RELATION BETWEEN BACTEREMIA AND DEATH IN MICE FOLLOWING X-RAY AND THERMAL COLUMN EXPOSURES[1]

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Invasion of the host by endogenous bacteria and increased susceptibility to infection following irradiation was observed over 20 years ago (Chrom, 1935; Lawrence and Tennant, 1937). A few reports (de Gara and Furth, 1945; Gowen and Zelle, 1945) appeared demonstrating increased susceptibility of irradiated animals to pathogenic organisms and the complication of radiation mortality by infection (LeRoy, 1947; Liebow *et al.*, 1949). Miller *et al.* (1950*a*, 1951) systematically investigated the incidence of endogenous intestinal bacteremia in mice following whole body irradiation with 450 and 600 r of X rays. Their data showed that the highest incidence of positive cultures occurred during the period of greatest mortality.

Numerous investigators have studied the efficacy of antibiotics in the treatment of post-irradiation infection (Howland *et al.*, 1949; Miller *et al.*, 1950*b*, 1952; Furth *et al.*, 1951). William and Congdon (1952) were able to modify infection after 900 r with bone marrow injection. More recently Marston *et al.* (1953) showed that intravenous injection of immature spleen homogenates complemented the action of streptomycin and increased the resistance of sublethally irradiated mice to *Proteus* infection.

Although several studies have demonstrated a relationship between infection and mortality following midlethal doses of radiation, there have been few studies which included doses over the entire LD_{50}^{30} range. Gonshery *et al.* (1953) observed essentially a linear relationship between dose and time of death in the dose ranges 550 to 800 r. Recently Vogel *et al.* (1954) reported studies of bacteremia in mice irradiated with fast neutrons and Co^{60} γ radiation which suggested that the relation between bacteremia and mortality may be one of cause and effect.

The purposes of the present study were: (1) to correlate specifically the incidence of bacteremia with mortality, and to determine the time of appearance of bacteria in heart blood and various tissues or organs following thermal column (predominately thermal neutrons) and X-ray exposures over the median lethal dose range; (2) to study the incidence of bacteria in heart-blood and tissues following a wide range of doses. The second study was carried out to determine whether there was any invasion of tissues or organs by intestinal bacteria in animals radiated with doses in the so-called "acute intestinal death" range. A few reports (Osborne *et al.*, 1952; Gonshery *et al.*, 1953) have indicated that infection following doses above 1000 r was rarely observed before death. Silverman *et al.* (1954) reported a high incidence of infection in mice exposed to high doses of neutrons and to 1200 r of whole body X-irradiation. Since Quastler *et al.* (1951) found that radiation doses of 1,200 to 10,000 r, delivered to the gut alone or to the whole body, elicited the stable 3- to 4-day survival time characteristic of the acute intestinal death, it was of interest to see if intestinal bacteria invaded the tissues at any time prior to death from such high doses. One dose (23,000 r) administered in the present study was considerably in excess of that necessary to elicit "acute intestinal death." This dose was employed to determine whether bacterial invasion occurred prior to very early death caused by the direct effect of very high doses of radiation on the central nervous system (Quastler *et al.*, 1951; Langham *et al.*, 1954).

METHODS AND PROCEDURES

Adult female CF_1 mice, approximately 12 weeks of age and weighing between 20 and 25 g, were

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used throughout the study. All animals were randomized and assigned to exposure groups on the basis of random number tables.

All X-ray exposures were carried out with the radial beam of the 250-kvp G. E. Maxitron which was operated at 30 ma. Without added filtration the radiation had an HVL of 2 mm of copper. The target-to-cage distance was 75 cm and the dose rate for all doses (except those of 10,000 and 23,000 r) was 50 r/min. The 10,000 and 23,000 r doses were delivered at a target-to-cage distance of 18.5 cm and at a dose rate of 247 r/min. Inverse square relationship does not apply because of variation of dose with angle between cage and target plane. All dose measurements were made with calibrated Victoreen 100-r ionization chambers.

The thermal column exposures were carried out in the thermal column of the Los Alamos "Water Boiler" as described by Brennan *et al.* (1954). Fifteen mice were exposed at a time at a dose rate of about 1.37 rem[3]/sec.

Cultures of heart blood were taken aseptically as follows: The mice were anesthetized with ether, after which the chest cavity was opened and the heart exposed and punctured with a sterile needle attached to a tuberculin syringe. One drop of blood, equivalent to 0.04 to 0.05 ml, was placed in each of several tubes containing brain-heart infusion agar. These were incubated at 37 C and checked for visible growth at 24 and 48 hours. After an additional 48 hours of incubation, they were streaked on blood agar plates and a final reading was made. Anaerobic cultures were not done, as Miller *et al.* (1950*a*, 1950*b*) have reported no significant number of anaerobes present in mice after radiation.

When tissue or organ specimens were taken, the animal was anesthetized and the abdomen and thoracic regions were swabbed with zephiran to moisten the hair and minimize contamination. After a heart-blood specimen was taken, the abdomen was opened and the entire chain of mesenteric lymph nodes, a section of liver and nearly all the spleen were cultured in brain-heart infusion agar. The capsules of all specimens were cut in several places before they were dropped into the culture tubes. All positive cultures were confirmed by streaking on blood agar plates.

[3] 1 rem based on MLD measurements in the mouse (Brennan *et al.*, 1954).

To study the correlation of bacteremia with mortality, 30 mice per point were exposed to 400, 475, 560, 670 and 800 r of X-ray and 480, 520, 570 and 616 rem of thermal column radiation. Thirty nonirradiated controls were included in the study. Following exposure the animals were housed 10 to the cage and given Purina laboratory chow and water *ad libitum*. On the ninth day after exposure 15 animals per point were chosen at random and sacrificed for heart-blood cultures. The remaining animals were checked daily for lethality. The data were analyzed for per cent bacteremia and the incidence of bacteremia on the ninth day was compared with the per cent mortality at the beginning of the eleventh day. Mortality was followed for 30 days and median survival times and LD_{50}^{30} values calculated.

To determine whether invasion of tissues by endogenous bacteria could be correlated with dose and time after dose in the LD_{50}^{30} range, 45 to 75 mice per point were exposed to 640, 780 and 950 r of X-ray and 480, 540, 600, 670 and 675 rem of thermal column radiation. The number of animals exposed at each dose level depended on the number of days, postradiation, on which blood and tissue cultures were taken. Ten animals per point per day were sacrificed for this purpose several days before, during and after the median survival time. Fifteen animals per point were used for lethality studies. The time required for a generalized systemic invasion to occur once bacteria invaded one of the tissues was also noted.

The days on which the heart blood and tissues were cultured are shown in the section on Results. Fewer than 10 animals were cultured at some of the later times because of the incidence of mortality. At 600 and 670 rem, only heart blood was cultured.

Ten control animals were cultured on the seventh and fifteenth days after exposure.

Forty-five to 60 mice per point were exposed to 3,000, 10,000 and 23,000 r of X-ray and 3,000 rem of thermal column radiation for the high dose studies as described earlier. Following the 3,000- and 10,000-r doses, heart-blood and tissue cultures were made from 15 animals per day (or from the remaining survivors) during the first 3 days after exposure. Following the 23,000-r dose, blood and tissue cultures were taken at 12 and 24 hours after exposure. Ten control animals

were sacrificed and simultaneously cultured with each group of exposed animals.

RESULTS

When the probits of per cent bacteremia 9 days postradiation and mortality 11 days postradiation were plotted against dose of X-ray and thermal column radiation, the respective lines of regression shown in figures 1 and 2 were obtained. An analysis of variance of the data indicated no significant difference in the regression coefficients of the lines of regression for the bacteremia and mortality responses to each type of radiation. There was, likewise, no significant difference in the means of these lines for each type of exposure. It was possible therefore to represent both the bacteremia and mortality responses by a single probit line for each type of radiation.

These data indicate that the 11-day LD_{50} for X rays was 725 r and that for thermal column radiation was 560 rem. The relative potency of pile thermal column radiations (compared to 250-kvp X rays) for the production of 11-day lethality in mice (and for production of bacteremia at 9 days) is, therefore, approximately

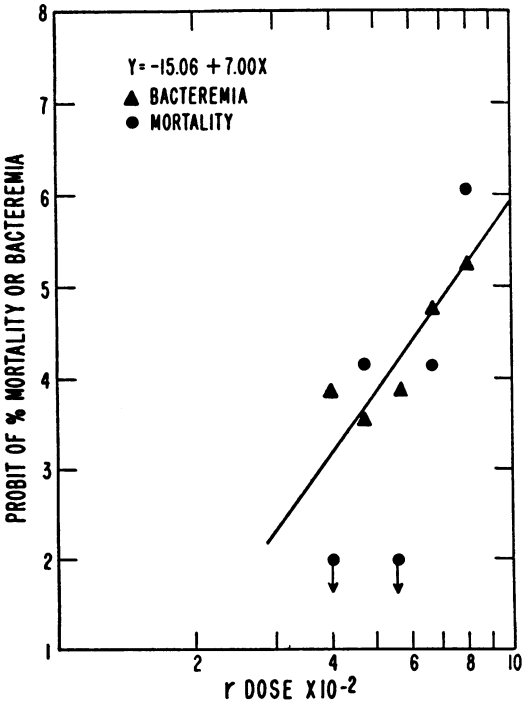

Figure 1. Variation of 11-day mortality and 9-day bacteremia with dose of x-ray.

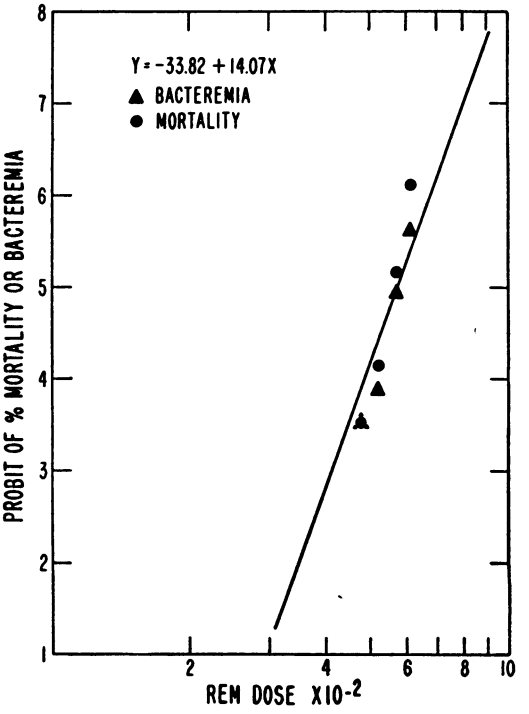

Figure 2. Variation of 11-day mortality and 9-day bacteremia with dose of thermal column radiation.

1.4 (Harris, 1954). The LD_{50}^{30} dose of X rays was 560 r and that for thermal column radiation was 520 rem, which indicates a relative potency level for the latter approaching 1.2.

A summary of per cent positive tissue and heart-blood cultures taken on predetermined days after exposure to the various doses of X-ray and thermal column radiation is shown in figures 3 and 4. The days selected for culturing were chosen, based on previous experience, to center around the median survival time. The data in table 1 show the median survival times of separate groups of randomized CF_1 mice following exposures to various doses of X-ray and thermal column radiation. These data show that median survival time may vary slightly between groups.

In general, the results as represented in figures 3 and 4 show that the highest incidence of positive tissue and heart-blood cultures occurred during the period surrounding the median survival time, which was the period of greatest lethality in the case of each individual dose. This, of course, would be anticipated from the previous results obtained in the mortality-bacteremia study.

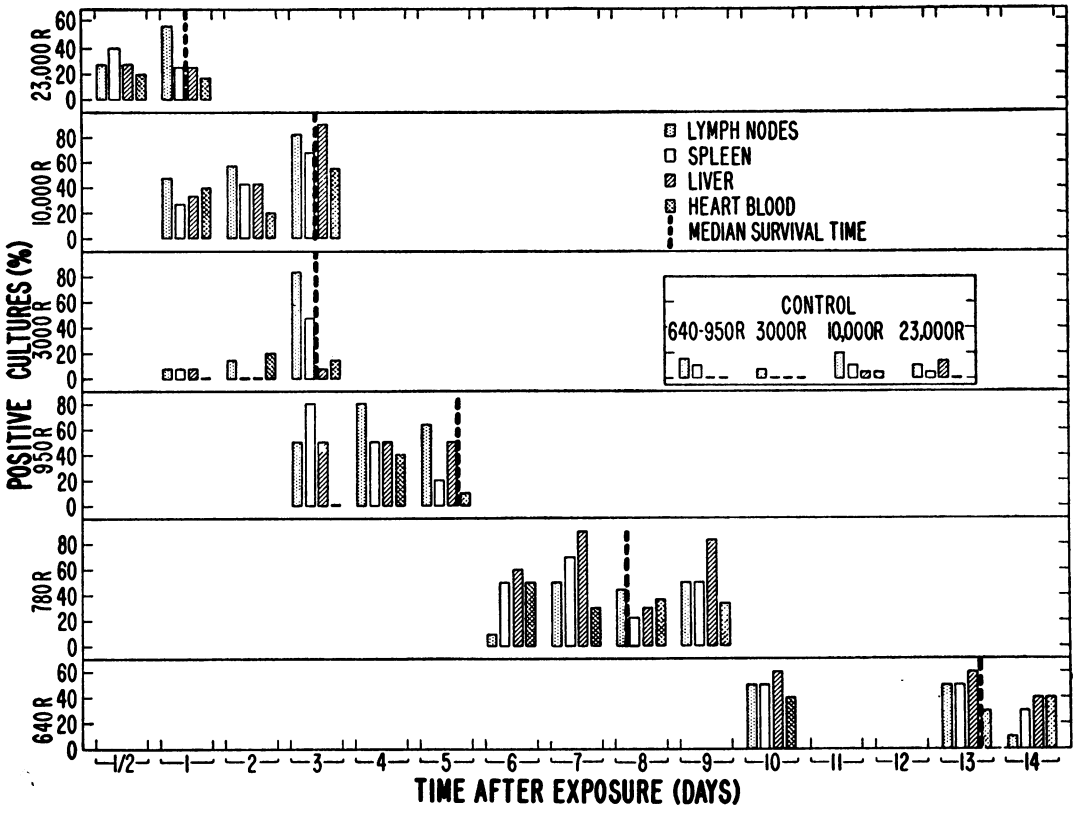

Figure 3. Time-dose relationship of positive cultures in heart blood and tissues of mice after X-ray

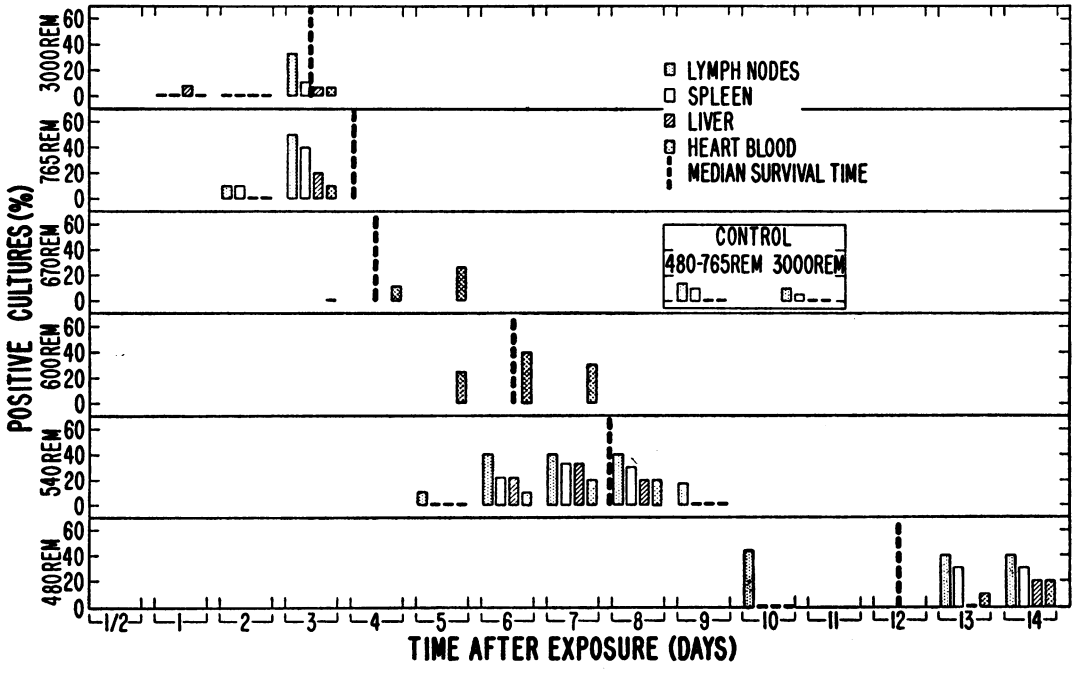

Figure 4. Time-dose relationship of positive cultures in heart blood and tissues of mice after thermal column radiation.

TABLE 1

Summary of median survival times on five groups of CF_1 Mice

Dose	Group				
	I	II	III	IV	V
X ray					
475 r		9.0			
500					
560		13.0			
600	11.0				
640			11.0	13.8	10.0
670		12.5			
700	9.3				
780			8.8	8.2	8.7
800	7.0	7.0			
900		6.1			
950			5.8	5.7	5.0
Thermal Column					
480 rem			13.5		9–10
520		14.0			
540			8.4	8.0	7–8
570		7.4			
600			7.0	6.6	5–6
616		5.5			
670			4.3	4.5	4–5
765				4.1	3–4

As a rule, the mesenteric lymph nodes were the first tissues to become positive, followed by the spleen and liver. Filtration of the bacteria into these tissues obviously can occur for several days before the generalized septicemia occurs, although with the higher doses this interval is shortened. Particularly, with the higher doses of X rays, the liver and spleen occasionally showed a higher per cent of positive cultures than the mesenteric lymph nodes.

At doses of 765 rem and 950 r, with the median survival times of 4 to 6 days, positive heart-blood cultures were not found for the first 3 days, although tissue cultures were definitely positive by the third day. Following doses of 765 rem and 950 r, there were insufficient numbers of survivors to provide heart-blood and tissue cultures at 1 to 2 days following the median survival time as planned.

Figures 3 and 4 also summarize the data showing the per cent of positive heart-bloods and tissue cultures of the mice receiving 3,000 r of X-ray and 3,000 rem of thermal column radiation respectively. The survival time of animals that received 3,000 r or rem and 10,000 r was 3 to 4 days and for those that received 23,000 r it was only 30 to 36 hours.

After 3,000 r of X-ray and 3,000 rem of thermal column radiation, heart-blood cultures were essentially negative for the 3 days before death. By the third day, however, 40 to 80 per cent of the mesenteric lymph node cultures were positive.

There was a change in the time of occurrence of bacteremia in animals exposed to the highest dose (10,000 r) in the so-called "acute intestinal radiation death" range. Tissue and heart-blood cultures became positive in some instances as early as 24 hours after exposure and continued to rise until the mice were dead at 3 to 4 days.

After 23,000 r of X rays (in the dose range where the median survival time is again dose-dependent and death is considered as caused by central nervous system damage) bacteria appeared in the tissues and in 20 per cent of the heart-bloods as early as 12 to 13 hours post-radiation.

The data showing the number of positive control cultures are presented in figures 3 and 4 as an average of all control cultures for each group of separately randomized animals.

DISCUSSION

Examination of the data showing per cent of bacteremia at 9 days and per cent lethality at 11 days following doses of X-ray and thermal column radiation in the LD_{50}^{30} range indicated that any animal that died in the first 11 days after exposure died with a bacteremia present. In comparing the results after X-ray with those following thermal column exposures, it appears that similar mechanisms are operating for the production of bacteremia and lethality at these times, even though the relative potency of pile thermal column radiations compared to 250-kvp X-rays for production of lethality and bacteremia is 1.4.

There is no doubt that infection contributed heavily to mortality, but this occurs as a result of factors involving host defense mechanisms. That infection is not the sole cause of death can be illustrated by the work with antibiotics, alone or in combinations. Although antibiotics decrease the mortality in mice at this early period, they cannot do so completely, even if the mice show negative cultures (Gonshery *et al.*, 1953). As

Lorenz and Congdon (1954) have pointed out, mice given daily injections of streptomycin after exposure to 900 r total body irradiation live longer, but they all die in 30 days, later deaths being due to hemorrhage.

On the other hand, 30-day lethality following lower doses of radiation was decreased by streptomycin (Miller *et al.*, 1950*b*) which indicates that bacteremia is a contributing cause of death at these lower doses. Also, Gordon and Scruggs (1953) radiated germ-free rats and found survival times in the LD_{50}^{30} dose range that were over 10 days longer than those of non-germ-free animals radiated with the same doses. Vogel *et al.* (1954) reported that streptomycin prolonged the survival time of mice exposed to fast neutron doses of 222 to 261 rep, but did not reduce mortality. It is also of interest that the incidence of bacteremia is not such a prominent phenomenon in dogs, rabbits or guinea pigs (Lorenz and Congdon, 1954) as it is in mice.

The data in the present study indicate that there is a direct relationship between mortality and bacteremia in animals irradiated with doses below 1,000 r for at least 11 days after exposure. If this infection can be prevented by antibiotics or other means, the chance for survival over this period increases—which indicates that bacteremia is a contributory cause of death at this time.

The per cent of positive tissue and blood cultures was greatest during the days surrounding the median survival time. The median survival time, and thus the onset of bacteremia, was extremely dose-dependent. The lower the dose, the greater was the period of time between exposures and appearance of positive tissue cultures. Following exposure to 540 rem of thermal column radiation, it was possible to show the exact days when the bloods and tissues became positive and when they again became negative after the initial wave of deaths surrounding the median survival time. This observation showed that endogenous bacteria were present in the heart blood and tissues only during the early wave of death.

As a rule, the mesenteric lymph nodes were the first tissues to show a positive culture followed by the liver and spleen, then heart blood. In the higher doses, especially after X-ray, liver and spleen cultures showed a higher per cent of positive results than did the lymph nodes. After these high doses it is possible that the nodes were so atrophic they were unable to retain any bacteria.

Many investigators (Pierce, 1948; Burrow and Tullis, 1952; Chase *et al.*, 1951) have shown that intestinal mucosa damaged by radiation is essentially repaired in 3 to 5 days. The present study showed that the incidence of infection following radiation doses in the LD_{50}^{30} range occurred at about the median survival time which, in most instances, was well over the time of maximum intestinal damage. A similar situation was demonstrated by Hammond *et al.* (1954) who showed that the susceptibility of mice to orally induced Pseudomonads after 550 r total body irradiation was greatest on the eleventh day post-irradiation.

The problem of the exact origin of endogenous infection is as yet not completely clear. If the damage to the gut is repaired in 3 to 5 days post-irradiation, when do the organisms leave the gut? If they pass through the intestinal wall during the time of maximum damage, then at lower doses there is a considerable period when defense mechanisms must be operating in the local lymphatic tissues to prevent the organisms from multiplying and spreading until a later time. If this is true, then the organisms should be detected by culturing the lymph nodes.

In a recent review Bond *et al.* (1954) advanced a hypothesis of Gordon and Miller (*personal communication*) that enteric organisms normally cross the bowel and do not multiply until the host defenses break down. From this hypothesis, one is led to believe that initial gut damage contributes little to bacteremia. Although this seems unlikely, evidence to the contrary has yet to be presented.

After exposures of 3,000 r of X-ray and 3,000 rem of thermal column radiation, there were still very few positive heart-blood cultures by the third day. However (as in the case of 950 r and 765 rem) some cultures from the mesenteric lymph nodes and spleens were positive. These results are comparable to those found by Gonshery *et al.* (1953) after 1,400 r. Osborne *et al.* (1952) have reported that in the acute intestinal radiation syndrome (after 3,000 r), bacteremia was nonexistent or occurred just before death and was of no critical importance.

No reports have been found on bacteremia following doses over 3,000 r. The results following 10,000 r show positive tissue and heart-blood

cultures present by 24 hours. The number of positive tissues continued to rise until death of all the animals on the third day postirradiation.

Following 23,000 r, positive spleen and heart-blood cultures were observed quite early and continued so until death of the animals. In general, bacteremia was more prominent, occurred earlier in the tissues, and spread rapidly in animals exposed to very high doses. The presence of bacteria in this situation is difficult to evaluate, as Bond *et al.* (1954) recently indicated. It is doubtful if there is any direct connection between the presence of bacteria in the tissues and mortality; but it does indicate, without a doubt, a rapid collapse of host resistance.

SUMMARY

The data correlating the percentage of bacteremia at 9 days with percentage lethality at 11 days, following doses of 400 to 800 r of X-ray and 480 to 616 rem of thermal column radiation, showed that all mice that died the first 11 days after exposure had a bacteremia present at the time of death. Although bacteremia occurs as a result of more complex primary factors involving host defense mechanisms, there is little doubt that the endogenous infection is a contributory cause of death in mice irradiated with median lethal doses of X- and thermal column radiation.

The results suggested that similar mechanisms were involved in the production of bacteremia and lethality at 11 days following both X- and thermal column radiation, although the relative potency of the two radiations for production of lethality and bacteremia was 1.4.

At doses of 640 to 950 r of X ray, and 480 to 765 rem of thermal column exposure, the percentage of positive tissue and blood cultures was greater during the days surrounding the median survival times. The lower the dose, the greater the period of time between exposure and appearance of positive cultures.

None of the animals in the LD_{50}^{30} range of doses showed any significant positive blood cultures for the first 3 days postirradiation. This was also true for tissues except for those animals that received 950 r and 765 rem, in which positive tissue cultures appeared on the third day and continued so until death.

In most instances, the mesenteric lymph nodes were the first tissues to become positive for endogenous bacteria. This was followed by the liver and spleen, and lastly the heart blood.

After 3,000 r or rem of X- and thermal column radiation, blood cultures were essentially negative for the 3 days before death; however, some lymph nodes and spleen cultures were positive. At 10,000 r some of the tissues and heart-blood cultures were positive by the first day and continued so until the death of the animal.

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