Technical Papers

The Incidence of Bacteremia in Mice Subjected to Total Body X-Radiation[1]

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Infection has been described as one of the features of radiation sickness (*1–4*) but its role has not been systematically investigated heretofore. This paper reports a study of such infection.

Methods. Male Swiss mice,[2] weighing 18 g to 22 g and obtained from an inbred stock, were subjected to total body x-radiation in a single exposure, delivered at 20 kv, 15 ma, at a distance of 27 in., using ½-mm copper and 3-mm Bakelite filter.[3] Their LD_{50} (30 days) was approximately 400 roentgen units. They were housed in a room kept at a temperature of 73°–78° F.

TABLE 1

SUMMARY OF EXPERIMENTS

Dose r	No. mice irradiated	Mice cultured	Total cultured
600	585	20 per day, 2nd–15th days after irradiation	280
450	1042	35 per day, 2nd–18th days after irradiation	595

Two series of mice were studied: one received 600 r, the other 450 r. Each series consisted of a number of groups, all the members of which were irradiated on a single day. Beginning the second day after irradiation, several (usually 5) mice in a group were killed and cultured daily until the desired number of cultures for each postirradiation day had been obtained (see Table 1). Of 585 mice irradiated with 600 r, 280 were killed and cultured; of 1,042 irradiated with 450 r, 595 were killed and cultured.

Cultures. None of the cultures reported here was made on mice that had died. Each mouse to be cultured was etherized and autopsied immediately. The chest was opened under aseptic precautions and heart blood aspirated with a capillary pipette. A drop (approximately 0.05 ml) was planted on the surface of nutrient agar and blood agar plates and carefully spread with a sterile loop. In the 450-r series, the remainder of the heart blood was inoculated into 5 ml of brain-heart infusion broth. The spleen was removed and cultured in brain-heart infusion broth. In the 600-r series, the spleen was also cultured on nutrient agar and blood agar plates, but in the 450-r series, only broth cultures were made.

Anaerobic cultures of blood from 25 hearts in each series were made in Brewer's fluid thioglycolate media. Blood and spleen of 62 additional animals in the 450-r series were cultured in tubes of brain-heart infusion media under NaOH and pyrogallic acid. Only one true anaerobe was recovered; it was isolated from the spleen of a mouse on the 16th day after irradiation.

Control cultures on normal mice. Blood and spleens of 35 normal mice from the same stock, cultured by the routine procedure for the 450-r series, were all sterile. The spleens of 17 additional mice were ground individually in a Waring Blendor and cultured aerobically and anaerobically. All of these cultures were also sterile.

TABLE 2

RESULTS OF BLOOD AND SPLEEN CULTURES ON MICE SUBJECTED TO 600 R TOTAL BODY X-RADIATION*

Days after irradiation	Daily death rate† %	Blood and spleen positive No. of mice	Spleen only positive No. of mice	Blood *or* spleen positive %
2	0	1	0	5
3	0.3	1	0	5
4	0.5	0	0	0
5	2.0	1	0	5
6	4.0	9	0	45
7	3.0	11	0	55
8	6.0	10	0	50
9	9.0	17	0	85
10	7.0	12	0	60
11	6.0	15	0	75
12	4.0	11	0	60
13	3.0	8	1	45
14	2.0	6	0	30
15	0.6	3	3	30

* Twenty mice killed and cultured each day after irradiation. Cultures on mice that died spontaneously are excluded from these data.

† Percent of total number irradiated.

Results. In the 600-r series, a total of 280 mice were killed and cultured. Table 2 presents the results of the blood and spleen cultures and also the daily mortality rate based on the total of 585 mice receiving this dose of radiation. The highest daily incidence of positive cultures (85%) occurred on the 9th day, which was also the day on which the greatest number died (9%). A rough estimate of the severity of the bacteremia was obtained from colony counts of plate cultures seeded with a single drop of blood. Seventy percent of the positive cultures contained more than 50 colonies and 35% of them innumerable colonies.

[1] This investigation was initiated as part of the U. S. Army Contract No. W49-007-MD-425 and has been continued under Contract No. At(11-1)-46 between the U. S. Atomic Energy Commission and the University of Chicago.

[2] From an old colony maintained by the Maple Grove Rabbitry, Springfield, Missouri.

[3] All of the mice were irradiated at the Argonne National Laboratory, with the assistance of Mr. Joseph Trier and Mr. Emil Johnson. The authors are indebted to them and also to Dr. Austin M. Brues, director of the Biology Division of the Argonne National Laboratory, for many helpful suggestions.

TABLE 3

RESULTS OF BLOOD AND SPLEEN CULTURES ON MICE SUBJECTED TO 450 R TOTAL BODY X-RADIATION*

Days after irradiation	Daily death rate† %	Blood and spleen positive No. of mice	Spleen only positive No. of mice	Blood *or* spleen positive %
2	0.2	1	2	8
3	0	0	0	0
4	0	1	5	17
5	2.0	0	3	8
6	1.0	4	1	14
7	4.2	3	1	11
8	4.3	8	1	25
9	4.6	4	2	17
10	6.0	15	4	54
11	3.6	15	2	48
12	4.0	15	2	48
13	3.0	13	3	45
14	1.0	12	1	37
15	0.8	8	3	31
16	0.2	10	3	36‡
17	0.1	3	0	8
18	0	5	1	17

* Thirty-five mice killed and cultured each day after irradiation. Cultures on mice that died spontaneously are excluded from these data.

† Percent of total number irradiated.

‡ One anaerobe.

In the 450-r series, a total of 595 mice were killed and cultured. Table 3 shows that a high incidence of positive cultures occurred during the period of greatest mortality, although the maxima were not as great as in the preceding series. The daily mortality rate was based on the total of 1,042 mice irradiated in this series. The bacteremia was not as severe; approximately 70% of the positive cultures developed more than 50 colonies but only 20% contained too many colonies to count.

TABLE 4

CLASSIFICATION OF MICROORGANISMS RECOVERED FROM HEART BLOOD OR SPLEEN OR BOTH

	%
Paracolobactrum	42
Coliform	22
Proteus	13
Pseudomonas	9
a Streptococcus	6
Unidentified Gram-negative rod	3
Alcaligenes	2
Anaerobes	0.3

Identity of the organisms. The cultures were identified by the customary methods. The relative frequence of the various species is given in Table 4. In a systematic survey of the flora at different levels of the intestinal tract of normal mice it was found that all of the species listed in Table 4 occurred regularly in the large bowel with the exception of *Pseudomonas*. This organism was found only occasionally. It is clear from these findings that the lower intestinal tract was the reservoir from which invasion of the blood stream occurred. In both the 600-r and 450-r series, 91% of the cases of bacteremia were caused by a single organism; in the remaining 9% no more than two species were present. This fact strongly suggests that multiplication of the organisms was occurring in the blood stream.

It was during the second week after irradiation, the period of greatest mortality, that bacteria from the lower intestinal tract invaded the blood stream and produced bacteremia, often of great severity. The maximum incidence of such infections in mice exposed to 600-r or 450-r x-radiation was 85% and 54%, respectively.

References

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The Effect of Cobalt on the Microbial Synthesis of LLD-active Substances

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Shorb (*7*) reported an unidentified growth factor in liver extract, LLD, which is required by *Lactobacillus lactis* Dorner. Crystalline vitamin B_{12}, a cobalt coordination complex isolated from liver (*5*), was found capable of satisfying the LLD requirement of this organism (*8*). It has also been shown that *Lactobacillus leichmannii* responds to vitamin B_{12} (*2*). Recently Rickes *et al.* (*6*) have reported the isolation of crystalline vitamin B_{12} from culture broths of a grisein-producing strain of *Streptomyces griseus.* In addition, they reported the presence of LLD-active substances in culture broths of other microorganisms. Other investigators have also observed the presence of materials having growth-promoting activity for *L. leichmannii* in various microbial broth cultures (*1, 4*). During an extensive screening program we have observed that large numbers of microorganisms are capable of synthesizing LLD-active substances. Chemical extraction data and paper strip chromatography carried out on some of these broths have shown them to contain vitamin B_{12}.

Various medium modifications were investigated in an attempt to increase the microbial synthesis of vitamin B_{12}. This paper presents the data obtained from a study on cobalt supplementation.

Medium. The medium employed in these studies was composed of 1% N-Z-Amine (Type A),[1] 0.3% Difco beef extract, and distilled water to volume. The medium was adjusted to pH 6.8–7.0 with NaOH, dispensed in 40-ml aliquots per 250-ml Erlenmeyer flask, and sterilized at 15 lb pressure for 20 min.

Inoculum. The various microorganisms were carried on nutrient agar slants. A 48-hr shake flask culture was used as inoculum in experiments with the grisein-produc-

[1] An enzymatic digest of casein manufactured by Sheffield Farms, Inc.