CHANGES IN THE UNCONDITIONED AND CONDITIONED REFLEX LEUKOCYTIC REACTIONS FOLLOWING ROENTGEN IRRADIATION

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At this time, the high sensitivity of the nervous system, especially its central divisions, to the action of penetrating ionizing radiation is an established fact [1, 4-8].

There is basis for postulating that the reflex reactions of the blood system undergo major changes in association with radiation exposures. In a number of recent works [2, 9, 10], data were presented on varying distortions of the leukocytic reactions in irradiated animals. However, certain authors hold an opposing opinion.

This work presents data from a study of unconditioned and conditioned reflex leukocytic reactions on a long term experimental basis, carried out alternately on 6 dogs, before and after their single-exposure total roentgen irradiation in varying doses. A total of 140 experiments were performed (89 before and 51 after irradiation), and 1016 different blood studies were carried out.

EXPERIMENTAL METHOD

The animals were of both sexes, of mixed breeding, weighed 9.5-17 kg, and were kept in the laboratory animal room in individual cages. The experiments were performed 3-4 times a week in an isolated room, always in the morning, several hours before the animals were fed. We attempted to maintain strictly stereotypic conditions in the set up. In the course of the experiment, blood was drawn from the same transverse incision in the marginal vein of the ear. The first drops of blood were removed with a gauze tampon. In a number of the cases, in order to prevent clotting of the blood at the incision site, we applied a piece of cotton moistened with heparin solution.

Over the course of 1-2 weeks, the dogs were first accustomed to the experimental apparatus. In this case, we studied the associated background fluctuations in the leukocytic composition of the blood. When we attained a stable picture for the leukocytic background, we began the experiments, in the course of which we tallied the total number of leukocytes and the leukocyte formula. We also investigated other peripheral blood indices in the dogs periodically (erythrocyte count, hemoglobin concentration, sed. rate), as well as a sternal puncture sample of the bone marrow.

The method for producing the conditioned leukocytic reflex with electrocutaneous stimulation was the following. The dog was led into the experimental room and freely fixed to a stand with loose straps. Lead electrodes were applied to the shaved lateral surface of the posterior paw, using gauze strips moistened with physiological saline, after which blood was drawn prior to application of the stimuli. As a rule, incision of the vein and drawing of the blood was carried out without any apparent reaction of the animal; nevertheless, in determining the starting data, we took 2-3 blood samples. In order to exclude definitively the influence of the pain factor associated with incision of the vein, in each experiment we began in 3 of the dogs with a preliminary study of the background fluctuations in the leukocytic composition of the blood. In this case, in the course of a half hour 8 blood samples were drawn from the animals, following the schema of the basic experiment presented below. Subsequently, we connected an electrometronome with a rhythm of 300 beats per minute. After 5 seconds, the action of the metronome was connected with an electrocutaneous stimulation, causing a faradic current with a force of 25-90 volts, a frequency of 80 alt/sec, and an impulse duration of 3 msec. The current was supplied from the ASM-2 apparatus for muscle stimulation. The duration of joint action of both stimuli was equal to 10 seconds. When the stimulus actions were

stopped, the blood was studied after 1, 2, 3, 4, 5, 10, 15 and 30 minutes. In the course of the experiments on developing a conditioned leukocytic reflex, we studied the dynamics of an unconditioned reaction. Periodically we also tested the isolated action of the conditioning stimulus without reinforcement, making it possible to follow the dynamics of the conditioned leukocytic reflex. After establishing the character and magnitude of the unconditioned leukocytic reaction, and developing and strenghthening the conditioned leukocytic reflex, the animals were subjected to a single-exposure, total roentgen irradiation.

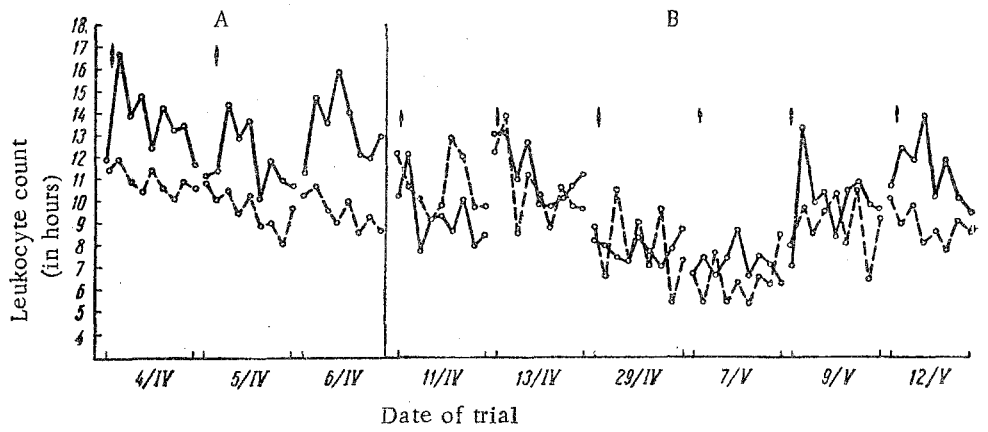

Fig. 1. Distortion of the unconditioned and conditioned reflex leukocytic reaction, plus the leukocytic background picture (broken line), in the dog Trus, as a result of a single – exposure roentgen irradiation with a dose of 50 r. A) Before irradiation; B) after irradiation. Arrow pointing up – M-300, arrow point down – electrocutaneous stimulation. Points on the curves: 1st) before the action of the metronome; 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th) at 1, 2, 3, 4, 5, 10, 15, and 30 minutes after stimulation respectively.

In dogs (2 in each group were irradiated in the RUM-3 apparatus, under the following conditions: first group – focal distance of 100 cm; 0.5 mm Cu + 1 mm Al filters; force of 180 kv; current intensity of 15 ma, output of 9.5 r/min; dose of 600 r. Second group – focal distance of 100 cm; 1.5 mm Cu + 3 mm Al filters, force of 200 kv; current intensity of 10 ma; output of 2 r/min; dose of 50 r. Third group – focal distance of 500 cm; 1 mm Cu filter; force of 213 kv; current intensity of 10 ma; output of 0.1 r/min; dose of 3 r.

Subsequently, we carried out a clinical investigation and observation of the condition of the animals. We studied the leukocytic reactions in the first hours, days, and more remote time intervals, after the irradiation.

EXPERIMENTAL RESULTS

The electrocutaneous stimulation caused a motor protective-defense reaction, the intensity of which depended upon the intensity of the stimulus and the individual characteristics of the animals. With weak stimulation of the animals in the first experiments, we observed a general unrest, and the subsequent motor reaction was limited to one flexion of the paw on which the electrodes were attached. Strong stimulation caused a violent motor protective-defense reaction.

In both cases, in the first minutes after stimulation, a neutrophilic leukocytosis developed, with a slight shift to the left due to an increase in the number of forms with rod-shaped nuclei (Fig. 1A). With strong pain (nociceptive) stimulation, we noted an additional elevation in the number of cytolysis cells and plasma cells.

The first signs of conditioned reflex changes in the leukocytic composition of the blood were noted after 4-9 combinations. Final formation of the conditioned leukocytic reflex took place as a result of 5-14, and in one dog – 22, combinations of electrocutaneous stimulation with the action of the conditioning agents. At this time, the conditioned reflex changes in the leukocytic composition of the blood completely corresponded to the unconditioned ones both quantitatively and qualitatively. With stereotyped conditions maintained in the experiment, the unconditioned and conditioned reflex leukocytic reactions were sufficiently constant.

After roentgen irradiation, in addition to preservation of the motor protective-defense reactions we observed a change in the dynamics of the unconditioned and conditioned reflex leukocytic reactions. This took place in all series of the experiments, with presence of clinical and hematological symptoms of radiation injury or without such symptoms.

In the first series of experiments, with total irradiation in a dosage of 600 r, the animals developed the characteristic picture of acute radiation sickness, concluding with the death of the dogs on the 6th-12th day after the exposure. In the 1st-2nd day, the leukocyte count fell by 40-50%. The leukocyte formula showed an absolute lymphopenia and neutropenia, with a shift to the right, and the presence of morphological changes in the neutrophiles typical for radiation sickness (hypersegmentosis, fragmentosis, hyperchromatosis of the nuclei, et al). One to two days prior to the death of the animals, the leukocyte count fell by 70-80%, as compared with the original level, and reached 3500 – 1500 per mm^3 of blood. Studying the bone marrow, we noted a picture of destruction, with substitution of the functional parenchyma by fat tissue. A scanty number of reticular and plasma cells were observed in individual clusters, and there were also a small number of erythroblasts.

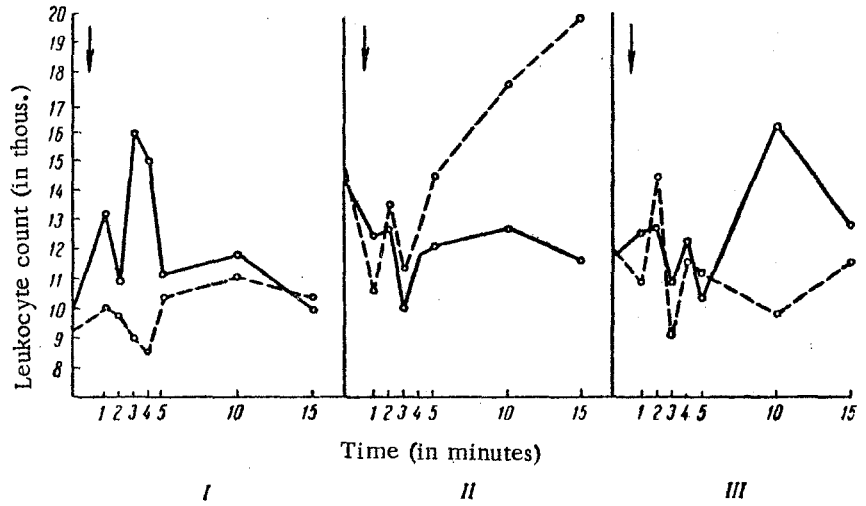

Fig. 2. Distortion of the unconditioned reflex leukocytic reaction and the leukocytic background picture (broken line) in the dog Laskovyi, after total roentgen irradiation with a dose of 3 r. I) Before irradiation; II) 2 hours after irradiation; III) twenty-four hours after irradiation. Arrow pointing downward – electrocutaneous stimulation.

In the first days after irradiation, the unconditioned and conditioned reflex leukocytic reactions in both animals were markedly changed. With action of the stimulators, there was no special rise in the leukocyte count like that seen prior to irradiation; on the contrary, there was even a tendency toward leukopenia. The amplitude of the fluctuations in the leukocyte count before and after action of the stimuli was decreased. Reduction of the wave-like character in the changes of the leukocyte count was the most characteristic peculiarity of this series of experiments.

Distortion of the leukocytic reactions was combined, in these experiments, with the presence of coarse morphological changes in the peripheral blood and the bone marrow. Thus, the greatest interest was aroused by the two subsequent series of experiments, in which the dose of external irradiation was significantly lower, and the morphological changes were minimal or totally absent.

In the second series of experiments, both dogs were irradiated with a dose of 50 r. Careful clinical investigations and observation of the animals' state did not reveal any deviations from the norm. On the 3-4th week, the leukocyte count fell by 20-30%, chiefly due to the neutrophiles, and to a smaller degree, the lymphocytes. Investigation of the sternal punctures did not disclose any essential changes in the hematopoiesis of the bone marrow. On the 5th-6th week, the leukocyte count returned to normal.

The unconditioned and conditioned reflex leukocytic reactions were completely distorted from the first days after the irradiation. Instead of the usual leukocytosis (in the first minutes after exposure to the stimuli) there took

place a tendency toward lowering of the leukocyte count, as a result of which the leukocytograms were upset and inverted in the first days after the irradiation (Fig. 1B). On the 3rd-4th week after the irradiation, all the altered leukocytic reactions developed against the setting of a leukopenia. Figure 1 shows that the background fluctuations in the leukocyte count (original level, determined before initiation of the experiment) after the irradiation completely lost their previous regularity. Their amplitude was rather great. The first signs of normalization of the original background picture, and restoration of the unconditioned and conditioned reflex leukocytic reactions were noted only on the 5th-6th week after the irradiation. The last graph in that figure shows that the conditioned leukocytogram generally corresponds at that point to the dynamics of the same reaction before the irradiation. The background fluctuations in the leukocyte count are also completely normalized.

Mean Indices of the Reflex Leukocytic Reactions in Dogs (Leukocyte Count in Thousands)

Dog, dose	Reaction	Before irradiation			After irradiation		
		M	± m	P	M	± m	P
Trus, 50 r	Background picture	9,9	0,2		9,1	0,4	
	Unconditioned	13	0,4	< 0,01	9,8	0,49	> 0,2
	Conditioned	13,4	0,5	< 0,01	8,4	0,77	> 0,3
Laskovyi, 3 r	Background picture	9,6	0,17		12,8	0,75	
	Unconditioned	11,6	0,33	< 0,01	12,5	0,4	> 0,3

Note. Standard error calculated with reference to the background data.

In the third series of experiments, the dogs were irradiated with a dose of 3 r. According to the data in the literature, this degree of radiation does not give rise to any noticeable changes in the activity of the hematopoietic organs or in the morphological composition of the peripheral blood. A dose of 3 r is regarded as completely tolerable and relatively harmless, and practically speaking, can be considered safe for humans. Follow up and observation of the state of the animals showed them to be absolutely healthy.

The leukocytic reactions were studied in the first 1-2 hours, and also 24 hours, after the single-exposure roentgen irradiation. The background fluctuations were characterized in this case by great amplitude, and chiefly, by the absence of any regularity in the leukocyte count changes. Along with an increase in the lability of the leukocytic background, we observed a marked change in the leukocytic reactions. As illustration, we present the data obtained on the dog Laskovyi (Fig. 2). Figure 2 shows that at the first test, 2 hours after irradiation, the configuration of the curve representing the dynamics of the unconditioned reflex leukocytic reaction was completely altered. Instead of the usual leukocytosis, the number of leukocytes in the first minutes after stimulation progressively fell. The dynamics of the leukocytic reaction were also changed 24 hours after the irradiation, except that the initial leukopenia was already absent.

Thus, in this series of experiments, as in the foregoing one, the reflex leukocytic reactions of the blood system were altered in the first days (and even hours) after irradiation.

Hence, as a result of single-exposure total roentgen irradiation, animals manifest a change in their unconditioned and conditioned reflex leukocytic reactions. The dynamics of the background fluctuations in the leukocyte count, entering into the course of the experiments, also lost their regularity. Depending on the intensity of the radiation exposure, the leukocytic curves were characterized by a reduced (600 r) or, inversely, an increased wave-like character (50 r, 3 r). In the first minutes after stimulation, as a rule, instead of the usual leukocytosis we observed a decrease in the leukocyte count. These functional disturbances could take place with obvious preservation of the effectors of the blood system. Thus, distortion of the leukocytic reactions in the first hours and days after the irradiation either preceded the appearance of clear morphological changes (50 r), or were observed in the absence of such changes (3 r).

The data from a statistical treatment of the investigation results (see table) testify to the statistical significance of the changes in the leukocyte count seen in the experiments on the intact animals. After the irradiation, there were no statistically significant differences between the original (background) data and the material obtained from conditioned and unconditioned stimulations.

Distortion of the leukocytic reactions with small doses of radiation are apparently caused by a disturbance in the neuro-hormonal, regulatory functioning of the organism. With painful stimuli, the reflex leukocytic reactions are accomplished through the participation of humoral, in particular hormonal, factors; in the first order, these include the hormones of the hypophysis and the adrenals. In this investigation, we were unable to resolve that aspect of the question. Our observations only allow us to come to one, extremely general, conclusion: with total irradiation of animals, the reflex reactions of the blood system are disrupted, and these changes take place both with lethal and essentially harmless, tolerable doses of roentgen irradiation.

Our data, as well as the data in the literature [2, 10], testify to the fact that, in comparison with the usual morphological investigations of the blood, functional hematologic tests can serve as a finer indicator of radiation injury.

The dissociation between the motor phenomenon of the protective-defense reaction and the change in the leukocytic composition of the blood is explained by a selective disturbance in the activity of the blood system, which represents a characteristic peculiarity of radiation injury.

Reflex regulation of an organism's life activity after irradiation has been studied chiefly in the case of conditioned secretory and motor reflexes. Studying the reflex reactions of the blood system as the most "radiosensitive" system will deepen and widen our concepts on the effects and limits of radiation action on the organism of man and animals.

SUMMARY

Experiments were staged on 6 dogs. A study was made of the unconditioned and conditioned-reflex leukocytic reactions (with electrocutaneous reinforcement), as well as of the background variations of the leukocyte count prior to and after a single total x-ray irradiation of the animals with the doses of 600, 50 and 3 r. During the first day (hours) after the irradiation, an inversion of the leukocytic reactions and of the leukocytic background was noted. During the first minutes after the stimulation leukopenia developed, instead of leukocytosis (as it was in the case prior to the irradiation). Variations in the leukocyte count were characterized by a decreased (600 r) or, conversely, increased (50 r, 3 r) amplitude. With low irradiation doses functional disturbances of the blood system activity could occur in the peripheral blood and bone marrow in the absence of morphological changes. It is quite possible that the functional hematological tests may be of diagnostic significance in radiation affections.

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