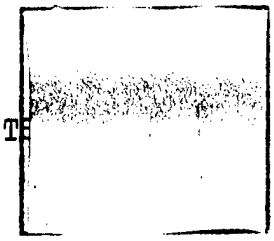

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- USSR -

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EXPERIENCE IN THE APPLICATION OF MYELOCYTOTOSIC SERUM IN RADIATION SICKNESS

By M. F. Sbitneva (Moscow)

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Scientific director -- Professor P. D. Gorizontov, corresponding member of the Academy of Medical Sciences USSR.

For the purpose of treating radiation injury various agents have been used among which a definite place has been given to hematopoietic stimulants. We had the task of testing out myelocytotoxic serum (MCS) in experiments on dogs, because it is well known that in small doses it exerts a definite stimulating effect on hematopoiesis normally and in anemias of various origin. The possibility has not been excluded either of a more extensive effect of it on other organs and systems of the body.

MCS was prepared by means of immunizing rabbits with dog bone marrow. The titer of specific antibodies in the serum obtained varied from 1 : 150 to 1 : 1200. The titer of hemolysins with respect to the dog erythrocytes was 1 : 5 to 1 : 12.5. For the purpose of proving the specificity of the MCS a number of serological studies were made (complement-fixation reaction with specific and nonspecific antigens, the complement-fixation reaction following adsorption of MCS with specific and nonspecific antigens, the reaction of dissolving cells). The biological activity of various doses of MCS (0.05, 0.1 and 1 cubic centimeter/kilogram) was shown in experiments on seven healthy dogs. The

data obtained are in agreement with the data in the literature (2, 3, 4 and others).

In the analysis of investigations on the application of large doses of MCS in healthy animals (5, 6, 8, 11 and others) as well as the results of our own experience attention has been directed to a certain similarity in the effect of a large dose of MCS and of ionizing radiation on hematopoiesis. This similarity is expressed in the occurrence of a pronounced leukopenia, in a reduction in the total number of erythrocytes and in the hemoglobin of the peripheral blood, an increase in the percentage of cells found in a state of degeneration, the occurrence of degenerative forms of neutrophils (chromatinolysis, hypochromatosis and others). After the injection of a large dose of MCS, as in radiation sickness, a notable loss of nucleated elements in the bone marrow was observed, with a disturbance of the normal interrelationship between the erythroblastic elements and the myeloid elements because of an increase in the polychromatophilic normoblasts. In our opinion, this fact may be of a certain significance in understanding the pathogenetic mechanism of development of hematological changes in radiation sickness.

The work was carried out on 38 dogs exposed to a total-body single effect of X-rays (doses of 500 and 400 r) and to fractional effects from doses of 10 and 20 r a day until a total dose of 500 r had been received.

A single irradiation of the dogs was accomplished on an aggregate three-tube apparatus with a tube voltage of 180 kv, a current of 20 ma, filters of 0.5 mm Cu and one mm of Al; distance from the axis of the tubes to the center of the trunk of the dog, 90 centimeters; dose rate of 12-15.8 r/min. In the case of fractional irradiation an RUM-3 apparatus was used; the voltage at the tube and filters was the same. The current was 15 ma; the distance from the anode to the center of the dog's trunk was 140 centimeters; the dose rate, 2-3.2 r/min.

Clinical manifestations of radiation sickness were found in all the dogs. Before irradiation and at definite intervals after it (on the fifth, 10th, 14th, 17th, 20th, 25th, 30th, 35th, 40th and 45th days in the case of acute radiation sickness and once a week in the case of fractional irradiation) a total blood analysis was made. Sternal marrow punctures were made less often: on the 10th, 20th, 30th and 45th day in the case of acute radiation sickness and once or twice a month in the case of fractional

irradiation.

The application of MCS in acute radiation sickness (dose 500 r) calculating 0.01 cubic centimeter/kilogram with a subcutaneous injection and 0.02 cubic centimeter/kilogram with intravenous injection on the second, third and fourth day after irradiation did not prevent the development of leukopenia and even aggravated the course of the disease somewhat. Evidence of this was the shorter latent period of the disease, the acceleration of death of the treated dogs, the occurrence of a considerable percentage of plasma cells of the myeloid series with pycnotic nuclei in the peripheral blood.

In checking the data of the pervious experiment with a reduction of the dose to 400 r we obtained similar results. Therefore, the application of MCS in such doses at the early stage of the disease should be considered contraindicated.

The use of such hematopoietic stimulants as cytotoxic sera is probably effective at later stages of radiation sickness, at a time when the first signs of blood regeneration have already appeared.

In subsequent experiments with the acute effect of X-rays (400 r), the time of administration of the serum was postponed to the end of the second and beginning of the third week of the sickness (11th, 13th, 16th day of the experiment), at a time, according to hematological and pathological data, regenerative phenomena occur in the bone marrow. The dose of serum administered in this and in subsequent experiments was 0.01 cubic centimeter/kilogram with a titer of the specific antibodies of 1 : 400. After injecting the MCS on the 11th, 13th and 16th day after the irradiation a somewhat more rapid recovery of the total number of leukocytes was observed by comparison with the controls (Figure 1), a certain increase in the percentage of young forms of myeloid series, beginning with the 20th day of the experiment; the absence of predominance of the absolute lymphocyte count over the neutrophil count which is characteristic of radiation sickness; a notable increase in the number of reticulocytes (up to 15-25 per thousand) on the 25th-30th day.

After a daily fractional irradiation using 20 r per day for a month until the time a total dose of 500 r was received, where the hematopoietic disturbances developed gradually and are not so deep-seated, as in acute

Radiation sickness, MCS was used in two series of experiments. In the first series the serum was injected into five dogs a week after concluding triple irradiation at intervals of two days. In the treated dogs a more rapid recovery was observed in the erythrocyte count (by the 70th day; in the controls, by the 120th day), an earlier normalization of the ratio of the erythroblastic to the myeloid elements (by the 56th day; in the controls, by the 120th day).

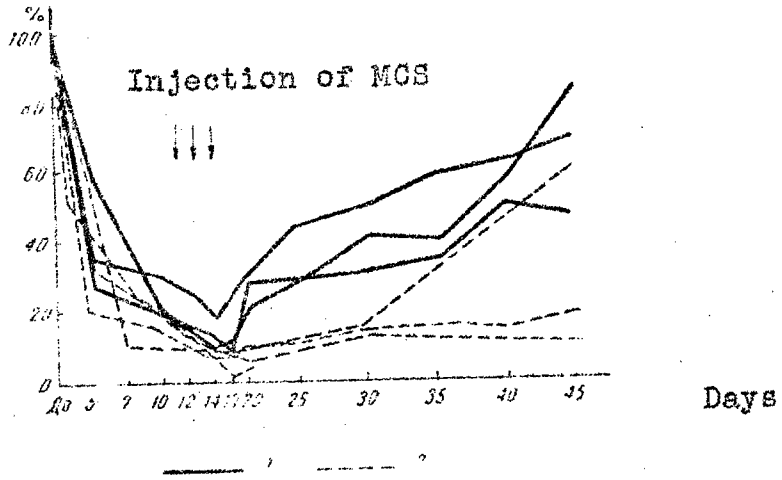

Figure 1. Change in the Total Leukocyte Count (in Percentages of the Original) in Control Dogs and in Those Treated with MCS After Irradiation (400 r).
1 -- Treated; 2 -- control.

In the second series the serum was injected into three dogs during the process of continuing irradiation after they had received a total dose of 300 r (on the 18th, 22nd and 26th days of the experiment). In all three treated dogs the anemia was much more pronounced than in the control dogs. The erythrocyte count decreased by 29-58 percent from the original level whereas in the control dogs the maximum reduction amounted to 29 percent. At the height of the relative erythrocytopenia (on the 29th-56th day) a poikilocytoisis was observed with anisocytosis of

the erythrocytes with the appearance of polychromatophilic macrocytes. Beginning with the 21st day after the onset of irradiation the appearance of a shift to the left in the differential count down to the myeloblast and myelocyte level was noted in the dogs treated with MCS along with a reduction in the total number of leukocytes to 67-89 percent of the original level. At the end of the irradiation period (28th day) the content of erythroblastic elements in the bone marrow increased somewhat in comparison with the myeloid elements (index of ratio of erythroblastic elements to myeloid elements 0.82-1.56). However, in dogs treated with MCS, in contrast to the controls, the number of regenerative forms in both of the red and white series not only did not decrease but, on the other hand, even increased by comparison with the original data. There was also an increase in the number of cells showing nuclear division (Figure 2). However, the activation of hematopoiesis was brief: by the 56th day a marked reduction occurred in the young forms and a decrease in mitotic activity.

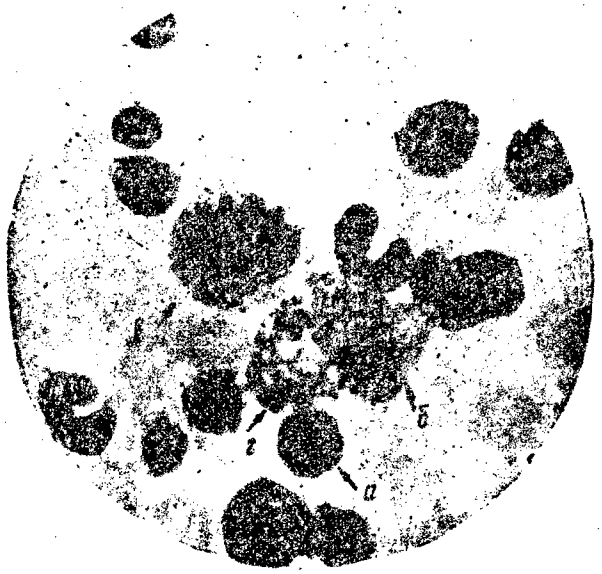

Figure 2. Microphotograph From Bone Marrow Smear of Experimental Dog in Second Series on the 28th Day. There are many formed elements in the smear. Cells are seen showing nuclear division (a, b, c) and which are actively phagocytic (d).

The third series of experiments was performed on seven dogs (four treated and three control). The dose of irradiation given each time was reduced to 10 r; the total dose remained as before, 500 r; the MCS was injected five times after the total dose of 300 r had been attained (on the 37th, 40th, 43rd, 46th and 50th days). In addition, the dogs were given multiple vitamins in the form of two dragées per day for a week before the irradiation and during the course of it. (One dragée contains the following vitamins: A, 1250 units, B1 and B2 each in a dose of 0.5 milligrams; C, 10 milligrams; D, 250 units; PP (nicotinic acid) 3 milligrams).

The change in the dose of irradiation given each time from 10 to 20 r, without changing the total direction of the hematopoietic reaction to the effect of ionizing radiation, produced a lesser degree of reduction in the various peripheral blood indices. Thus, after a daily fractional irradiation in a dose of 10 r per day until a total dose of 500 r had been received the maximum reduction in the total erythrocyte count in the control dogs did not exceed 12 percent, that is, it actually fitted within the framework of normal variations. The reduction in the total leukocyte count reached 46-73 percent of the original level. With a dose of 20 r each time the reduction in the total erythrocyte count amounted to 29 percent. The leukopenia was more severe (total leukocyte count fell by 68-92 percent).

At the same time, in the treated dogs which had been given multiple vitamins and MCS the reduction in the erythrocytes and leukocytes during irradiation were somewhat less pronounced than in the control animals. Apparently, the prolonged administration of multiple vitamins was of definite importance, which was also confirmed by the results of an additional control experiment on four dogs. The MCS, used against a background of a less severe injury to hematopoiesis, probably provided a more rapid recovery not only of the erythrocytes but also of the leukocytes (Figure 3).

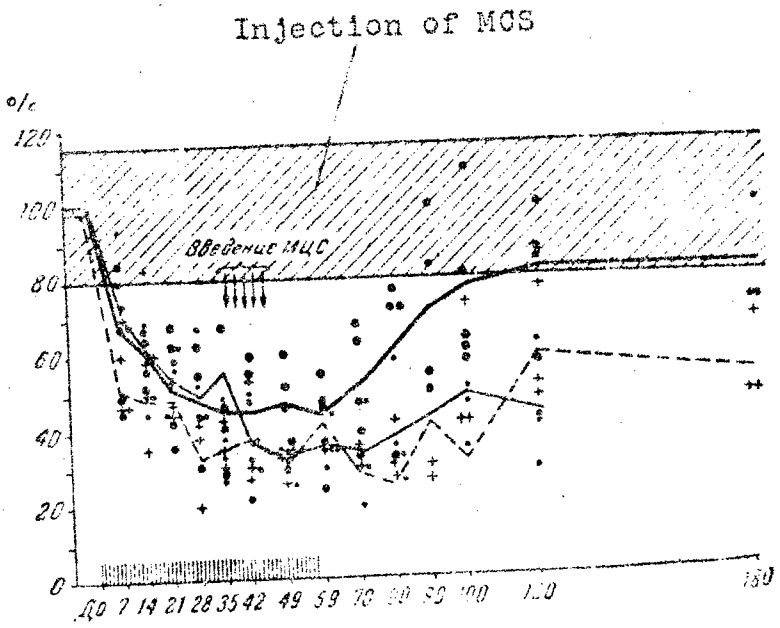

Figure 3. Change in the Total Leukocyte Count in Dogs of the Third Series. The two straight parallel horizontal axes show the limits of variation in the leukocyte count before beginning the experiment. The arrows in the horizontal axis show the period of irradiation. The heavy line shows the change in the average number of leukocytes for the group of dogs treated with MCS; the broken line, for the group of pure controls (irradiation alone); the solid thin line, for the group of controls which had been given multiple vitamins. The dots and crosses represent individual data for the leukocyte count in the treated and control animals respectively.

Based on what has been presented there are grounds for the belief that MCS exerts an influence on the acceleration of the processes of transformation of the poorly differentiated bone marrow elements into blood cells, leading to a notable increase in the content of young regenerating forms in the bone marrow chiefly of the myeloid series. At the same time, MCS apparently accelerates the washing out of cellular elements from the bone marrow into the blood stream. Evidence of this is the

appearance of immature forms down to the myeloblast level in the peripheral blood.

It may be supposed that MCS exerts an effect also on the interrelationship between the erythroblastic and myeloid elements. Such a conclusion stems from the following facts: 1) using an injection of a "blocking" dose of MCS (one cubic centimeter/kilogram intravenously) into a healthy dog a change in the normal interrelationship between the erythroblastic and myeloid elements in the direction of an increase in the former was observed in the bone marrow a day after the injection. The ratio of erythroblastic elements to the myeloid elements increased to 1.45; 2) in experiments on dogs following a single and fractional irradiation the injection of MCS in stimulating doses produced a more rapid normalization of the altered ratio of erythroblastic to myeloid elements in comparison with the controls.

A similar conclusion concerning the effect of MCS on the acceleration of maturation processes of cellular elements in the bone marrow and a normalization of the ratio in erythroblastic to myeloid elements can be reached by analyzing the results of the research of F. E. Faynshteyn (2), although the author himself does not emphasize this. Thus, he writes that before the use of MCS in patients with a hyporegenerative [hypoplastic] normoblastic anemia a reduction was observed in the leuko-erythroblastic ratio because of an increase in the percentage of erythroblasts and normoblasts. After the use of the myelotoxin a reduction in the cells of the erythroblastic series was observed.

Conclusions

1. The injection of MCS into dogs in a dose of 0.01 cubic centimeter/kilogram on the first to fourth day after a single total-body irradiation (400 and 500 r) impoverishes the hematopoietic process.

2. The use of MCS in acute radiation sickness (dose of 400 r on the 11th, 13th and 16th day of the experiment calculating 0.01 cubic centimeter/kilogram) exerts a slight effect on the acceleration of the recovery of the total leukocyte count and an increase in the percentage of young forms of the myeloid series, beginning with the 20th day after irradiation.

3. Injection of MCS three times in a dose of 0.01

cubic centimeter/kilogram a week after concluding the fractional irradiation (in a dose of 20 r per day and a total dose of 500 r) produces a more rapid recovery in total erythrocyte count of treated dogs, the earlier normalization of the ratio of erythroblastic to myeloid elements.

4. The use of MCS in fractional irradiation with a dose of 20 r per day after a total dose of 300 r has been received produces signs of overstimulation of myelocytopoiesis and more deep-seated disturbances of erythrocytopoiesis during the process of continuing irradiation.

5. The injection of MCS against a background of a less severe injury to hematopoiesis (with a fractional irradiation in a dose of 10 r per day and a total of 500 r and a prolonged administration of multiple vitamins) brings about a more rapid normalization of the hematopoietic processes in experimental dogs.

6. The intravenous injection of MCS in a dose of one cubic centimeter/kilogram produces changes in the hematopoietic system in healthy dogs which superficially resemble the changes in acute radiation sickness.

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