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EXPERIMENTAL ANTHRAX INFECTION IN IRRADIATED ANIMALS

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EXPERIMENTAL ANTHRAX INFECTION IN IRRADIATED ANIMALS

-USSR-

[Following is the translation of an article by A. P. Krasil'nikov and N. A. Israitel' in the Russian-language publication Meditsynskaya Radiologiya (Medical Radiology), Vol 4, No 6, 1959, pages 56-60. Tables have been appended at end of report.]

From the Microbiology Department (Professor B. Ya. El'bert, Chairman) of the Minsk Medical Institute.

The present article contains the results of studies on the course of experimental anthracic infection in irradiated animals. We were able to find no data on this subject in the literature.

We investigated the following problems: 1) the natural resistance of irradiated animals to experimental infection depending on the time of infection following irradiation, the radiation dose, and the point of introduction of the material; 2) the time of appearance and duration of bacteremia and degree of microbial semination of various organs; 3) the quantitative characteristic of bacteremia and microbe accumulation in the liver.

The experiments were carried out on white mice weighing 17-22 grams. The animals were irradiated in a wooden box in groups of 21 with the aid of an RUM-3 apparatus. Conditions of irradiation: focal distance -- 60 cm, filters -- 0.5 mm Cu and 1 mm Al, current -- 15 milliamperes, voltage -- 180 kilovolts, dose rate -- 19.8 r (roentgens); total radiation dose -- 342.54 roentgens. Deviations in intensity of radiation for individual animals did not exceed 0.1 r/min.

The work was carried out on a weakened variant of the anthrax bacillus -- the first Tsenkovskiy vaccine. The animals were infected with a smear of a 24-hour agar culture which was first centrifuged for 2 minutes at 1000 rev/min in order to obtain a homogeneous suspension. The resulting homogeneous suspension was dissolved physiologically to 1 billion microbe bodies (enteric standard) from which the required solutions were then prepared. The microbe suspension was introduced into the animals subcutaneously after irradiation. Infection was carried out 1, 3, 7, 12, and 21 days after irradiation. Observation over the infected animals lasted 5 days. The certification of the cause of death as the anthrax bacillus in this series, as well as in subsequent experiments, was controlled by liver sample culturing in Petri dishes with sarcopeptonic agar; the development of radiation sickness was certified by means of leucocyte counts.

The sensitivity of animals to the anthrax bacillus was judged according to the LD_{50/5} value determined by the Reed-Munch method. The statistical treatment of the data followed the method described by B. S. Bessmertrnyy.

Upon infection 24 hours after irradiation the LD_{50/5} value for the experimental animals (75,900 cells) was somewhat less than the LD_{50/5} for the control animals (640,000 cells) although the difference did not exceed probable limits. Upon the infection of mice 3 days following irradiation, there was a clearly expressed drop in the resistance of the organism to anthrax infection. The LD_{50/5} value for the irradiated mice in this experiment was equal to 178,000 cells (probable limits 76,000-381,000 cells), while the LD_{50/5} for the control animals was 25.2 million cells (probable limits 16.6-39 million cells). Thus the irradiated animals, according to the most conservative estimates, were 46 times as sensitive to the anthrax bacillus than the unirradiated mice in the same group. The inhibition of resistance of the organisms of irradiated animals was even more strongly manifested upon their infection on the seventh day following subjection to ionizing radiation, i.e., at the height of the radiation sickness. The LD_{50/5} value for the irradiated animals in this case was 11,300 cells, and for the control specimens -- 3.17 million. The maximum LD_{50/5} for irradiated animals was 170 times smaller than the minimum LD_{50/5} value for the control specimens. On the 12th day following irradiation, the resistance of the irradiated mice to anthrax infection returned to the initial level; the LD_{50/5} values for the irradiated animals (178,000) and the control animals (252,000) were very close. Finally, on the 21st day after irradiation (LD_{50/5} for irradiated specimens -- 1.6 million, for control specimens -- 219,000) the mice were even somewhat more resistant in comparison with the control mice.

In the study of white mice irradiated with X-rays (550 r dose) to anthrax infection at various stages in the development of radiation sickness, the experimental technique was the same as in the previous series. The only differences were in the total radiation dose; the animals were subjected to radiation for 25 minutes (total dose -- 550 r) under the same conditions as described above.

The animals were infected 24 hours before irradiation, as well as 1, 3, 7, and 14 days after irradiation. Because of the high sensitivity of some groups of animals, it was not possible to determine the LD₅₀ for them. For this reason, in order to determine the sensitivity of the animals to anthrax infection, we used the LD_{100/5} value calculated according to A. Ya. Boyarskiy's method of straight-curve smoothing (as is known, the Reed-Munch method is inapplicable in this case).

With increasing radiation dosage, the degree of resistance inhibition was even more manifest, although the experiments revealed basically the same regularities in the development of the infectious process as during the action of smaller doses of ionizing radiation (342 r).

Upon infection of animals 24 hours prior to irradiation, the LD_{100/5} values for the irradiated and control groups differed but little from one another. In animals infected 24 hours after irradiation, there was a marked tendency toward the reduction of resistance of the irradiated animals to anthrax infection (LD_{100/5} for irradiated animals was less than 100 cells, and for the control animals -- 458 million cells). The same shifts in the resistance of the organism were observed in the experiment in which infection was performed on the 7th day following irradiation. The resistance of mice to anthrax infection likewise remained substantially below the control level 14 days following irradiation with the indicated dose of X-rays.

The results obtained did not make it possible to detect any restoration or tendency toward restoration of the resistance of animals to the studied infection with this radiation dose. At the same time, with a lesser dose (342 r), the resistance of the irradiated and control animals by the 14-21st day following irradiation was found to even out. It was impossible to establish the regularities of the course of the anthrax disease against the radiation sickness background (with a 550 r dose) in the later stages, since with the indicated dose most of the animals were dead by the 20-21st day. The third series of experiments had as its purpose the determination of the effect of radiation on the development and course of infection in white mice with various methods of introducing the infection. The animals were irradiated under the same conditions as in the first series of experiments (total radiation dose -- 342 r). On the 7th day following irradiation the animals were infected subcutaneously, intranasally, and perorally. Intranasal infection was performed under a light ether anesthesia; peroral infection was introduced directly into the stomachs of hungry animals (0.5 ml dose) with the aid of a ventral catheter. The unit of sensitivity of the animals to infection, as in the first series of experiments, was the LD_{50/5} value.

The results of this series of experiments show that the LD_{50/5} value for irradiated animals with the subcutaneous and intraventral methods of infection was 28 and 84 times less, respectively, than for the control animals. The experiments likewise showed that upon the introduction of very large doses of the anthrax bacillus directly into the stomach it is sometimes possible to produce anthracic sepsis (bacteriological control positive in all cases), although the occurrence of the disease depends a great deal on the individual peculiarities of the organism. Also not excluded is the possibility of the penetration of microbes through the damaged mucous membrane of the digestive tract, such as may occur upon the insertion of a ventral catheter.

We may also conclude from the resulting data that mice are more sensitive to the subcutaneous injection of infectious material than to intraventral infection. This was also observed with the infection of mice with Tsenkovskiy's second vaccine. With the intraventral method of infection the LD_{100/5} for the second Tsenkovskiy vaccine was equal to 250,000-25 million cells, and with subcutaneous injection -- to 10,000-100 cells.

The semination of organs, which served as an indicator of the intensity of development of the infectious process, was studied on white mice subjected to X-rays (550 r) and infected with a microbial culture (100,000 and 10 million microbe doses) on the 3rd day following irradiation.

The mice were autopsied 1, 3, 6, 9, 12, 24, 36, 48, and 60 hours (4 animals each time) after infection; specimens were taken from the inguinal lymph node, spleen, kidneys, blood, brain, and bone marrow, and cultured on Petri dishes with sarcopeptonic agar (pH = 7.4). On the basis of results obtained with due account of microbial reproduction, the indices of organ semination were determined; these indices the percentage ratio of organs revealing growth of anthrax bacilli to all of the organs studied.

In order to characterize the microbial semination quantitatively, a determination was made of the microbe count in the blood and liver of mice irradiated and unirradiated at various times following infection: 3, 6, 12, 18, 24, 36, 48, and 60 hours. The infection dose in the various experiments was equal to 100 million and 10 million microbe cells. The radiation dose was 550 r.

The experiment was carried out on the 3rd day following irradiation according to the following procedure. Blood (0.1 ml) drawn with a pipette from the axillary sac was diluted in a 1:10 blood-water ratio. From this basic solution, weaker solutions in ratios of 1:10², 1:10³, 1:10⁴, 1:10⁵, 1:10⁶, etc. were prepared. Each of the indicated solutions in amounts of 0.1 ml was then placed in test tubes containing sarcopeptonic agar. The detection of growth only in the first test tube indicated that 1 ml of blood contained about 100 microbe cells, and the first two -- about 1000, etc. The renal specimens were taken by means of 2-3 probes into the body of the liver with a Pasteur pipette, which yielded about 8-10 mg. The solution and culturing procedures were the same as in the case of the blood specimens. Only in the first test tube did the growth correspond to 1000 microbe cells in 1 g liver; the growth in the subsequent ones yielded 10,000, etc.

The results of this series of experiments, shown in Tables 1 and 2, were calculated with due regard for the "doubled error" (probability 19/20). The studies showed that the dissemination of anthrax bacilli through the organism of irradiated animals occurs much earlier than in the control animals. Thus, most of the irradiated mice had anthrax bacilli in many of their organs 12 hours after infection. In the control animals, microbes in the organs were as a rule detected only 24-36 hours after infection (with a dose of 10 million microbe cells) or only after 36-48 hours (100,000 microbe dose).

The semination of the irradiated animals with microbes, as follows from the cited data, has a more constant character and takes in a greater number of organs than in the control-group animals. The total index of organ semination for the irradiated mice was 45.6, and for the control mice -- 12.3. A particularly large difference in the semination indices was noted in the earlier stages of the disease. Thus, in the period 3-18 hours after infection the semination index in the group of irradiated

animals was 12 times larger than in the control group, just 5 times larger after 24 hours, and only 2 times larger after 36 hours. A difference in microbial semination was observed in all 7 of the organs studied, as may be seen from Table 1. It was particularly large in the brain, bone marrow, and kidneys. We noted no changes in the duration of bacteremia and microbial semination of organs. Organ cultures from animals recovering from the disease on the 6th, 8th, 10th, and 12th days following infection did not once produce microbe growth, although a considerable number of mice was studied (100 irradiated, 100 control) from each group.

The rates of microbe reproduction in the organs of irradiated animals were incomparably higher than for the control specimens. This was especially clear in the early stages of the disease, when the liver and blood were found to contain enormously high microbe counts (Table 2).

The number of microbes in 1 g of liver for the irradiated animals 18 hours after irradiation was equal to $10^{10.2}$ (control = 0), after 24 hours -- $10^{13.09}$ (control -- $10^{7.09}$), etc. (Table 2).

In the later stages of the disease, when the protective powers of the organism had been fully inhibited by the exceptionally virulent anthrax bacillus, the difference in the intensity of organ semination evened out.

In the study of the quantitative characteristic of organ semination of white mice, we were obliged to work with new animals each time. Because of this, we attempted to duplicate the experiments on 8 guinea pigs and 4 rabbits. The material used in the study was blood from the heart as the only possible object of multiple (periodic) investigation. However, the anthrax bacillus was not detected either in the early or later stages of the disease. Shortly before death, the irradiated rabbits were found to contain a very large amount of extraneous flora.

We did not succeed in our experiments on the infecting dose, which we defined as the minimal dose sufficient to break down the barrier to microbe penetration into the organism, but without the development of the disease. The fact is that with small doses, the microbes were detected quite inconstantly, while large doses produced lethal infection.

Conclusions

1. A single overall irradiation of white mice with X-rays in sublethal doses (342 r) lowers the resistance of the animals to anthrax infection. This reduction is already evident one day following irradiation, is clearly manifest after 3 days, and reaches a maximum on the 7th day. Resistance returns fully by the 12th-21st day after irradiation.

2. The lowering of the resistance of the irradiated organism to anthrax infection is even more clearly manifest in experiments using a large radiation dose (550 r).

3. A lowering of the resistance of white mice subjected to ionizing radiation occurs with subcutaneous and intraventral infection;

with the intranasal and peroral methods of infection, no significant difference was observed in the reactions of the irradiated and control specimens.

4. Experimental anthrax infection during the course of development of radiation sickness ensues from a substantially lower dose of the infecting agent (a factor of hundreds of thousands), develops 1-1½ days faster, develops with the semination of a large number of organs and the accumulation in them of enormous numbers of microbes (in comparison with the control animals), with a death rate somewhat higher than that of the control group.

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Table 1

Индекс обсеменности органов белых мышей, зараженных микробной культурой на 3-и сутки после облучения в дозе 550 r				
Исследованные органы	Индекс обсеменности облученных животных	Индекс обсеменности контрольных животных	Разность между индексами	Требуемая минимальная разность при удвоенной ошибке
(B)	(C)	(D)	(E)	(F)
(G) Кровь	44,4	16,6	27,8	19
(H) Печень	50,0	13,8	36,2	19
(I) Селезенка	44,4	13,8	30,6	19
(J) Лимфатический узел	44,4	16,6	27,8	19
(K) Почка	41,7	8,3	33,4	16
(L) Костный мозг	50,0	8,3	41,7	16
(M) Головной мозг	44,4	8,3	36,1	16
(N) Все органы	45,6	12,3	33,3	6,8

Взято из «Таблиц достоверности статистических показателей» и «Числа наблюдений» А. Я. Боярского.

Legend:

A = Index of Organ Semination in White Mice Infected With Microbe Culture on the 3rd Day Following Irradiation With a Dose of 550 r;
 B = Organs examined; C = Semination index for irradiated animals;
 D = Semination index for control animals; E = Difference between indices; F = Required minimal difference with doubled error /see note/; G = Blood; H = Liver; I = Spleen; J = Lymph node; K = Kidneys;
 L = Bone marrow; M = Brain; N = All organs; O = (/Note:/ Taken from A. Ya. Boyarskiy's Authenticity Tables for Statistical Indices and Number of Observations).

Table 2

A. Темп размножения микробов у облученных животных			
Группы B	Время вскрытия после заражения, часы C	D. Количество микробов, lg 10	
		E. в 1 мл крови	F. в 1 г печени
1. Облученные H. Контрольные	3	—	—
2. Облученные H. Контрольные	12	3,17965 —	4,11394 —
3. Облученные H. Контрольные	18	5,23096 —	10,22194 —
4. Облученные H. Контрольные	24	7,11394 3,00094	13,00094 7,00094
5. Облученные H. Контрольные	36	8,52394 12,22194	11,22394 9,22316
6. Облученные H. Контрольные	48	Посторонняя флора (всего бактерий) 10,00736 12,10476	

Legend:

A = Rate of Microbe Reproduction in Irradiated Animals; B = Groups;
C = Time of autopsy following infection, in hours; D = Number of
microbes, lg 10; E = in 1 ml blood; F = in 1 g liver; G = Irradiated;
H = Control; I = Extraneous flora (entire bacilli).

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