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THE INFLUENCE OF X-IRRADIATION ON IMMUNE PHENOMENA IN THE COURSE OF EXPERIMENTAL TRICHINELLOSIS IN MICE *

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The influence of gamma and X-irradiation on immune processes has been the subject of many publications in recent years. Although this problem has been studied in trichinellosis by Semmerad (1937), Stoner and Hale (1952), Yarinsky (1962), Larsh et al. (1962), Kim (1966) and Kim et al. (1974), there is a lack of investigations on the behavior of various cells involved in immunity in the course of *T. spiralis* infection in irradiated host. An investigation on the effects of irradiation with 600 R on mast cells, rosette-forming cells in the immunoadherence test and lymphocytes responsible for DH [1] in mice infected with trichinellae is therefore justified. Comparison of the results with the concurrent histopathologic picture and numbers of parasites in the intestines and muscles permitted more precise determination of the influence of X-irradiation on the course of trichinellosis in mice.

Material and methods

Animals. The study was carried out on 190 male RIII × $C57B_1/F_1$ mice weighing 20 gm (±0.7), aged over 3 months, maintained under constant conditions throughout the experiment.

Irradiation. The mice were irradiated with a Siemens apparatus, type Med. s 34 c (No. 2964106, tube housing No. 129187) with a suble-

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[1] delayed hypersensitivity

thal whole-body dose of 600 R at 130 kV, 6 mA, distance 30 cm, filter 2.5 mm Al, half-layer (HVL) 0.6 mm Cu. Time of irradiation was 1766.5 sec, and strength of the dose under these conditions was 0.34 $R \cdot sec^{-1}$.

Infection. *T. spiralis* larvae were obtained by acting on the muscles of infected mice with a digesting solution (pepsin 10 g, conc. HCL 7 ml, H_2O dist. 1,000 ml) at 40°C during five hours. A sieve containing comminuted muscles was placed on a funnel with its outlet inserted in a test tube, containing the digesting solution. Larvae collected in the sediment were washed several times with physiologic saline and suspended in 0.2% agar to give a concentration of 500 larvae/ml. Mice were infected by introducing 0.2 ml of the suspension (i.e. 100 larvae/mouse) into the esophagus.

Macrophage migration inhibition test (indirect method). The technique of macrophage migration inhibition test, we used based on the method described by Rajapakse and Glynn (1970) and Marsman and van de Hart (1973).

Three days before the test, healthy mice were injected intraperitoneally with 2.5 ml of sterile paraffin oil. On the day of the test, the mice were killed by exsanguination, and 5 ml of Parker fluid (containing 10% calf serum and 10 γ/ml kanamycin) cooled to 4°C was injected into the peritoneal cavity. After vigorously massaging of the abdomen of the mice, the fluid was aspirated into test tubes. Cells obtained by centrifuging were washed twice with Parker fluid and used in the test.

At the same time, lymphocytes were secured from the tested animals. To this end, the mesenteric lymph node was teased, combined with a small amount (2-3 ml) of Parker fluid, rubbed through a double nylon filter (Blood Filter, "Technochemia", Warsaw) and centrifuged. The sedimented cells were washed twice with Parker fluid and combined with macrophages from healthy mice in the proportion of 1 : 4. Capillary tubes filled with this mixture were centrifuged. The parts of the capillaries containing the cells were cut off and placed in a Mackeness chamber filled with Parker fluid with or without trichinella antigen (lyophilized extract of *T. spiralis* larvae, deprived of lipids, containing 5 mg% protein), diluted 1 : 400. After 24 hr incubation at 37°C the migration zones under magnification on a projection trichinoscope (× 50) were copied on paper and measured planimetrically. Percentage migration was calculated by the equation:

$$\frac{\text{aver. area of migration with antigen}}{\text{aver. area of migration without antigen}} \times 100 = \% \text{ of migration with antigen}$$

Immunocytoadherence test. The reaction of lymphocytes with antigen-coated erythrocytes was set up by the method adapted for studies on trichinellosis by Płonka (1974) and Machnicka (1976).

Mast cells. Fixed sections of organs were prepared by the method of Ennerböck (1966a, 1966b). When staining intestinal preparations, Astra blue was applied for one hour. The method of counting the cells was described previously by Karmańska et al. (1971).

Histopathologic study. Preparations for histopathologic study were stained with hematoxylin and eosin.

Intestinal parasites. Comminuted intestines of each mouse were placed on a sieve in a funnel attached to a test tube containing the saline solution and incubated at 40°C for 18 hr. The sediment in the test tube was transferred to a watch glass and parasites were counted with a magnifying glass.

Results

All the mice were divided into four groups: two control groups (1 and 2) and two experimental (3 and 4). Group 1 mice were X-irradiated with 600 R (controls of irradiation), and group 2 mice were infected with 100 larvae/mouse (controls of infection). Groups 3 and 4 were irradiated with 600 R and infected with 100 *T. spiralis* larvae. Group 3 mice were infected 24 hr after exposure, and group 4 after 7 days.

Group 1 mice were killed on alternate days during 31 days. At autopsy, a lymph node, spleen and bone marrow were secured for histopathologic study. Mice of the remaining groups were killed at 16, 19, 22, 28, 31 and 60 days post infection (p.i.), and the macrophage migration inhibition and rosette tests were set up with cells from the mesenteric lymph node. Sections from the jejunum were examined for mast cells and histopathologically. In all mice that were killed, adult parasites were counted, and at 60 days p.i. also muscular larvae.

Macrophage migration inhibition test. Mesenteric lymph node cells from control mice (group 2) killed 16 days p.i. did not inhibit macrophage migration. Percentages of migration calculated in relation to migration without antigen amounted to 96.0%. However, at 19 days after infection the migration fields were reduced to 75.3%, and at 22 days to 65.0%. At 25 days p.i. inhibition of migration was less pronounced, 73.6%, and on the 28th experimental day 71.2%. Thirty-one days p.i. the macrophage migration area increased to 78.7%.

Lymphocytes from mice infected 24 hr after irradiation (group 3) examined between 16 and 28 days p.i. failed to inhibit migration of the peritoneal macrophages (the mean area of migration fields at 16 days p.i. was 100.0%, at 19 days 98.5%, at 22 days 117.2%, at 25 days 97.8%, and at 28 days p.i. 103.8%). However, at 31 days p.i. macrophage migration was inhibited to 89.8%.

Similar results were obtained with mice infected 7 days after irradiation (group 4). No inhibition of macrophage migration was observed until the 28th day p.i., and compared with the controls without antigen amounted to: on day 16 p.i. 115.0%, on day 19 — 107.6%, on day 22 — 104.4%, on day 25 — 101.2%, and on day 28 p.i. 97.6%. On day 31 p.i. the mean area of migration dropped to 77.1%, showing distinct inhibition at this time (Fig. 1).

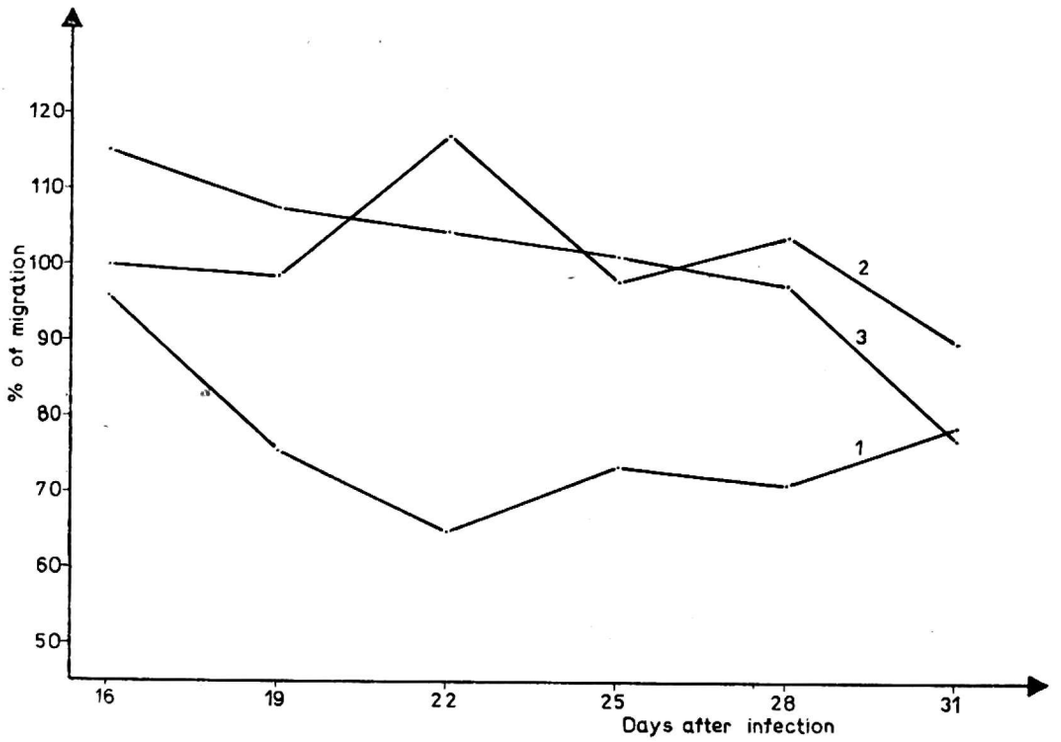

Fig. 1 Macrophage migration inhibition test
1 — group 2 (control), *2* — group 3, *3* — group 4

Immunocytoadherence test. In control mice killed 16 days p.i. (group 2), 10.2% of the cells from the mesenteric lymph node produced rosettes. On the 19th day p.i. this proportion dropped to 8.0%, on the 22nd day to 9.25%, and on the 25th day to 10.0%. On the 28th day 8.5% of the lymphocytes formed rosettes, and on the 31st day distinctly dropped to 5.5%.

In mice infected 24 hr after irradiation (group 3), the mean proportion of rosette-forming cells on the 16th day p.i. was 3.5%, and the same on the 19th and 22nd days p.i.: 3.25% and 3.5%. A slight increase to 4.5% was noted on days 25 and 28 p.i., and nearly the same (4.75%) on day 31 p.i.

In the group of mice infected 7 days after irradiation (group 4) on day 16 only 3.1% of the cells formed rosettes. After that, the proportion increased somewhat: on 19 and 22 p.i. to 5.0%, on day 25 to 5.2%, and on day 28 p.i. to 6.0%. The highest level of rosette-forming cells (6.5%) in this group of mice was observed on day 31 p.i. (Fig. 2).

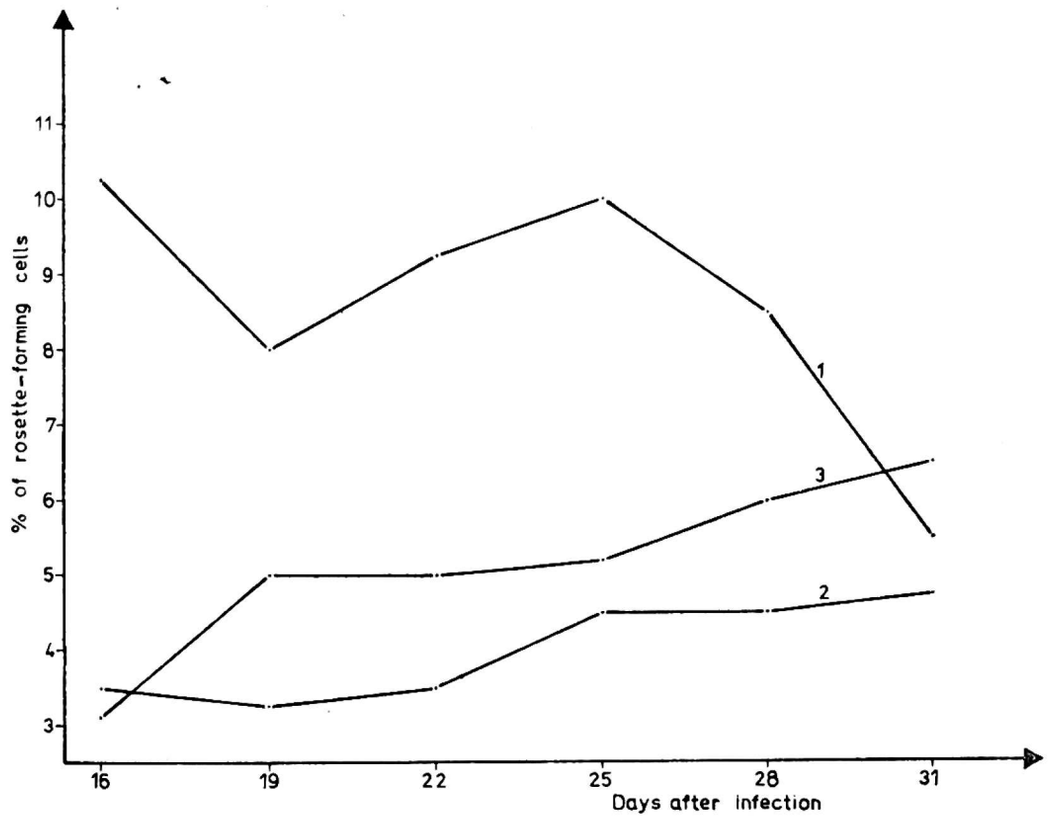

Fig. 2 Rosette-forming cells. Explanations as in Fig. 1

Mast cells. In control mice (group 2) on day 16 the mean number of mast cells was 288.9 per sq mm of intestinal mucous membrane. Subsequent counts showed a gradual drop to 196.9/sq mm on day 19, 150.7/sq mm on day 22 and 135.3/sq mm on day 25 p.i. A smaller number of mast cells was found in the last two examinations: 57.4/sq mm on day 28 p.i. and 48.4/sq mm on day 31 p.i.

Dynamics of the degranulating cells was similar. On day 16 p.i. there were 103.0/sq mm, but on day 19 p.i. fell to 93.5/sq mm. On day 22 p.i. the number of degranulated cells dropped further to 71.5/sq mm, and on day 25 p.i. to 64.9/sq mm. In subsequent counts, a further drop occurred to 16.5/sq mm on day 28, and 13.2/sq mm on day 31.

In the intestinal mucosa of mice infected 24 hr after irradiation (group 3) no stimulation of the mast cells was observed throughout the observation period. On days 16 and 19 p.i. the mean numbers of mast cells per sq mm was 1.1, on day 22 — 3.3/sq mm, on day 25 nearly 1.1, and on day 28 — 2.2/sq mm. On day 31 p.i. the level of mast cells rose to 11.0/sq mm.

In this group of mice very few degranulated cells were noted. The mean numbers on days 16 and 19 were 0.5/sq mm, on day 22 p.i. 1.6/sq mm, and on days 25 and 28 1.1/sq mm. However, at 31 days p.i. the mean numbers of degranulated mast cells increased to 7.7/sq mm.

In mice infected 7 days after irradiation (group 4), no stimulation of intestinal mast cells occurred such as is usually seen in the course of *T. spiralis* infections. On days 16 and 19 the mean numbers of mast cells per sq mm were 0.5, and on day 22 p.i. 1.6/sq mm. However, 25 days p.i. the number of mast cells in the intestinal mucosa rose to 8.8/sq mm, on day 28 to 11.5/sq mm, and on day 31 to 29.1/sq mm.

The dynamics of degranulation were similar. No degranulating cells were seen on days 16 and 19 p.i., and only 0.5/sq mm on day 22 p.i.; on days 25 and 28 p.i. the mean numbers of degranulated cells per sq mm were 4.4, and on day 31 p.i. increased to 19.2 (Fig. 3).

Histopathologic studies. In control mice X-irradiated with 600 R (group 1), the lymph nodes, spleen and bone marrow were studied histopathologically.

In the mesenteric lymph nodes of the irradiated animals, marked atrophy of lymphoid tissue replaced by proliferating and often edematous reticulum was noted. Loose lymphatic tissue was present only focally. Beginning on the 13th day after irradiation, the lymphoid tissue

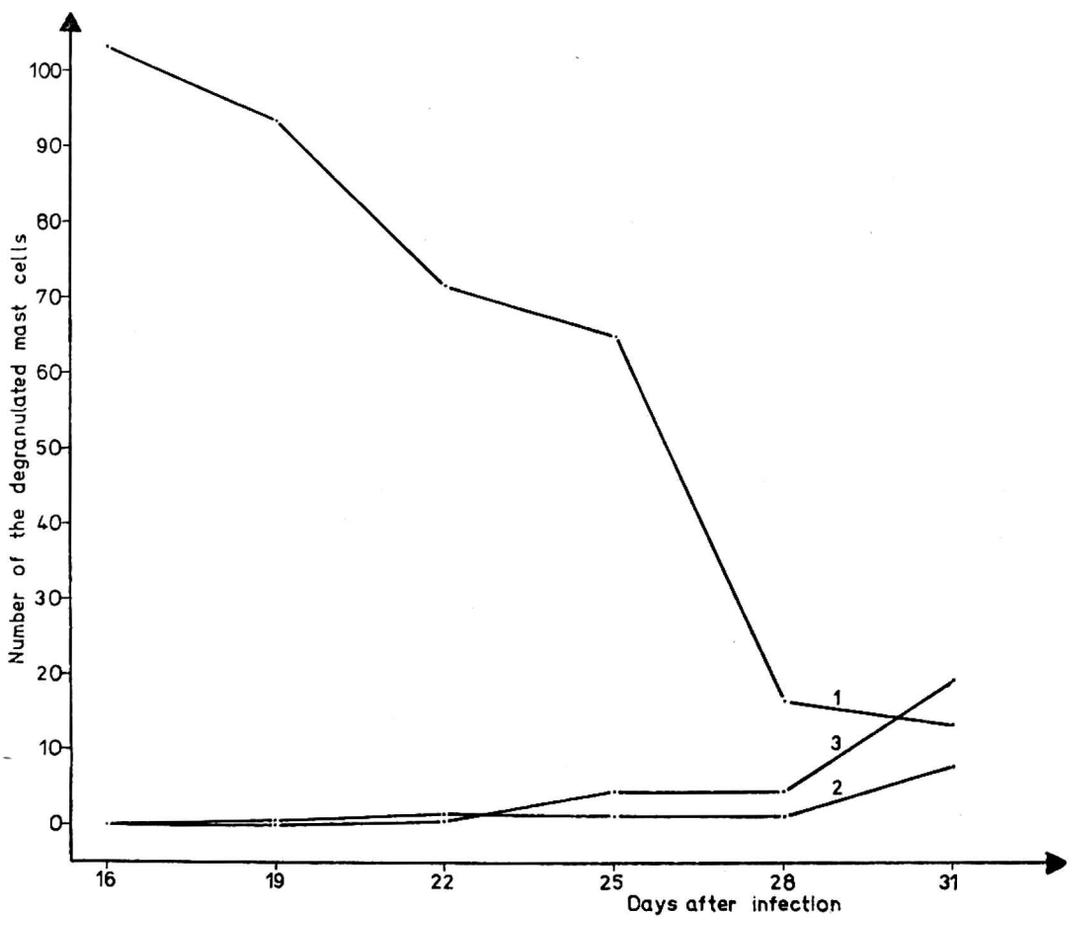

Fig. 3 Degranulated mast cells in jejunum. Explanations as in Fig. 1

of lymph nodes showed evidence of gradual reconstruction, at first diffusely with few lymphoblasts and plasma cells. After 15 days, the first germinal centers of lymphocytes appeared. After about 20 days normal structure of the lymph nodes was restored. In the spleen, X-irradiation caused focal proliferation of the reticulum and partial atrophy of the white pulp, in which Malpighian follicles were scarce and poorly visible. In the venously congested red pulp, besides hemosiderin grains, there were numerous megakaryocytes, usually damaged (showing adhesion or disintegration of the cell nuclei and homogenized cytoplasm). Beginning on the 13th to 15th experimental day, the spleen's structure began to return to normal, and after about 20 days contained fairly numerous lymph follicles surrounded by small lymphocytes. Megakaryocytes were less numerous and undamaged at this time.

In the bone marrow of the irradiated animals, young cells such as hemocytoblasts, proerythroblasts, promyelocytes and metamyelocytes were markedly damaged, showing advanced homogenization of the cytoplasm, complet colliquation of the nucleus, or only a faint shadow of the nucleus. Similar changes were observed in large bone marrow megakaryocytes and in some of the stellate cells of the reticulum. More mature cells, e.g. erythroblasts, normoblasts, band and segmented cells, were more or less intact. From the 13th day of the experiment, the bone marrow began to recover gradually, but even at 15 days after irradiation nuclei of the less mature cells showed poor staining, which improved after about 20 days.

In the group of control mice infected with *T. spiralis* (group 2), as early as 5 days p.i. the lymphatics were dilated, the stroma of the mucous membrane was moderately edematous, and the apical parts of the villi were hyperemic. Mucus production was somewhat enhanced, and infiltrates in which mononuclear cells predominated were fairly numerous. At this time plasma cells were seen too. At 10 days p.i. proliferation of Paneth cells were also observed. Fifteen days p.i. eosinophilic and neutrophilic leukocytes appeared in the infiltrates. After 22 and 25 days p.i. inflammation began to regress. In the infiltrates, which were now smaller than before, leukocytes predominated. The number of Paneth cells decreased. On days 28 and 31 p.i. no changes were remarked in the lamina propria of the intestinal mucosa.

In mice infected on the next day after irradiation (group 3) until the 28th day p.i. the cellular stroma of the lamina propria of the intestinal mucosa, mainly in small villi, was scanty. No inflammatory infiltrates were present. Paneth cells were hardly visible (or nearly absent). A few of the villi were hyperemic (mainly on days 25 and 28 p.i.). On the 31st day p.i. inflammatory infiltrates composed of lymphocytes,

histiocytes and leukocytes (so-called mixed infiltrates) began to appear in small numbers in about 1/3 of the villi. Moderate proliferation of Paneth cells was seen at the base of the glandular crypts.

In mice that were infected 7 days after irradiation (group 4), from days 19 to 25 p.i. some of the intestinal villi showed marked hyperemia. The stroma of about 50% of the larger villi contained small numbers of mixed infiltrates. At this time the lymphatic spaces were dilated and there was slight proliferation of Paneth cells. On days 28 and 31 p.i. only richly cellular stroma of some of the larger villi was remarked. Remnants of the inflammatory infiltrates and hyperemic capillaries were encountered sporadically.

Parasites. The intestines of the control animals (group 2) contained no parasites 16 days p.i.

In mice infected 24 hr after irradiaton (group 3), the mean number of parasites in the intestines on days 16 and 19 p.i. was 43.0, and on day 22 — 37.5, on day 25 — 37.0, and day 28 — 29.0 and day 31 p.i. the mean number of parasites was 31.0. Even 60 days p.i. the mean number of parasites was 6.5 per animal.

Similarly, in the group of mice infected on the 7th day after irradiation (group 4) adult trichinellae survived to the end of the experiment. On day 16 p.i. the number of parasites was 42.5, on day 19 — 37.5, on day 22 — 34.5, and on day 25 — 18.1. During further observation the numbers of parasites dropped, to 4.5 on day 28 p.i., to 6.0 on day 31 and after 60 days there were only 0.9 parasites per mouse left in this group (Fig. 4).

In the control mice (group 2) the mean number of muscular larvae 60 days p.i. was 17,100, compared with 140,000 in group 3 and 107,000 in group 4.

Discussion

The suppressive action of gamma and X rays on lymphocytes is known for many years. Whereas most of the cells exposed to moderate doses of irradiation do not die until the first or second post-irradiation mitosis (mitotic death), small lymphocytes can be killed without entering the mitotic cycle (interphase death), (Trowell, 1952). B lymphocytes are more sensitive to irradiation than T lymphocytes (Keuning et al., 1963; Anderson, Warner, 1975; Kataoka, Sado, 1975). Hence, irradiation exerts a greater effect on the humoral response (Uhr, Scharff, 1960; Archerson, Loevi, 1966; Volkman, Collins, 1968). However, Kelly et al. (1973) found that whole-body irradiation of rats with 400 R was

followed quickly (after 4-6 days) by regeneration of the plaque-forming cells, but not of the lymphocytes responsible for DH; doses of at least 750 R were needed to prevent reconstitution of antibody-producing cells and to damage the bone marrow cells effectively. We, too, have found that X-irradiation of mice with 600 R (the highest dose after which RIII × $C57B_1/F_1$ mice survived) markedly damages bone marrow cells as well as lymphatic cells.

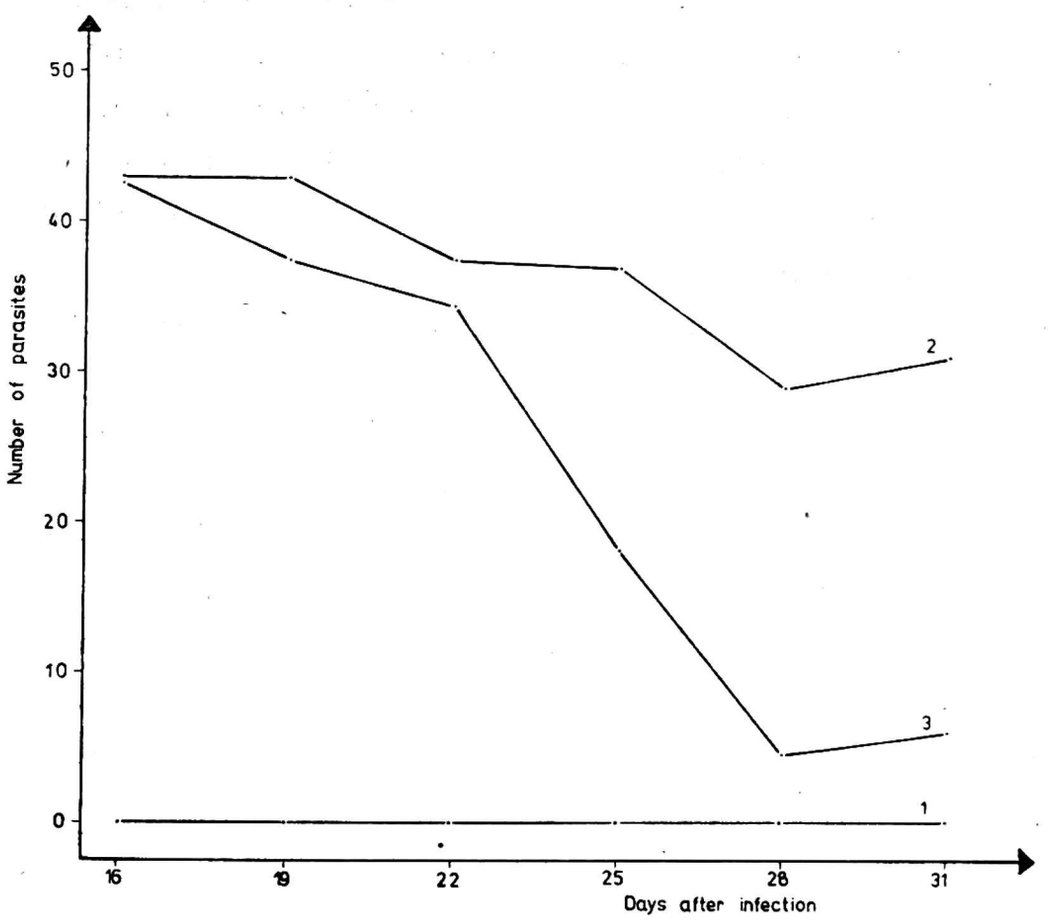

Fig. 4. Mean number of *T. spiralis* in the intestines. Explanations as in Fig. 1

Macrophages behaved quite differently in the body of irradiated mice (Cheers, Waller, 1975). In mice exposed to 850 R, these cells were activated because, according to the authors, in irradiated mice macrophage-activating factors could be released by the lethal action of irradiated lymphocytes. This different (frequently dose-related) action of gamma or X rays on various types of cells has been taken advangage of in studies on immune mechanisms under experimental conditions.

The first observations on the influence of X rays on the course of trichinellosis in rats were made by Semmerad (1937), who noted that in animals irradiated with 1,000-2,250 R (250 R per day) fewer larvae developed than in the controls. However, the experiments of Stoner and Hale (1952) showed increased susceptibility to *T. spiralis* infection

in mice irradiated with ^{60}Co in doses of 550-650 rep (roentgen equivalent physical). In addition, immunity of these animals to reinfection was destroyed, and a sharp drop in the number of circulating leukocytes in the peripheral blood occurred.

Subsequently, Yarinsky (1962) studied the influence of X-irradiation (250-650 R) on mortality of mice infected 24 hr after irradiation. He found significant differences only in the group irradiated with 650 R and attributed the evidently higher mortality among the irradiated mice to easier penetration of the damaged intestinal wall by bacteria.

In 1962 Larsh et al. reported that whole-body irradiation of sensitized mice with 450 R markedly diminishes the inflammatory reaction which occurs in the intestinal wall after challenge, resulting in longer survival of mature trichinellae. According to Keuning et al. (1963) lymphocytes stimulated once by antigen or mitogens sharply lose their sensitivity to irradiation.

Several years later Kim (1966) showed that development of delayed-type hypersensitivity to *T. spiralis* antigen in guinea pigs was not inhibited by total body X-irradiation with up to 200 R when administered 18 hours prior to sensitization with antigen plus adjuvant. An X-ray dose of 200 R, however, seemed to inhibit partially the appearance of antibodies in some animals for 21 days following sensitization as determined by the PCA test, and seemed to inhibit the appearance of precipitating antibodies altogether. In further experiments, Kim et al. (1974) noted that X-irradiation (200 R) indeed suppressed the immune activity in recipients (delay in the appearance of PCA and precipitating antibodies).

In our studies, we used a higher dose of X rays (600 R), to which mice were exposed before administration of antigen, i.e. before infection. In the group 3 animals (infected 24 hr after irradiation) T lymphocytes and intestinal mast cells were completely suppressed up to 28 days p.i. Antibody-producing cells were also suppressed as no rise in titers occurred such as was observed in controls, and after 28 days no inflammatory infiltrates were found in the intestinal mucosa. Stimulation of all elements was delayed until the 31st day p.i. (i.e. 32 days post irradiation). Throughout the experimental period the intestines contained large numbers of adult trichinellae, which were detected even at 60 days p.i., and the number of muscular larvae increased greatly (more than 8-fold compared with controls). It seems that in mice exposed to 600 R all defense mechanisms active in the course of trichinellosis were eliminated for about 20 days.

The results in group 4 confirmed those in group 3. Return to normal resembled that in group 3, and the number of parasites was proportionately lower.

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WPŁYW NAPROMIENIOWANIA PROMIENIAMI X NA ZJAWISKA IMMUNOLOGICZNE ROZWIJAJĄCE SIĘ W PRZEBIEGU DOŚWIADCZALNEJ WŁOŚNICY MYSZY

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Myszy szczepu RIII $\times$ C57B$_1$/F$_1$ o wadze około 20 g, w wieku 3 miesięcy, napromieniowano dawką 600 R. Następnie jedną z grup zarażono larwami włośni po 24 godz. od napromieniowania, inną zaś po 7 dniach. W obu grupach dawka zarażająca równa była 100 larw/mysz. Zwierzęta uśmiercano w 16, 19, 22, 25, 28, 31 i 60 dniu po zarażeniu (p.z.). Z komórkami węzła chłonnego krezkowego nastawiano odczyn zahamowania migracji makrofagów oraz test immunocytoadherencyjny (z krwinkami barana opłaszczonymi antygenem włośniowym). Z wycinków jelita czczego sporządzano preparaty histologiczne oraz preparaty dla obserwacji komórek tucznych. Ponadto liczono pasożyty dorosłe, a w 60 dniu p.z. także larwy mięśniowe.

W wyniku przeprowadzonych doświadczeń w obu grupach zwierząt obserwowano hamowanie wszystkich badanych procesów immunologicznych, a także dłuższe niż u zwierząt kontrolnych przeżywanie włośni dorosłych oraz dużo większe ilości larw mięśniowych.