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Changes in the Blood Count of Growing Rats Irradiated with a Microwave Pulse Field

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ABSTRACT

A group of 20 male rats of mean initial body weight of 65.53 g were irradiated for 7 wk (5 days per wk, 4 hr per day) with an electromagnetic pulse field of the following parameters: working frequency 2,736.5 MHz; repeated frequency 395 Hz; pulse width 2.6 μsec; vertical polarization; mean power density 24.4 mW/cm^2; accuracy of measurement ± 6%.

The rectal temperature of experimental animals increased during irradiation by a maximum of 0.5°C. Blood was taken before irradiation, at the end of the 1st, 3rd, 5th, and 7th wk of irradiation, and at the end of the 1st, 2nd, 6th, and 10th wk after irradiation was completed.

The parameters under study included the hematocrit value; number of leukocyte differential count in both absolute and relative proportions; activity of alkaline phosphatase in neutrophil leukocytes; and body weight increase. The results were compared with parallel data obtained from a control group of 20 animals and evaluated by Student's *t* test at a significance level of 1%.

In the second half of the irradiation period the experimental animals exhibited significantly lower mean hematocrit values, lower numbers of leukocytes, and lower absolute numbers of lymphocytes. These changes disappeared gradually within 10 wk after completed irradiation. Activity of alkaline phosphatase in neutrophil leukocytes was significantly increased in the 1st wk of irradiation and dropped transiently after the irradiation. In the post-irradiation interval experimental animals displayed significant decline in rate of body weight increase.

The level of the other examined parameters did not differ from the controls.

DURING THE LAST THIRTY YEARS there has been a marked development and increase in the use of military, industrial, and consumer equipment and devices that emit a large variety of nonionizing electromagnetic energies.

Very high frequency (30-300 MHz) is used in FM broadcasting, television, air traffic control, and navigation; ultra high frequency (0.3-3 GHz) is used in television, microwave ovens, citizens band broadcasting, telemetry, tropo scatter, and meteorological radars. Super high frequency (3-30 GHz) is used in satellite communication and airborne weather radars. Recommended maximum permissible intensities for electromagnetic radiation are a bit different in Western European countries (including USA and Canada) and Eastern European countries.[1] Future research will bring more exact data that will allow more precise determination of maximum permissible intensities for electromagnetic radiation.

Several years ago we had the opportunity to examine

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two groups of persons exposed to electromagnetic radiation, e.g., 82 employees of radio transmitting stations (frequency 0.3-30 MHz, mean exposure 14.4 yr, mean intensity of electromagnetic field 80 V/m) and 58 employees of television transmitting stations (frequency 30-300 MHz, mean exposure 7.2 yr, mean intensity of electromagnetic field 2.9 V/m). The health condition of examined persons was assessed on the basis of complete examination in which the data from case history and the results of the following examinations were evaluated: electrocardiogram, x-ray of chest, BWR (Bordet-Wassermann reaction), BSR (blood sedimentation rate), analysis of urine, blood pressure, blood count including thrombocyte count, liver tests, protein spectrum in blood, glycemia curve, opthalmological test, neurological test, electroencephalogram, psychiatric, and psychological examination, and gynecological examination of women. The results were compared and statistically evaluated with control groups. No signs of damage due to electromagnetic radiation were found in the examined persons.[2-3]

The data on the effect of electromagnetic radiation on hematopoiesis are often disparate and contradictory.[4-8] Unfortunately, we had no opportunity for a complete examination of a sufficient number of persons who had worked long enough in a pulse microwave field under well-known hygienic conditions. It was therefore expedient to find out if a microwave pulse field of power density corresponding to levels approved in some countries as nearly harmless[1] can evoke, in several weeks of exposure, changes in the blood count of irradiated animals. In spite of the fact that it is impossible to transfer results of animals' examinations directly to human medicine (especially in physical injury), we had no choice but an experiment with animals.

Microwave energy sources are being more and more widely used for both civilian and military purposes; consequently, young people can be exposed to this kind of irradiation, especially in military service. For this reason rats in our experiment were irradiated during their maturity.

Materials and Methods

A total of 20 male rats (*Rattus norvegicus*, f. albus, Wistar strain, Konarovice breed) were irradiated for 7 wk (5 days per wk, 4 hr per day) with a pulse electromagnetic field of the following parameters: working frequency 2,736.5 MHz; repeated frequency 395 Hz; pulse width 2.6 μsec; vertical polarization; mean power density 24.4 mW/cm^2, measured with ± 6% accuracy. Irradiation antenna was a rectangular horn 2 m long.

During irradiation the animals were placed singly in perforated plexiglass boxes situated above each other according to equal power density planes. The relative position of the boxes was changed daily according to a predetermined "seating order." The air in the irradiated space was cooled by a fan. Air temperature was measured daily both at the housing site and in the irradiated space, and, in addition, at the site where controls were housed during irradiation. The rectal temperature was measured in a selected group of rats before exposure and at 1-hr intervals during the irradiation.

During the irradiation a group of 20 control rats were also placed singly in plexiglass boxes in the irradiation room but outside the electromagnetic field. When not being irradiated, all animals were placed in boxes in groups of five and were given pelletted food and water ad libitum.

The mean initial body weight of exposed rats was 65.53 g ($s = 5.63$, $s_{\bar{x}} = 1.26$), while that of the controls was 63.36 g ($s = 4.53$, $s_{\bar{x}} = 1.01$). Body weight increase was measured at 3-wk intervals.

Venous blood was withdrawn from the animals by an incision into the lateral caudal vein. First samples were taken before the onset of irradiation, and others were collected at the end of the 1st, 3rd, 5th, and 7th wk of irradiation and at the end of the 1st, 2nd, 6th, and 10th wk after termination of the exposure. Blood was always taken in the morning before irradiation.

The hematocrit value was determined by a micromethod using 0.006 to 0.007 ml of blood in a Polish Microhematocrit centrifuge, type 316 with evaluator. The centrifugation was carried out for 4 min at 13,000 rpm, one set of centrifuged samples including 10 samples from irradiated animals and 10 samples from the controls.

Leukocytes were counted by a conventional flask method immediately after blood collection. Differential count was appraised from 200 cells of the preparation. The counts of neutrophil leukocytes and lymphocytes were determined in both relative and absolute proportions; the counts of other elements of the white blood count were determined in relative proportions only.

The activity of alkaline phosphatase was determined according to Hayhoe and Quaglino.[9] A semiquantitative evaluation was done with 100 neutrophil leukocytes from every smear. The following 4-score system was adopted to appraise the activity: 0, cells with no reaction; 1, elements with a slight smoky tint or with a hint of fine granulation; 2, elements with more intense tint or granulation; 3, higher activity demonstrated by solitary clusters of coarse granulation; 4, massive coarse granulation indicating the highest activity. In view of the insufficient objectivity of this classification, significant differences were taken to be only those between groups 1 and 4; smaller differences between neighboring classes were disregarded.

The results obtained in irradiated animals were compared with data from the controls and evaluated by Student's *t*-test at a significance level of 1%.

Results

Rectal temperatures rose during irradiation, with a maximum after 3 hr. The mean temperature rise was 0.3°C, the maximum increase was 0.5°C, as compared with the initial level.

Body weight increase is illustrated in Figure 1. Prior to irradiation, experimental animals are seen to have a higher mean body weight than control rats. This statistically insignificant difference disappeared in the 3rd wk of irradiation, and the body weight increase of the control animals began to consistently exceed that of the experimental animals. The difference attained a significant level in the 1st wk after the termination of exposure.

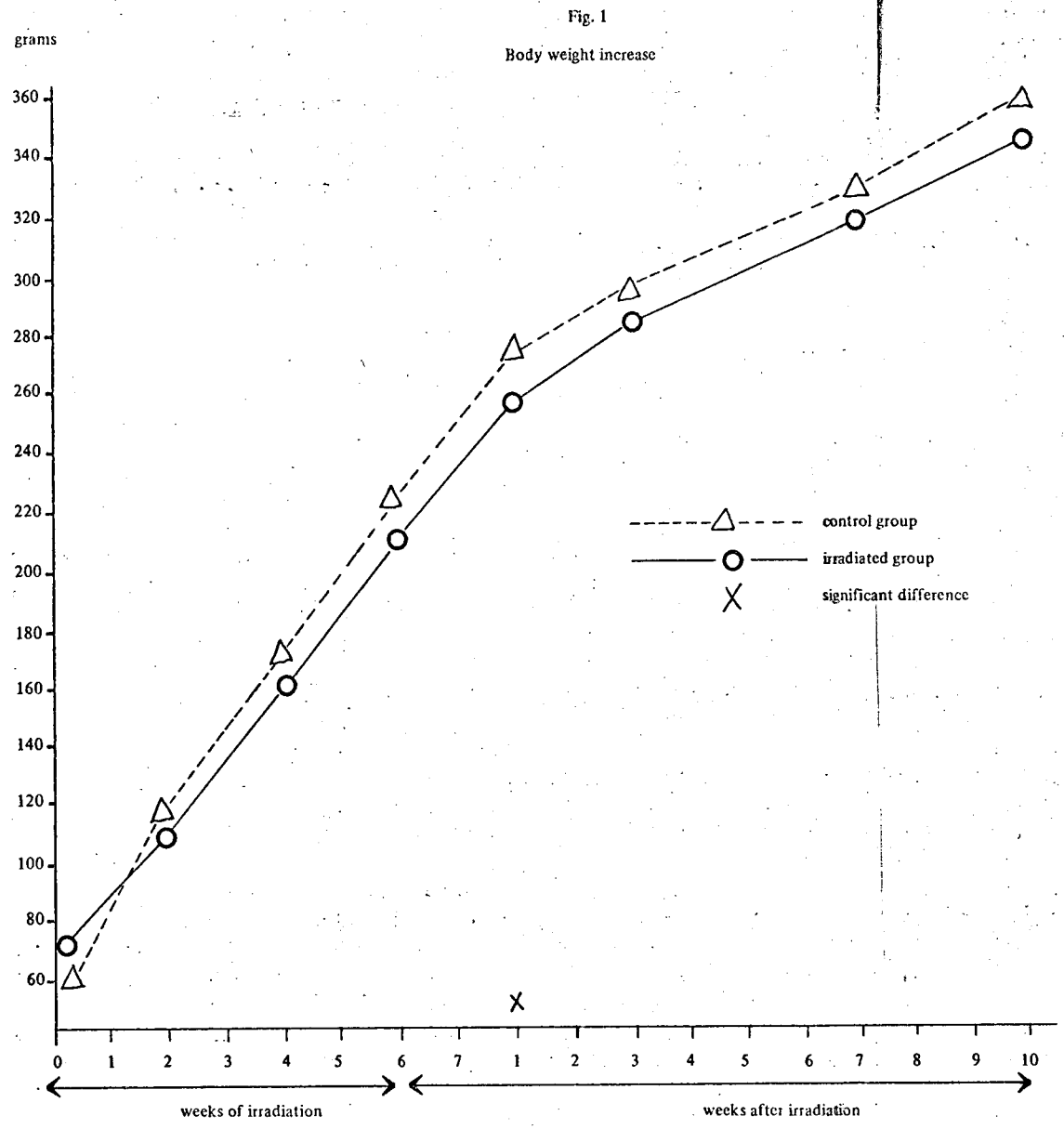

Fig. 1. Body weight increase of growing rats in course of irradiation and during next 10 wk after irradiation.

The mean hematocrit value of the experimental animals was somewhat higher before irradiation than that of the control rats. Figure 2 shows that, beginning with the 3rd wk of irradiation, experimental animals exhibited a statistically significant reduction of hematocrit values that persisted even after termination of the treatment and finally disappeared in the last sampling, i.e., 10 wk after irradiation.

The mean number of leukocytes (Fig. 3) was insignificantly lower in experimental rats than in controls. Beginning with the 3rd wk of irradiation this difference disappeared, but a significant drop in the leukocyte count of irradiated animals occurred from the 5th wk of irradiation on. This significantly lower leukocyte count persisted even after irradiation and disappeared only after 10 wk.

Figure 4 shows that the curve illustrating the absolute lymphocyte count has an analogous character. The mean value of the absolute count of neutrophil leukocytes in irradiated animals was lowered only once, namely, in the last week of irradiation. The statistical evaluation revealed no significant differences in the percentage proportion of the individual elements of the white blood count.

Figure 5 illustrates the activity of alkaline phosphatase and shows its statistically significant transient increase at the end of the 1st wk of irradiation and a transient drop immediately after irradiation.

Discussion

Data on the effect of microwave radiation on living organisms are numerous in the literature, but comparatively scant attention has been given to changes in the blood count. The results of experimental and clinical studies often differ.[4-8] This is due not only to a different approach

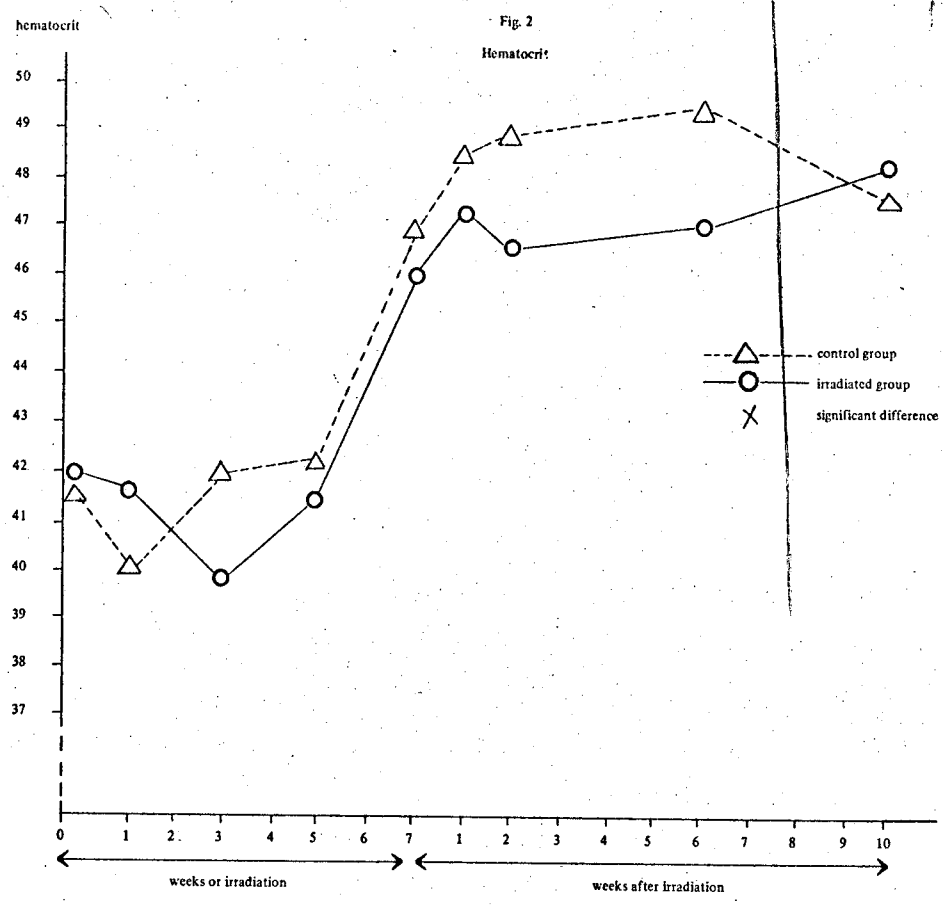

Fig. 2. Value of hematocrit of growing rats in course of irradiation and during next 10 wk after irradiation.

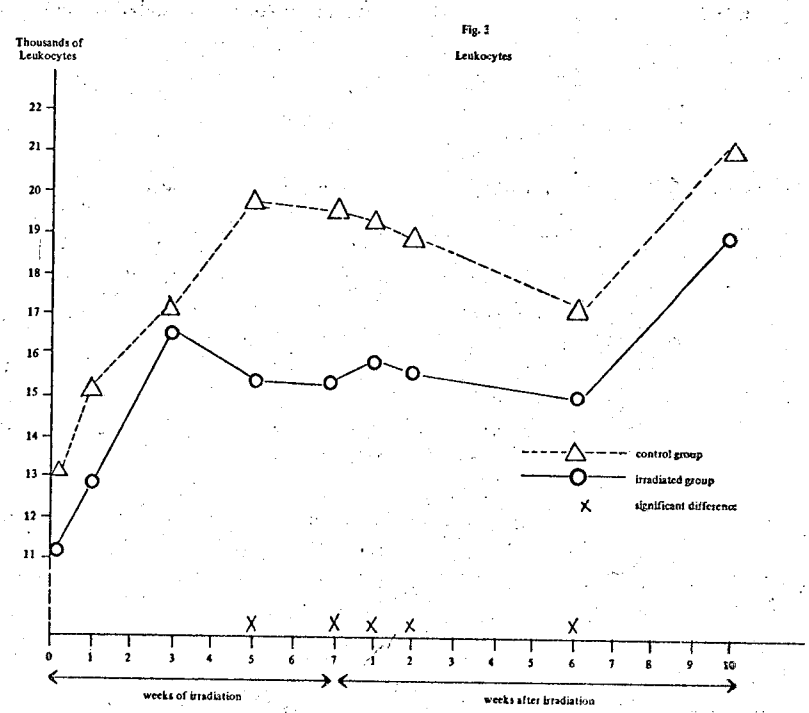

Fig. 3. Changes in leukocyte count of growing rats in course of irradiation and during next 10 wk after irradiation.

Fig. 4

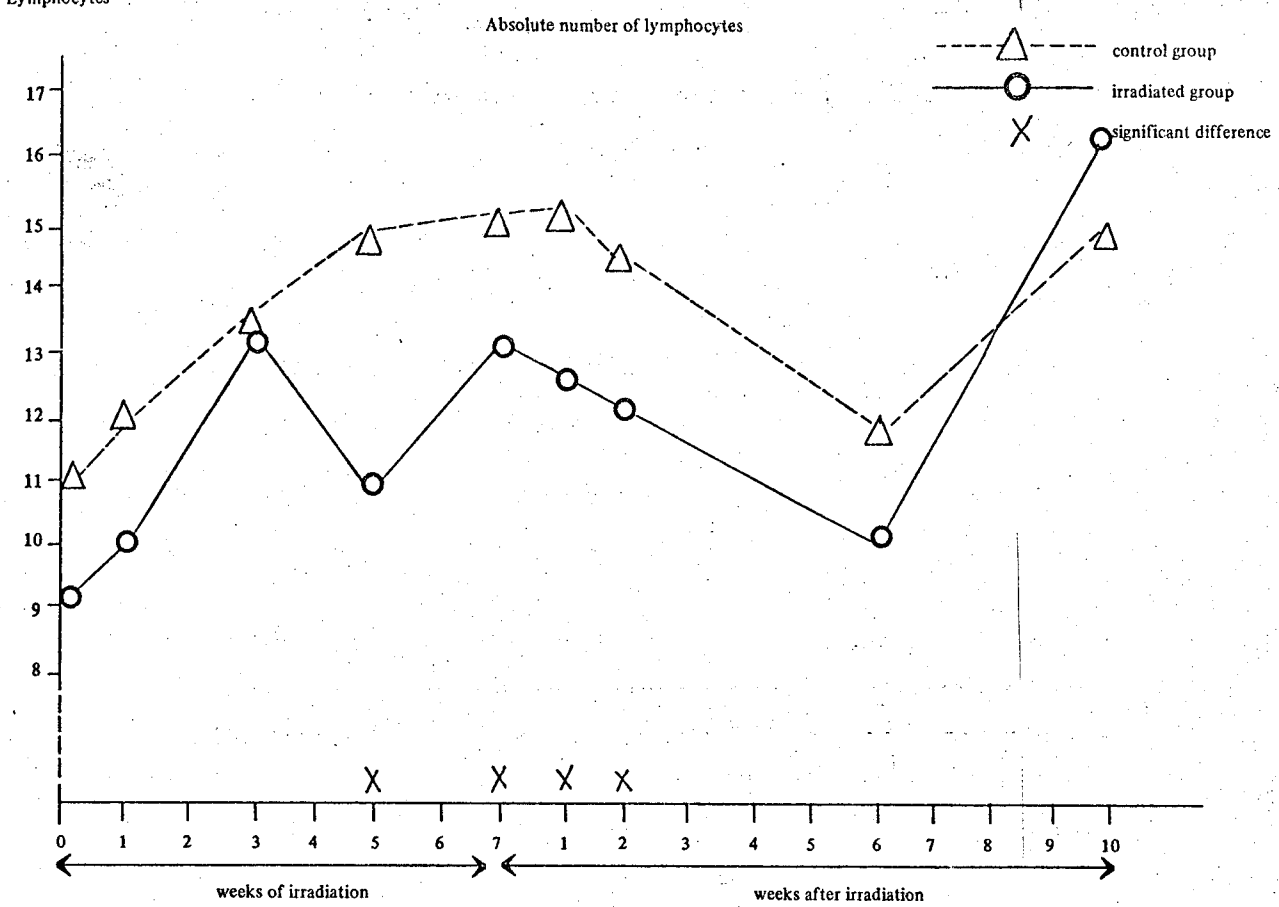

Fig. 4. Changes in absolute value of lymphocyte count in growing rats in course of irradiation and next 10 wk after irradiation.

to data evaluation used in some older papers but mainly to differences in the type of electromagnetic radiation used, intensity and length of exposure, and the species of experimental animals.

Baranski[4] succeeded, by a prolonged microwave irradiation of rabbits, in evoking stimulation of lymphopoiesis in bone marrow, lymph nodes, and spleen. He reported certain anomalies in the nuclear structure of lymphocytes and erythrocytes, and mitochondrial aberrations. Czerski[6-7] described a blastotransformation of lymphocytes irradiated with microwaves in vitro. Yagi et al.[10] irradiated a rabbit's limb locally with high doses of microwaves (1.3 W/cm^2). The site of irradiation exhibited anatomical changes similar to those found in aplastic anemia; the peripheral blood count was characterized by a drop in lymphocyte count. The mechanism of action of radiation on hematopoiesis is likely, however, to be different from that evoked in other studies, since the power density of the microwave field used in Yagi's study was several orders of magnitude higher than otherwise used.

Sokolov,[11] studied the blood counts of professionally exposed persons; the power density of the microwave field in these cases was of the order of units mW/cm^2 or less. The counts were characterized by a slight decrease to erythrocyte and leukocyte counts; differential formulas of white blood elements showed no significant alterations.

An experiment similar to those reported in this paper had been performed in cooperation with Moscow colleagues. Adult male rats were irradiated for 14 wk under the same daily regime. The frequency of the microwave source was 3,000 MHz, repeated frequency was 300 Hz, pulse width 2.5 μsec, and mean power density was 1 mW/cm^2. Accuracy of measurement was ± 30%. The same parameters were studied by identical hematological methods; in addition, the count and morphology of nucleoli in lymphocytes were investigated. Evaluation by Student's *t* test showed no significant differences between irradiated and control animals.

Changes in leukocyte count are attributed by Czreski[6] to a shift of blood elements between the blood stream and the tissues and to changes in the distribution of body fluid. In our opinion, the data in existing literature, including

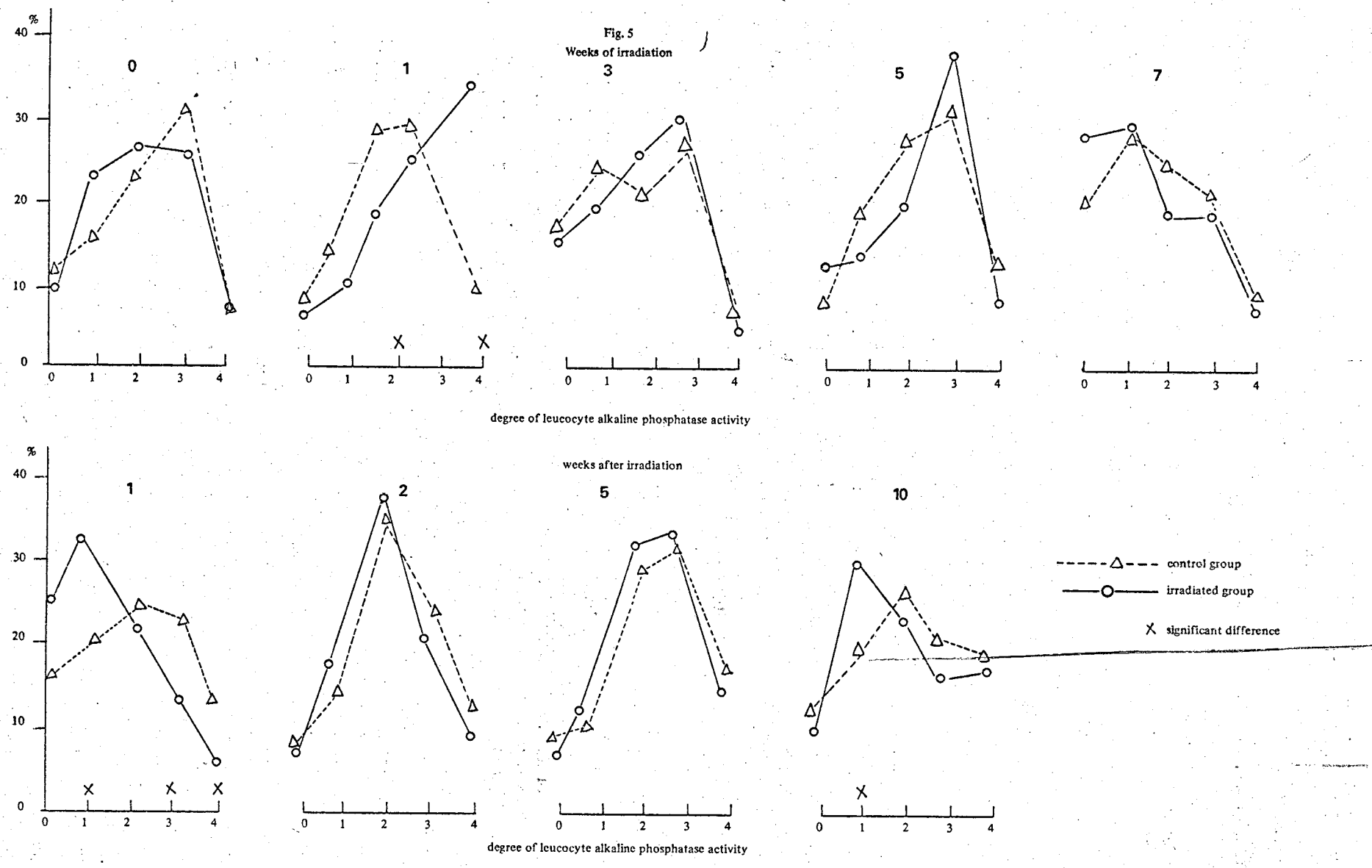

Fig. 5. Value of alkaline phosphatase activity in neutrophil leukocytes of growing rats in course of irradiation and during next 10 wk after irradiation.

our results, do not provide sufficient information for a tangible interpretation of experimentally determined changes.

The initial drop in hematocrit value in both control and irradiated animals can be attributed to artificial anemization resulting from frequent blood withdrawal in very young rats. The phenomenon disappeared with increasing intervals between individual blood samplings and with the growth of animals during adolescence. The retarded increase in the hematocrit value of irradiated rats during their adolescence, beginning in the second half of the irradiation period and persisting for several weeks after irradiation, is both conspicuous and reproducible. It can be explained only as being brought about by the irradiation.

In his experiments on rabbits irradiated with microwaves, Czerski[6-7] observed changes in the metabolism of plasma iron and its transfer and incorporation into erythrocytes. The changes resulted in hypochromic anemia. Michaelson[12] found a shorter life span of erythrocytes in repeatedly irradiated dogs.

The increase in alkaline phosphatase activity of leukocytes is ascribed by some authors to a protection mechanism. The transient rise in the activity of the enzyme after the onset of exposure and the significant drop after termination of the treatment signifies that the enzyme may be affected by radiation.

Whether the observed changes in the peripheral blood count are brought about by the heat effect of microwaves or by other effects remains to be decided by specialists. As observed in our experiments, the rectal temperature of irradiated rats increased by as much as 0.5°C. The rise could not have been due to the transfer of the animals to a small, confined place, since control rats housed in the same manner showed no temperature increase.

Our study was not aimed at exploring animal behavior; still, our attention was attracted by the conspicuous obedience of irradiated animals, which willingly entered the box where their blood was taken. This obedience and placid behavior disappeared after irradiation. None of the animals showed any signs of disease during the irradiation; no autopsy was done after the experiment.

Our study thus provides an answer only to the first part of the question posed at the beginning. A microwave pulse field of given intensity affected both the white and the red blood count in peripheral blood in its volume component. So far, it is not clear if the growing organism is more sensitive to microwave radiation than the adult one; no analogous experiment has been done on adult rats under identical conditions. Nevertheless, a microwave pulse field of a 10-fold lower intensity was found to cause no changes in the hematological parameters under study even if the total exposure time was twice that used in our experiment.

Conclusion

Growing rats irradiated for 7 wk with a microwave pulse field of mean power density of 24.4 mW/cm^2 showed a significant drop in hematocrit value and leukocyte and lymphocyte counts as compared with those of control animals. These differences disappeared only several weeks after the end of treatment. The activity of alkaline phosphatase in neutrophil leukocytes exhibited transient changes at the beginning and at the end of radiation.

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The authors thank Mrs. Jirina Nová, technician in the Clinic of Occupational Diseases, for skilled technical assistance.

Submitted for publication May 4, 1977; revised; accepted for publication June 20, 1977.

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