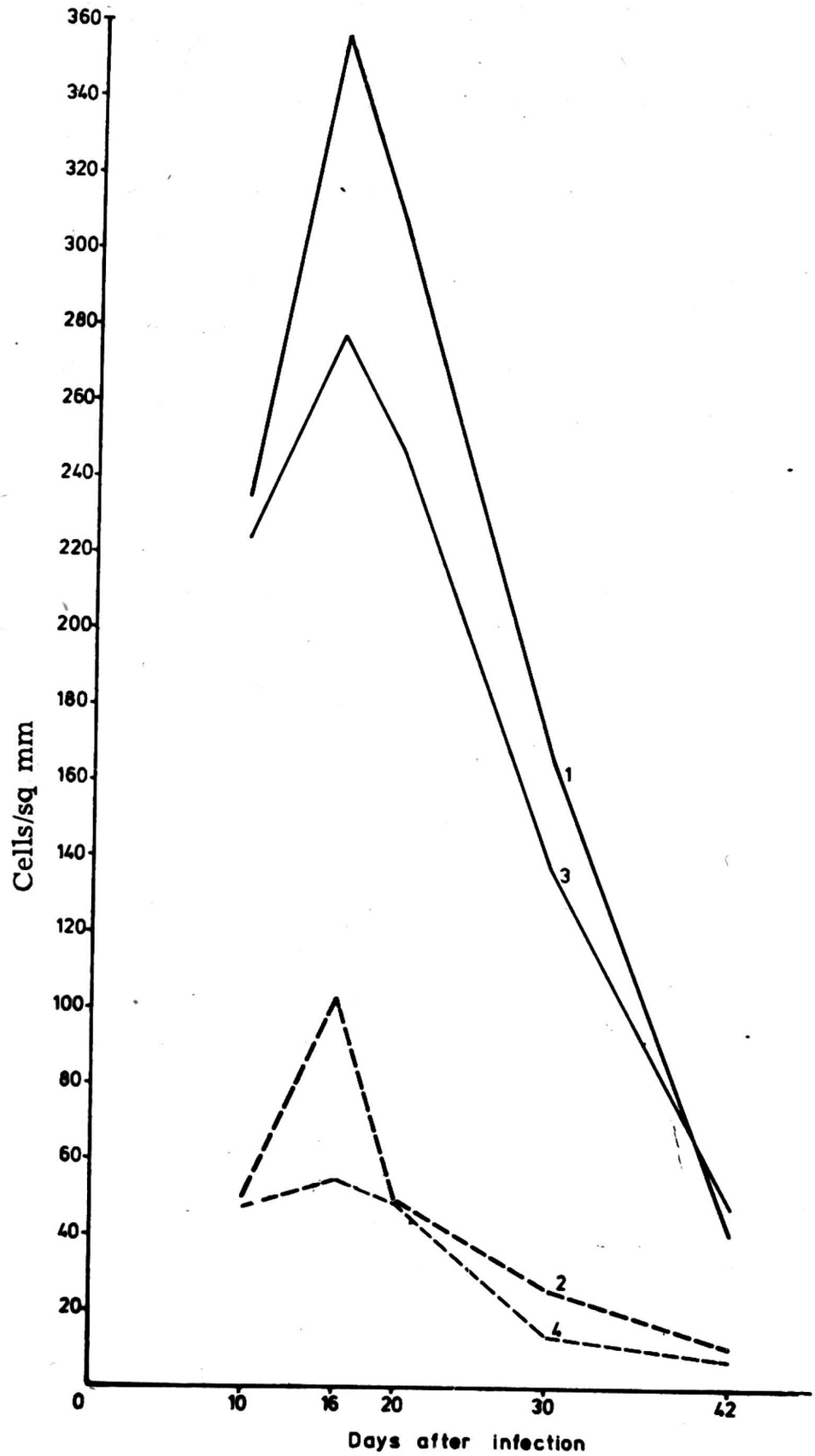

Fig. 1. Mast cells in jejunum

1 — control group: mast cells/sq mm, *2* — control group: degranulated mast cells/sq mm, *3* — experimental group: mast cells/sq mm, *4* — experimental group: degranulated mast cells/sq mm

Histopathologic studies.

In the control mice, 10 and 16 days p.i., there was serous edema of the lamina propria of the intestinal mucosa, dilatation of lymphatics, hyperemia of capillaries of the intestinal villi, increased mucus production, and a moderate number of inflammatory infiltrates composed of lymphocytes, histiocytes and neutrophilic and eosinophilic leukocytes. Leukocytes were most numerous in the central area of the mucous membrane, although a few mixed infiltrates were seen also near its base. On day 20 p.i. the stroma of the villi was still markedly edematous, but inflammatory changes were less pronounced (slight hyperemia, fewer infiltrates). On day 30 p.i. only the larger villi of the jejunal mucosa showed a richly cellular lamina propria and moderately dilated lymphatics. By day 42 p.i., the intestinal mucose showed no changes. Between days 10 and 20 p.i. proliferation and swelling of Paneth cells with increased content of eosinophilic granulations was seen.

In the jejunum of the experimental group of mice, between days 10 and 20 p.i. the lamina propria of villous mucosa showed marked serous edema (but not in all villi) or dilatation of the lymphatic spaces. The villous capillaries and larger blood vessels at the base of the mucous membrane and in the submucosa were packed with blood. The goblet cells of the mucous membrane contained increased amounts of mucus, and the superficial epithelium was detached and desquamated into the intestinal lumen in some places. The mucous membrane contained numerous inflammatory infiltrates composed mainly of lymphocytes, histiocytes and neutrophilic and eosinophilic leukocytes. On day 30 p.i. intensity of inflammatory changes subsided, but edematous stroma and cellular infiltrates with numerous polymorphonuclear leukocytes in some but not all of the villi were still present. The leukocytes polymorphonuclear were most numerous in the middle and lower parts of the mucous membranes. By 42 days p.i. there were practically no more inflammatory infiltrates, but some of the intestinal villi were still edematous and hyperemic. Between days 10 and 20 p.i. the Paneth cells showed proliferation and swelling.

Parasitological studies. In the intestines of control mice (group 1) the mean number of adult parasites 10 days p.i. was 76.5, and on day 15 p.i. 46. On day 20 no parasites were encountered.

In mice treated with histamine and teophylline (group 2) on 10 day p.i. mean number of adult trichinellae reached 93, on day 16 — 46 and on day 20 p.i. 19. On day 30 p.i. their number fell to 0.5 and after that no more parasites were detected (Fig. 2).

2. The masseter muscle.

Mast cells. In the muscles from control mice (group 1), 10 days p.i. the mean number of mast cells was 5.2/sq mm, and on day 16 p.i. 5.2/sq mm. However, on day 20 p.i. the number of mast cells in the muscle rose to 16.2/sq mm. On day 30 p.i. the mean number of mast cells was 14.5/sq mm, and the maximum number, 21.5/sq mm, was noted on day 4.2 p.i.

Mobilization of the mast cells was acompanied by increased degranulation. Whereas at 10 days p.i. the mean number of degranulated cells was 2.3/sq mm, and on day 16 p.i. 1.7/sq mm, the number of degranulated cells rose to 6.4/sq mm on day 20 p.i., and on day 30 p.i. to 7.0/sq mm, dropping on day 42 p.i. to 6.4/sq mm.

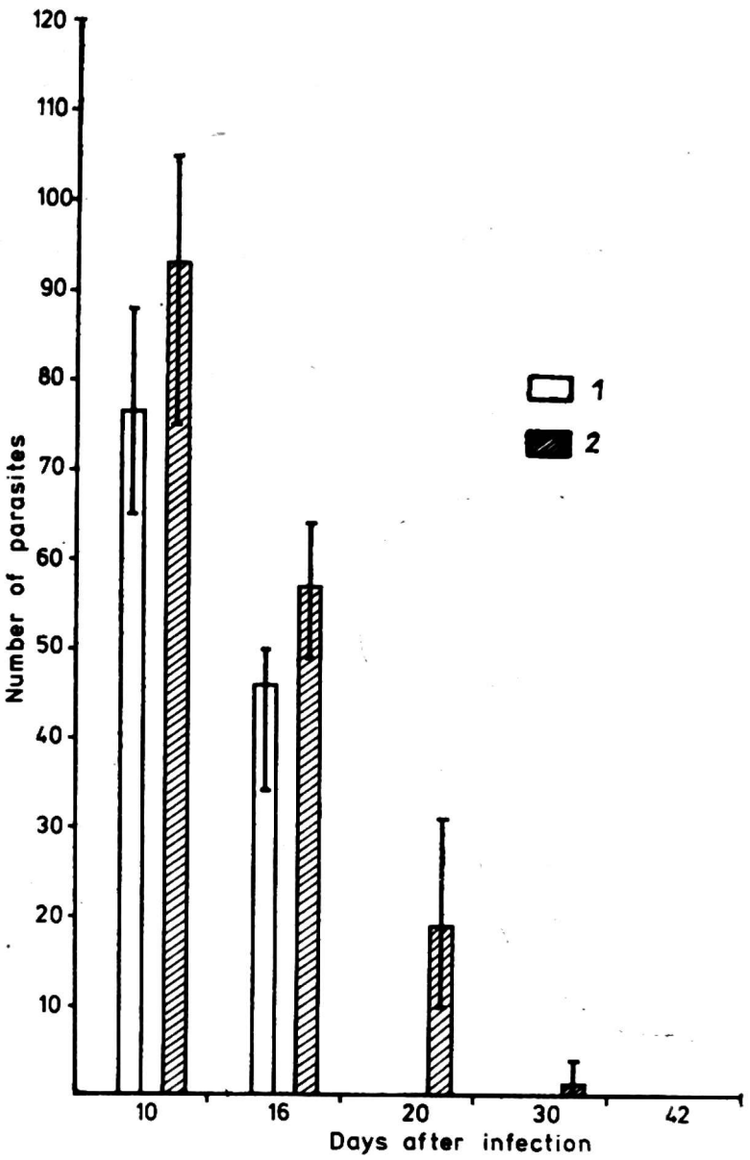

Fig. 2. Mean number of *T. spiralis* in the intestines
1 — control group, *2* — experimental group

In the muscles of mice treated with histamine and theophylline (group 2) mast cells underwent almost no mobilization. On day 10 p.i. the mean number of mast cells was 7.5/sq mm, on day 16 p.i. 4.6/sq mm, and on day 20 p.i. 7.5/sq mm. At 30 days p.i. the number of mast cells dropped to 6.4/sq mm, but on day 40 rose to 8.7/sq mm.

The course of degranulation in muscular mast cells in mice treated with the drugs was also lower than in the control group. On day 10 p.i. the mean number of degranulated cells was only 0.6/sq mm, and on day 16 p.i. 1.0/sq mm. A somewhat higher number of degranulated cells was seen after day 20 p.i., namely: at 20 days 2.3/sq mm, at 30 days 3.5/sq mm, and at 42 days 1.7/sq mm (Fig. 3).

Histopathologic studies. In the control mice (group 1), on day 10 p.i. the masseter muscle contained few basophically altered muscle fibers with proliferation and edema of the sarcolemmal nuclei. Intermuscular inflammatory infiltrates were scarce, composed of only a few cells,

mainly lymphocytes and histiocytes Muscular larvae began to appear 16 days p.i., accompanied by fairly numerous inflammatory infiltrates containing eosinophilic leukocytes, especially in larger infiltrates. On days 20 and 30 p.i. the number of larvae and inflammatory infiltrates increased, with distinct predominance of eosinophilic leukocytes, for-

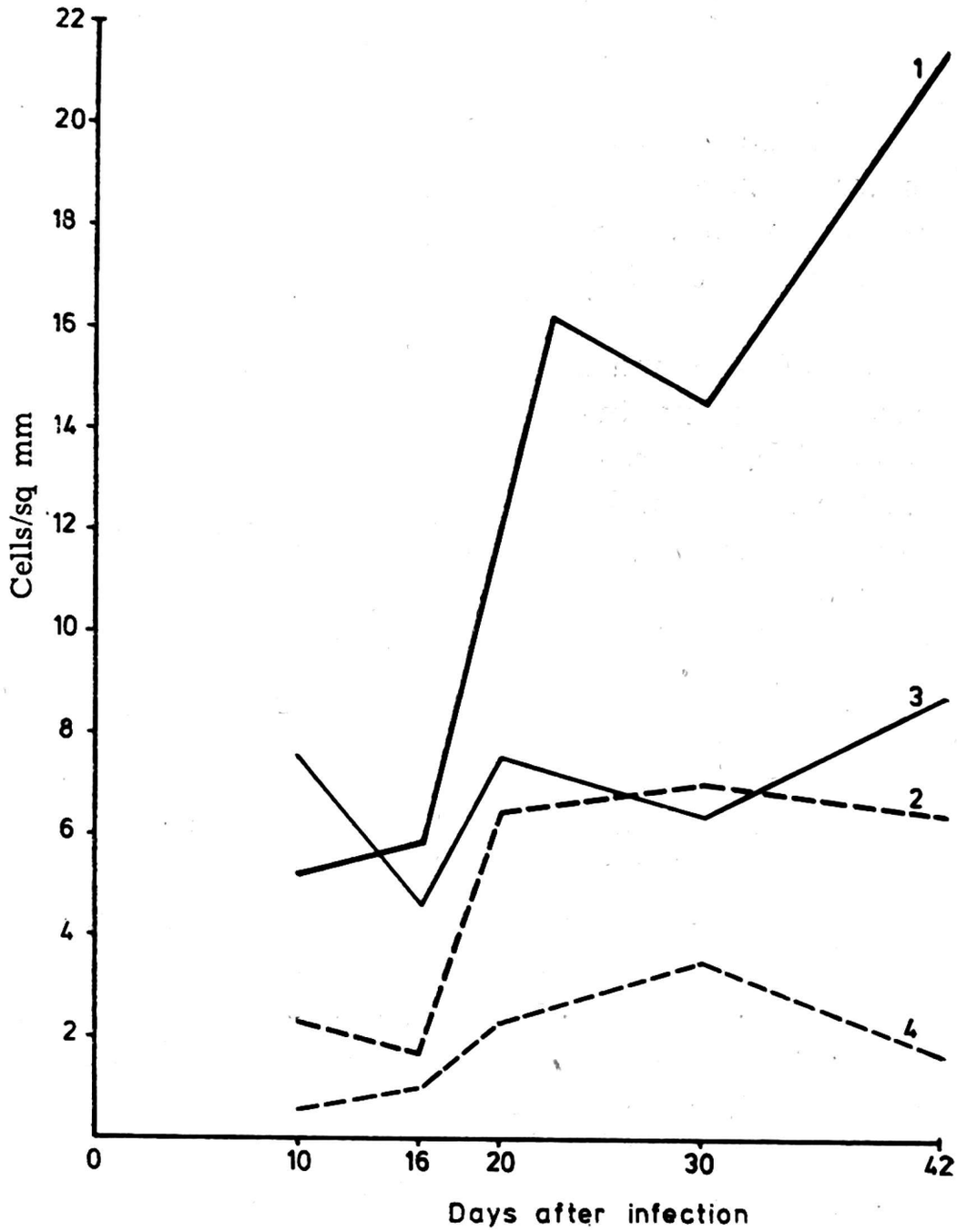

Fig. 3. Mast cells in m. masseter. Explanations as in Fig. 1

ming granulomas of various size in place of dying larvae. On day 42 p.i. fewer infiltrates and granulomas were seen around the curling larvae, compared with the preceding periods (days 20-30). The infiltrates, now mainly mixed, i.e. composed of lymphocytes, histiocytes, fibroblasts and polymorphonuclear leukocytes were mostly grouped around the larval capsules, and less often formed strands between the muscle fibers. Some

muscular larvae were unaccompanied by any infiltrates. Between 30 and 42 days p.i. few muscle fibers were altered basophically.

The masseter muscles from experimental mice 10 days p.i. contained few basophilic muscle fibres with proliferation of edematous sarcolemmal nuclei. There were almost no inflammatory infiltrates, but sporadically intramusculary lymphohistiocytary cells were found. On day 16 and 20 p.i. appeared a few muscular larvae and numerous inflammatory infiltrates with preponderance of eosinophilic leucocytes. More and large eosinophilic granulomas around the dying parasites were also observed. On day 30 p.i. the number of infiltrates decreased somewhat. In the smaller infiltrates mononuclear cells (lymphocytes, histiocytes some fibroblasts) appeared and eosinophilic granulomas were still numerous. The picture of the muscle was similar 42 days p.i., only the infiltrates were les extensive and mononuclear cells more numerous. Small granulomas contained mainly eosinophilic leucocytes. Later (days 30-42 p.i.) basophilic fibres were increasingly scarce.

Comparison of the two groups of mice showed more numerous inflammatory infiltrates in group 2 than in the control group, although appearing somewhat later (after 16 days p.i.).

Parasitologic studies. The mean number of muscle larvae in the control mice (group 1) was 59,625 (S.D.=6,126. 4), and in the mice treated with histamine and theophylline (group 2), 30,400 (S.D.=4,118. 1).

Immunocytoadherence test. In the control mice (group 1) on day 10p.i. 5.5% of the cells produced rosettes. On day 16 p.i., however, there were already 7.5% and on day 20 p.i. 10.9%. The highest number of cells reacting with antigen-coated erythrocytes, 19.5%, was reached on day 20 p.i., but dropped to 9.5% on day 42 p.i.

In mice treated with histamine and theophylline (group 2), 10 days p.i. 2% of the cells produced rosettes. On day 16 p.i. this rose to 4.1% on day 20 to 5.3%, and on day 30 p.i. to 6.5%. At the last examination on day 42 p.i., only 2.75% of the cells were rosette-forming (Fig. 4).

Discussion

In our studies on the role of mast cells in the mechanism of expulsion of adult trichinellae, we used factors which act on growth or lowering of the levels of degranulated cells. By thus "guiding" the process of degranulation, we were able to reach some conclusions about the role of intestinal mast cells in the process of "self-cure". Since degranulation of mast cells depends on the intracellular level of cAMP,

regarded as a mediator of anaphylactic reactions, we selected preparations which raise or depress that level. A rise of intracellular cAMP enhances stability of the cells, and a drop causes their degranulation.

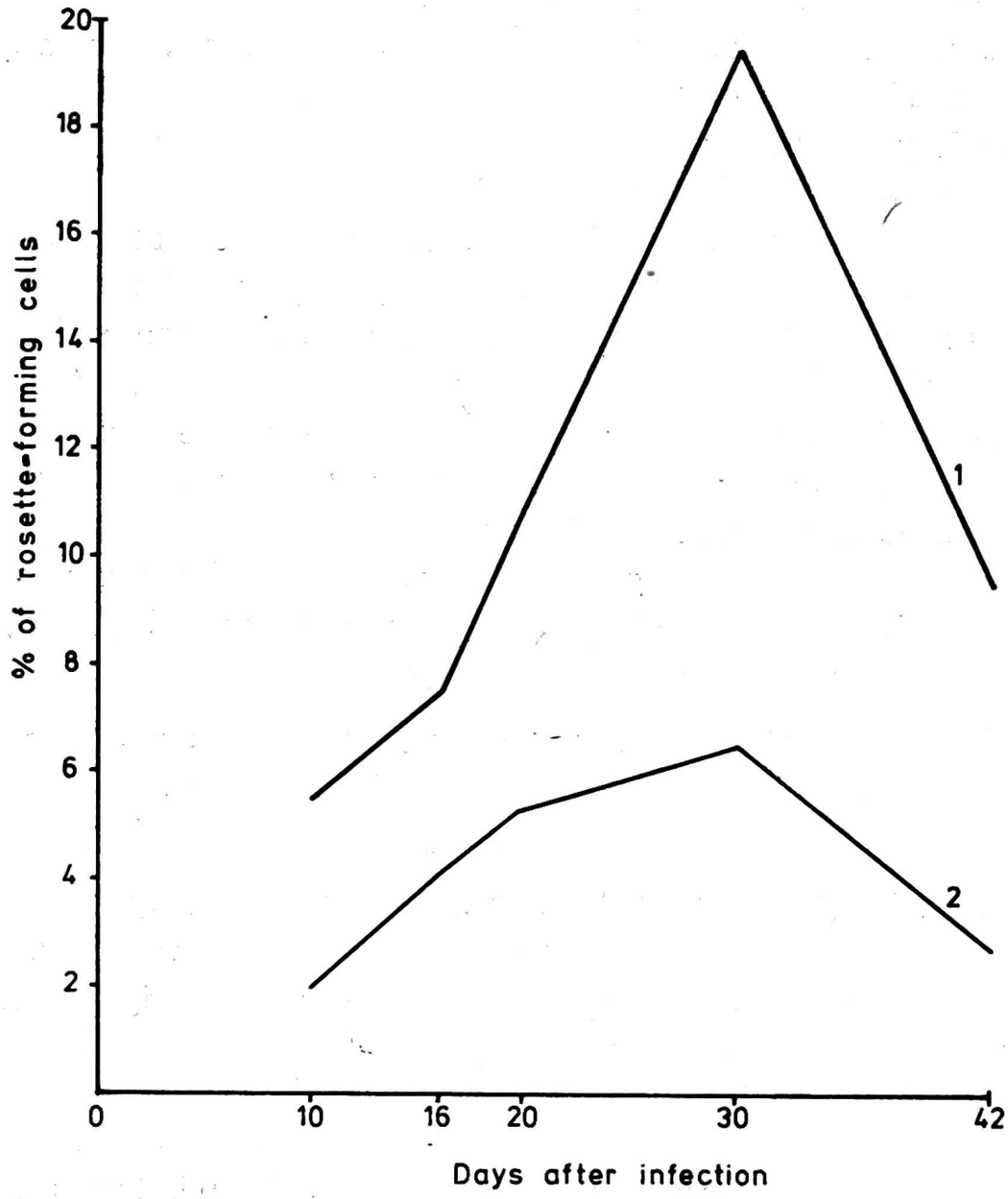

Fig. 4. Rosette-forming cells
1 — control group, *2* — experimental group

Levels of this nucleotide can be raised by stimulating adenyl cyclase (responsible for synthesis), or by inhibiting phosphodiesterase (on which degradation depends). In previous studies, we used histamine (Karmańska et al., 1974) or prostaglandin E_2 (Karmańska, Michalska, 1977a, b) which stimulate adenyl cyclase, respectively Intal (Michalska, Karmańska, 1976) or theophylline (Karmańska, Michalska, in printing b), which act on phosphodiesterase. In the present study, by acting simultaneously on both enzymes, we wanted to obtain maximal degranulation of the in-

testinal mast cells. The results of experiment with mice treated with histamine and theophylline showed that this treatment reduces degranulation by one half (at the peak of mobilization). Under these conditions, survival of parasites in the intestine was distinctly prolonged and was accompanied by greater histopathologic changes. On the other hand, in muscles, the smaller number of degranulating cells was due to accompanied by smaller number of muscular larvae and intense inflammatory infiltration. Our previous results on the influence of histamine (Karmańska et al., 1974) and theophylline (Karmańska, Michalska, in printing b) gave similar results.

Keller, Ogilvie (1972), who treated rats infected with *Nippostrongylus brasiliensis* with histamine also observed delayed expulsion of the parasites, and concluded that mast cells take no part in the process of "expulsion" based on release of histamine from mast cells. On the ground of these findings, however, we (Karmańska et al., 1974) concluded that they indicate a role of the mast cells in "self-cure". Inhibition of degranulation of mast cells by histamine probably lowers levels of other substances which can influence expulsions. According to our interpretation, the results of experiments with exogenous histamine indicate either that histamine plays no direct role in expulsion of parasites, or that the action of exogenous histamine is different from that of endogenous histamine. Both explanations don't exclude a role of mast cells.

In the present study, basides weaker mobilization and degranulation of mast cells, we also observed lowered levels of cells forming rosettes with antigen-coated erythrocytes.

Weissman et al. (1971), Diamantstein, Ulmer (1975), and Vischer (1976) also observed that raised levels of cAMP inhibit activity of other cells. Keller, Ogilvie (1972) have called attention to this fact in the mechanism of "self-cure". However, our latest studies on identification of T lymphocytes in sections of organs using the histochemical method of Mueller et al. (1975) have shown that in the course of trichinella infection T cells are stimulated after expulsion of the parasites from the intestine (Karmańska, Michalska, in printing a, 1977b). Consequently, experiments with preparations that modify cAMP levels would affect only the behavior of mast cells and antibody-producing cells, although Vischer (1976) considers this nucleotide has a much weaker effect on the behavior of B lymphocytes, compared with its effect on T lymphocytes, Moreover, administration of histamine raises not only levels of cAMP, but also those of cGMP (Mathé et al., 1974). According to Diamentstein and Ulmer (1974), cGMP stimulates B lymphocytes as well as T lymphocytes.

Finally, one must take into account that damage of parasites, hitherto attributed to antibodies (Ogilvie, Huckley, 1968) may be an effect of prostaglandins (Dineen, Kelly, 1976). In that case, the changes following elevation of intracellular cAMP in the course of the intestinal phase of trichinellosis would pertain chiefty to the mast cells.

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WPŁYW POŁĄCZONEGO DZIAŁANIA HISTAMINY I TEOFILINY NA KOMÓRKI TUCZNE, KOMÓRKI TWORZĄCE PRZECIWCIAŁA ORAZ PASOŻYTY W PRZEBIEGU DOŚWIADCZALNEJ WŁOŚNICY U MYSZY

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Autorki podawały histaminę i teofilinę myszom szczepu CFW (samcom o wadze około 20 g, w wieku 3 miesięcy) zarażonym 200 larwami *T. spiralis*. Preparaty stosowano między 1-30 dniem po zarażeniu (p.z.), co 12 godz., w następujących dawkach: histaminę — 30 mg/kg/dobę, teofilinę — 24 mg/kg/dobę. Zwierzęta zabijano w 10, 16, 20, 30 i 42 dniu p.z., po czym z komórkami płynu otrzewnowego nastawiano odczyn immunocytoadherencyjny. Jednocześnie wycinki jelita czczego i mięśnia żuchwowego pobierano do badań histopatologicznych oraz do badań w kierunku komórek tucznych.

Na podstawie uzyskanych wyników stwierdzono, że podawanie histaminy i teofiliny obniża mobilizację i degranulację komórek tucznych. Równocześnie w jelitach myszy, które otrzymywały preparaty, stwierdzono większe niż w kontroli zmiany histopatologiczne oraz opóźnione usuwanie dorosłych włośni. Podobnie większe zmiany histopatologiczne obserwowano w mięśniach zwierząt traktowanych histaminą i teofiliną, a liczba uzyskanych od nich larw była o wiele mniejsza. Ponadto w odczynie immunocytoadherencyjnym stwierdzono niższy niż w kontroli odsetek komórek tworzących rozetki.