MECHANISM OF THE INCREASE IN RADIORESISTANCE IN THE LACTOPROPHYLAXIS OF ACUTE RADIATION SICKNESS

(UDC 616-001.28-036.11-085.362.664-039.71-07:612.017:612.014.482)

N. V. Raeva and N. N. Klemparskaya

Moscow

(Presented by A. V. Lebedinskii, Active Member, Academy of Medical Sciences, USSR)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 58, No. 7, pp. 44-48, July, 1964
Original article submitted March 18, 1963

In investigating the influence of antigenic stimulation on the radioresistance of animals, principal attention has been paid to studying microbial vaccines [1, 2]. In separate experiments we also tested other substances, such as sterile milk or cream [2]. It is well known that lactotherapy is widely used for treating allergic illnesses [6].

Taking into account the important role of autosensitization in the pathogenesis of radiation sickness, we set ourselves the task of observing the radioresistance of animals injected with sterile milk before exposure to radiation. We had to determine the influence of the milk dose and administration time (the interval between the injection and the irradiation) and to attempt to establish a possible mechanism by which milk influences the course of radiation sickness.

EXPERIMENTAL METHOD AND RESULTS

The experiments were conducted on 64 guinea pigs and 30 dogs irradiated with Co^{60} γ-rays on an EGO-2 apparatus at a dose rate of 245-248 r/min (over a period of 70-82 sec).

Table 1 presents data on the influence of injection of various quantities of milk two weeks before irradiation on the survival rate among guinea pigs. The 1st and 2nd experiments involved animals of different batches and different mean weights and different irradiation doses were consequently employed. In the 1st experiment a dose of 300 r proved absolutely lethal for the control guinea pigs, which were injected with physiological solution, and for the animals which received 0.5 ml of milk. When the milk dose was increased to 1.5 ml one guinea pig survived, while when this dose was doubled two of the six animals were protected. In the second experiment, which was conducted on younger guinea pigs, the irradiation dose was reduced to 250 r and this led to the development of a mild form of radiation sickness. Only 3 of the 10 control guinea pigs died, while only 2 of the 30 which received milk succumbed. Statistical processing of the data on the survival rate among the guinea pigs which received a sufficient milk dose (1.5-5 ml) showed a reliable difference from the control group. In addition to the number of animals which died, another index of the severity of the radiation sickness is the change in their weight. By the 18th day there was a drop of 5 g in mean weight in the control group, while the mean weight in the experimental group rose by 7-22 g. By the 30th day the gain in weight for the experimental guinea pigs amounted to 50-56 g, while the weight of the control animals which survived remained at its previous level.

These data indicated the possibility of influencing radioresistance by preliminary parenteral administration of milk before irradiation. Experiments on guinea pigs are naturally not sufficiently demonstrative for studying the clinical characteristics of the course of radiation sickness: in this connection we conducted experiments on dogs, studying the clinical course of radiation sickness and the character of their hematological indices.

The dogs were given one or two subcutaneous or intramuscular injections of milk in a dose of 0.5-1 ml per kg of body weight before irradiation. The interval between the 1st and 2nd injections was 3-4 days, while the interval between the 1st injection and the irradiation differed, 4, 7, 30, and 40 days being employed. The irradiation dose was 300-350 r. A total of 30 mongrel dogs weighing from 6 to 25 kg were used in the experiment, receiving the usual vivarium diet. Twenty dogs were given milk before irradiation: 10 of these received no treatment after irra-

TABLE 1. Influence of a Single Intramuscular Injection of Sterile Milk 14 Days before Irradiation of Guinea Pigs with Co^{60} γ-Rays on the Outcome of Radiation Sickness

Date of experiment (1962)	Group of animals	No. of guinea pigs in groups	Irradiation dose (in r)	Mean wt. of guinea pigs (in g)	Quantity of milk (in ml)	No. of guinea pigs survived[2]	No. of guinea pigs died
8/III	1	6	300	380	0,5	—	6
	2	6	300		1,5	1	5
	3	6	300		3,0	2	4
	4	6	300		—[1]	—	6
17/VII	1	10	250	236	0,5	10	—
	2	10	250		3,0	9	1
	3	10	250		5,0	9	1
	4	10	250		—[1]	7	3

[1]The guinea pigs of the control group were given 3 ml of sterile physiological solution.
[2] Statistical processing of the data on the survival rate among the animals of the 2nd and 3rd groups of both experiments, which were given a sufficient dose of milk, and among the animals of both 4th groups showed a reliable difference with respect to the x^2 criterion;

$$x^2 = 8,4;\ P<0,01$$

The data on the two 1st groups were not processed, since the milk dose was not sufficient in the first experiment (8/III).

TABLE 2. Statistical Processing of Data on Dogs' Survival Time in Days (Student's Criterion)

Experimental conditions	No. of dogs	$M \pm m$	t	P
Control	10	13,2±0,435	—	—
Milk	9	17,1±0,806	4,3	<0,01
Milk + antibiotics	5	19,6±0,812	7,6	<0,01

diation (1st group), while 10 were given antibiotics and vitamins from the 1st to 20th day after irradiation (2nd group). Ten dogs did not receive milk, but were irradiated (3rd, control group). The antibiotics—oxytetracycline, streptomycin, and phenoxymethylpenicillin—were given orally in individual doses of 100,000-200,000 units twice daily; 0.6 g of vitamin C, 20 mg of vitamin B_1, and 0.5 mg of vitamin B_2 were given once daily. During the course of the experiment we evaluated the survival rate, survival time, general condition, body temperature, appetite, stool character, and manifestation of hemorrhagic diathesis. Every five days we made a clinical analysis of the peripheral blood; at the same time, the quantity of serotonin (which was extracted from the whole blood with acetone) was determined biologically on an isolated rat colon.

We established that there was a slight increase in survival rate and a reliable increase in survival time over the control group among the dogs which received milk (Table 2).

Only one of the 10 dogs innoculated with milk survived, but the survival times of the innoculated dogs were prolonged by 3-13 days in all variants of the experiment. All the control dogs died by the 13th day. It must be noted that the best results were obtained when two subcutaneous injections of milk were given 7-30 days before irradiation, with an interval of 3-4 days between injections.

Analysis of the general data for all the groups of irradiated dogs shows that five of the 10 dogs in the 2nd group survived; the other five dogs died after a mean survival time of 20 days. This effect cannot be attributed solely to

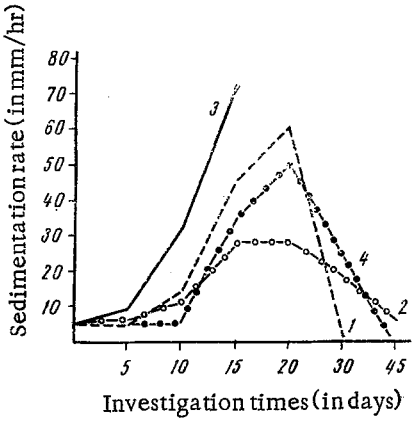

Fig. 1. Change in sedimentation rate of dogs of various groups after irradiation (mean data). 1) 1st group; 2) 2nd group; 3) 3rd, control group; 4) group of dogs treated with antibiotics only.

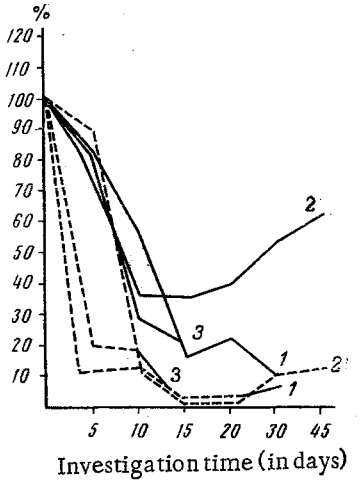

Fig. 2. Thrombocyte and serotonin counts and dynamics in the blood (as percentages of their initial values). A solid line indicates tee thrombocyte count and a dash line the sertonin count. The remaining designations are the same as in Fig. 1.

the administration of antibiotics and vitamins. Investigations which we had previously conducted showed that dogs irradiated with lethal doses of γ-rays and treated with antibiotics and vitamins alone, in accordance with the same scheme, survived in 50% of all cases, but became seriously ill; the dogs innoculated with milk easily withstood radiation sickness. Symptoms of radiation sickness set in later in the experimental dogs than in the control animals, while fewer subjects exhibited a given symptom at a certain time.

The greatest difference between the groups appeared in the animals' general condition and appetite. On the 10th-13th day a deterioration of general condition and adynamia set in all 10 control dogs and they refused to eat. Only a slight decrease in general mobility and appetite was noted in the eight dogs of the 1st group at this time while the general conditions of two of the animals remained unchanged. The dogs of the 2nd group maintained a completely satisfactory general condition and ate well, with the exception of two animals whose appetites decreased slightly. An intestinal disturbance—a partially liquid stool—usually set in on the 10th-13th day in the dogs of the 3rd, control group; only half the animals in the 1st group and two of the animals in the 2nd group exhibited a semi-solid stool.

From the 9th day onward, together with hemorrhages in the skin and subcutaneous cellular tissue, three of the dogs in the control group exhibited sanious discharges from the nose and mouth and contamination of the feces with blood, while in the 1st group the hemorrhagic phenomena were limited to petechiae and the dogs of the 2nd group exhibited no hemorrhages at this time. The animals of all groups, inclucing the experimental animals, exhibited marked changes in the cellular composition of their peripheral blood. The leucocyte dropped in all the dogs, to 30-40% of its mean initial value by the 5th day; it did not exceed 8% in the control group by the 10th day. but equalled 15-20% in the experimental group. These slight differences virtually disappeared by the 15th day. A tendency toward less marked disruptions was noted in the erythrocyte count and sedimentation rate of the innoculated dogs. Thus, the erythrocyte count on the 5th and 10th days was higher in the dogs innoculated with milk than in the dogs of the control group. As may be seen from Fig. 1, the sedimentation rate of the control dogs exceeded 30 mm per h (as against the normal 5-6 mm per h), while that of the experimental animals was 12-14 mm per h. At the time of death (on the 13th-15th day) the sedimentation rate of the control dogs reached 73 mm per h, that of the dogs in the 1st group was 45 mm per h, and that of the dogs in the 2nd group was 27 mm per h.

The differences in thrombocyte count merit attention. On the 10th day of illness this index was 28% of its mean initial value in the control animals and 56% of its initial value in the animals of the 1st group (Fig. 2). In the dogs of the 2nd group the thrombocyte count did not drop belwo 35% of its mean initial value. The changes in serotonin count were identical in the dogs of the 3rd and 1st groups. This count dropped sharply by the 5th day (to 10% of its mean initial value) and serotonin disappeared entirely from the dogs of all groups by the 15th day. An excep-

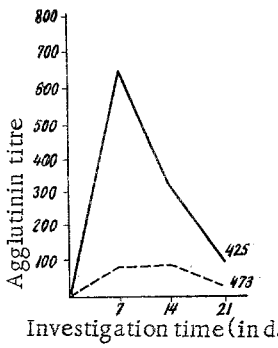

Fig. 3. Dynamics of titres of agglutinins to Breslau paratyphoid bacillus in a dog preliminarily given milk (No. 473) and in a control dog (No. 425). Both animals were innoculated with Breslau Paratyphoid vaccine.

tion was the dogs of the 2nd group; the quantity of serotonin in their blood on the 5th day amounted to 90% of its initial value.

The dependence of manifestations of hemorrhagic diathesis and the thrombocytic reaction on the quantity of serotonin in the blood has been pointed out in the literature [4]. We also stated that the maximum decrease in serotonin count coincided with the period of most marked manifestations of hemorrhagic diathesis. The serotonin count of the surviving dogs of the 2nd group remained at its initial level on the 5th day and has not dropped below 60% of this level on the 10th day; in the dogs of this group which died the serotonin count equalled 10% of its initial value on the 5th day and remained at this level on the 10th day.

The data which we obtained enable us to state that the lactoprophylaxis of radiation sickness is of some effectiveness.

What mechanisms form the basis for this phenomenon? It is well known that preliminary administration of "powerful" antigens (microbes or serum proteins) may suppress the development of the organisms reaction to subsequent administration of another antigen [3, 5]. The same concurrent relationships are observed between the action of vaccination and the organism's reactions to the tissue antigens liberated from cells under the influence of ionizing radiation. We may assume that there is a concurrent suppression of the immunological reaction to tissue antigens in the case of preliminary administration of milk as well.

We conducted supplemental experiments on 21 guinea pigs and 2 dogs. The guinea pigs were given two subcutaneous injections of sterile milk in a dose of 3 ml, with an interval of three days between them. Fifteen min after the 2nd injection two billion microbes of Breslau parathyphoid vaccine was injected intraperitoneally (11 guinea pigs); the 10 control animals were given physiological solution instead of milk and then innoculated with Breslau vaccine in the same dose. The concurrent suppression of antibody formation in the experimental guinea pigs took the form of a substantial decrease in agglutinin titres, which averaged 1:208 ±65 on the 7th day, while amounting to 1:688 ±65 in the control guinea pigs.

This phenomenon was especially marked in experiments on two dogs, one of which (No. 473) was first given two subcutaneous injections of milk in a dose of 1 ml/kg and then, 30 min later, an intravenous injection of microbial antigen (25 million microbes of Breslau vaccine). The other dog (No. 425) was given two subcutaneous injections of physiological solution and then an intravenous injection of the same dose of the same microbial antigen. As may be seen from Fig. 3, dog No. 425 exhibited the typical agglutination-formation curve, while antibody formation was somewhat depressed in the experimental animal (No. 473), so that the agglutinin titres did not rise beyond the normal antibody level throughout the entire observation period, despite the immunization.

It may be assumed that while administration of milk causes suppressions of the organism's reaction to "powerful" microbial antigens, it enters to an even greater extent into concurrent relationships with the action of tissue antigens and may itself have an inhibiting effect on autosensitization. Such concurrent relationships are also observed in following the changes in the serotonin leve of the blood. A considerable rise in the quantity of serotonin in the blood was noted in dog No. 425, while no such reaction was observed in dog No. 473.

LITERATURE CITED

1. N. N. Klemparskaya, V. F. Sosova, O. R. Nemirovich-Danichenko et al., Med. Radiol., No. 5, p. 65 (1957).
2. N. N. Klemparskaya and N. V. Raeva, Radiobiologiya, No. 1, p. 134 (1962).
3. M. A. Frolova, G. A. Klisenko, and L. I. Krasnoproshina, Byull. eksper. biol., No. 11, p. 62 (1958).
4. G. A. Chernov and M. O. Raushenbakh, gematol., No. 9, p. 3 (1960).
5. P. Abramoff, J. Immunol., Vol. 85, p. 648 (1960).
6. G. Kemmerer, Allergic Diatheses and Allergic Illnesses [Russian translation], Moscow-Leningrad (1936).