THE ROLE OF INTESTINAL BACTERIA IN THE RECOVERY FROM WHOLE BODY RADIATION*

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Exposure of the whole body to x-rays produces a degree of acute injury which is largely dose-dependent. When exposure reaches the lethal range, the death which follows within the next 30 days has been attributed to central nervous system damage (1), to gastrointestinal injury (2, 11), or to bone marrow failure and loss of resistance to infection, depending on the clinical manifestations of injury (3, 7, 10). The role of infection in these deaths has not been clearly defined, and the use of systemic antibiotic therapy has produced conflicting results (4, 5, 8, 9). Invading bacteria are isolated sporadically from the blood during the postirradiation period, particularly during the 2nd week after radiation, but correlation between the incidence of infection and survival rate is poor (6, 12). Since those bacteria that are found are usually of intestinal origin, a study was undertaken to evaluate the effect of suppressing the intestinal bacterial flora on the ability to recover following lethal radiation.

Materials

Groups of Wistar strain female rats, 6 to 8 weeks of age, and averaging 140 gm in weight, were exposed to a single dose of whole body x-irradiation delivered by a standard Picker therapeutic unit. Technical factors were 260 kv, 16 ma, Cu-Al filter with HVL 2.0 mm copper, and a target to skin distance of 70 cm. The dose rate in air was approximately 17 roentgens per minute.

Non-absorbable antibiotic, either neomycin sulfate or polymyxin B, was administered in solution daily by gavage to suppress the Gram-negative flora. Cultures of the stool were taken before starting such therapy, immediately before radiation, and every 4 days thereafter. The specimens for culture were incubated overnight in thioglycolate broth which was then used to inoculate Endo agar plates. The flora of untreated rats included *Escherichia coli* and other coliforms, *Proteus vulgaris, Escherichia freundii, Pseudomonas aeruginosa,* enterococci, and anaerobic organisms. In most rats, *E. coli* and the coliforms constitute the bulk of the Gram-negative aerobic bacteria which are eliminated from the stool by the antibiotic therapy. Although some animals continue to harbor a small number of *E. coli* in the gut, all were considered coliform-free if pigmented colonies were absent 24 hours after inoculation of the Endo agar plates.

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Procedures

Animals with normal or coliform-free intestinal flora were irradiated simultaneously and then housed together, sharing all environmental factors. There were no restrictions on activity or intake of Purina chow or water. Weights were recorded daily. Bacterial examination of liver, lung, spleen, and peripheral blood was performed at intervals on animals not used for determining the survival rate. Blood samples for counting white cells, and tissues for histology were taken from these same animals.

Animals with a normal intestinal bacterial flora were exposed to a single dose of whole body radiation ranging from 350 to 675 r. Mortality increased progressively with the dose of radiation delivered. The $LD_{50}30$ was determined from a probit plot of the data and shown to be 475 r (Fig. 1). Exposure to 550 r produced a uniform response: diarrhea, lassitude, huddling

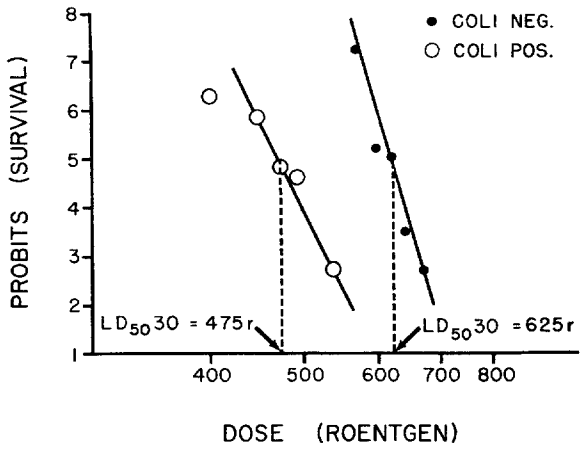

FIG. 1. Probit plot of survival after exposure to varying doses of radiation in rats bearing a normal coliform (positive) flora and in those made coliform-free (negative) with neomycin.

together, and ruffling of the coat developed on the 3rd day and were followed by bleeding from the nose within the next 3 to 5 days. The slope of the weight curve was consistently negative, and by the 10th day there was a loss of 25 to 30 per cent of the preradiation weight (Fig. 2). The mortality at the 550 r dose level was 100 per cent, with a median survival of 11.7 ± 2.85 days (Table I). This dose was the standard used in determining the effect of antibiotic therapy on survival.

Schedule of Antibiotic Therapy.—

A. Antibiotic Therapy Instituted Prior to Radiation.—This group of animals was gavaged daily with either neomycin (10 mg) or polymyxin B (6 mg) for 3 days before radiation, and daily for the 30 day observation period thereafter. Three days of treatment were usually required to render the rats coliform-free. When cultures indicated this result had not been achieved, radiation was delayed until further treatment was effective.

B. Antibiotic Therapy Started at Varying Intervals after Radiation.—In another group of rats with a normal intestinal bacterial flora, neomycin (25 mg daily) was given by gavage at varying intervals after radiation and continued throughout the 30 day observation period. Cultures of the stool were taken before radiation, after the antibiotic was started, and at 4-day intervals during the next 30 days.

C. Antibiotic Treatment Started Immediately after Radiation and Continued for Varying Periods.—In a third experiment, four groups of 30 animals, each with a normal bacterial flora, were radiated. Immediately thereafter they were given neomycin (25 mg) by gavage. This treatment was given once daily thereafter for 3, 7, 14, or 21 days. Stool cultures were prepared as in the preceding groups.

RESULTS

A. Antibiotic Instituted Prior to Radiation.—Table I shows the mortality associated with radiation dose levels varying from 350 to 675 r. Coliform-free

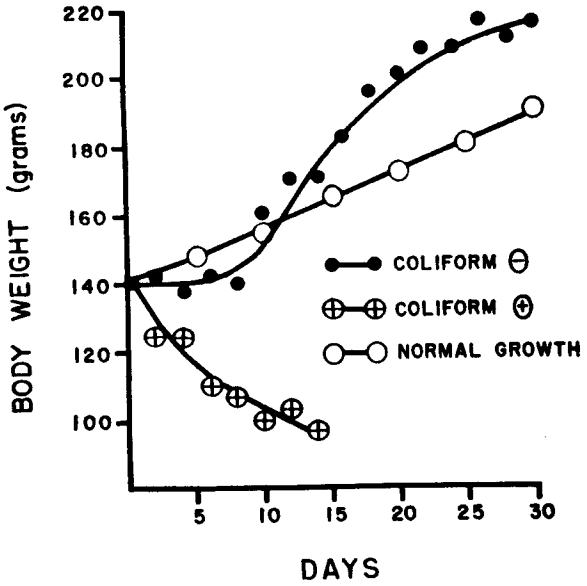

FIG. 2. The weight curves of coliform-free ⊖ and coliform-bearing ⊕ rats after exposure to 550 r whole body radiation are compared to the expected rate in normals.

rats developed diarrhea at the 625 r level and bleeding from the nose at the 650 and 675 r exposures. The $LD_{50}30$ for rats with a normal flora was 475 r; for coliform-free rats it was 625 r, a dose reduction factor of 1.32 (Fig. 1).

Eighty rats coliform-"free" before exposure to 550 r and maintained coliform-free thereafter for 30 days survived this radiation dose without mortality. They exhibited no diarrhea, bleeding from the nose, or other signs of illness and remained active, eating and drinking normally. After a 10 per cent loss of body weight during the first 5 days following irradiation, weight gain was again observed and approximated that of normal, non-irradiated rats in our colony (Fig. 2).

At the end of the 1st week after radiation, blood and tissue cultures from animals with a normal stool flora showed organisms of intestinal origin in 25

per cent of the specimens. Corresponding data from coliform-free rats rarely yielded positive cultures. Since the coliform-bearing rats died whether the blood and tissue cultures were positive or negative, and since the coliform-free animals lived, it would appear that survival related not only to preventing bacterial invasion, but also to the preservation of other defense mechanisms.

White blood cell counts were performed at frequent intervals in both groups (Fig. 3). Leukopenia of equal degree occurred in both groups during the 1st week following radiation. Thereafter, as the coliform-free rats gained weight and showed a more normal growth curve, the white cell count slowly returned toward normal. In the case of animals with a normal flora, the decline in both weight and white cell count persisted until death.

TABLE I

Relation of Radiation Dose to the 30 Day Mortality

Air dose	No.	Coliform-bearing, mortality	Median survival	No.	Coliform-"free," mortality	Median survival
r		*per cent*	*days*		*per cent*	*days*
350	30	10	17	—	—	—
450	30	20	13	—	—	—
475	35	46	14	—	—	—
500	31	55	13	—	—	—
550	114	100	12	80	0	—
575	—	—	—	30	0	—
600	—	—	—	30	20	15
625	—	—	—	30	47	13
650	—	—	—	30	93	10
675	—	—	—	30	100	10

Histologic examination of the tissues taken at frequent intervals from 15 minutes to 3 weeks following exposure showed no difference between groups during the 1st week after radiation. In the group receiving neomycin, a gradual regeneration of bone marrow and splenic pulp could be seen and, at the end of 3 weeks, these tissues appeared to be normal. Animals bearing coliform organisms showed little if any regenerative activity in these areas.

B. Antibiotic Treatment Started at Varying Intervals after Radiation.—Of those rats started on neomycin after radiation exposure, all survived when this treatment was started within the 1st hour after radiation. Sixty-seven per cent survived when the treatment was started 2 hours afterward, 60 per cent if the delay was 4 hours, 50 per cent if the delay was 8 hours, and 6 per cent if the delay was 30 hours (Fig. 4).

In every instance in which neomycin failed to render the stool coliform-free,

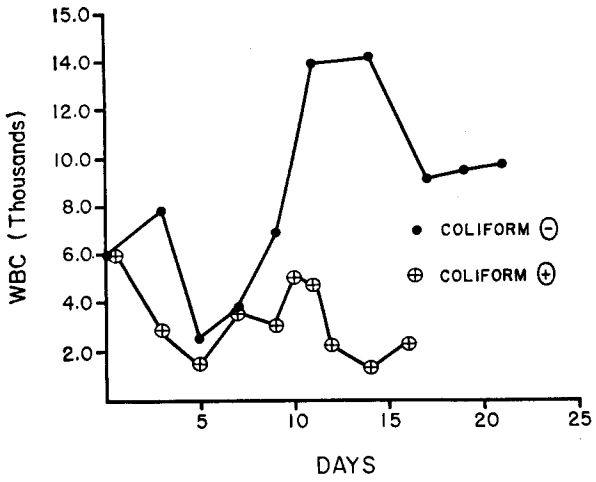

FIG. 3. Total white cell counts in peripheral blood of rats exposed to 550 r whole body radiation with ⊖ and without ⊕ change in the coliform flora of the intestinal tract.

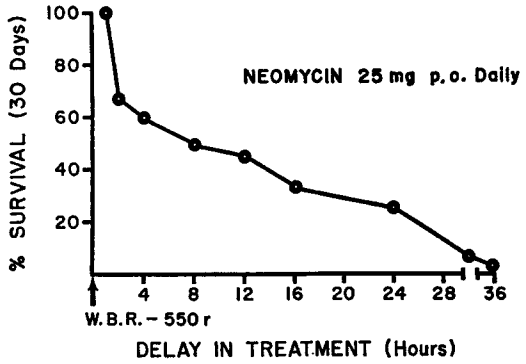

FIG. 4. The relationship of survival to delay in instituting neomycin treatment after exposure to 550 r whole body radiation (W. B. R.). Each point represents 30 animals. p.o., *per os.*

death occurred regardless of when the antibiotic was started, and whether before or after radiation.

C. Antibiotic Therapy of Varying Duration Started Immediately after Radiation.—When neomycin, started immediately after radiation, was continued for varying periods of time, the survival figures changed accordingly. The 30 day survival was 20 per cent for those treated for 3 days, 50 per cent for those

treated for 7 days, 70 per cent for those treated for 2 weeks, and 100 per cent for those treated for 3 weeks (Table II, Fig. 5).

TABLE II

The Effect of the Duration of Neomycin Treatment on Survival

Duration of neomycin treatment*	No. of animals	Survival	Median day of death
days		*per cent*	
3	30	20	16
wks.			
1	30	50	14
2	30	70	22
3	30	100	—

* 25 mg daily by gavage after radiation exposure.

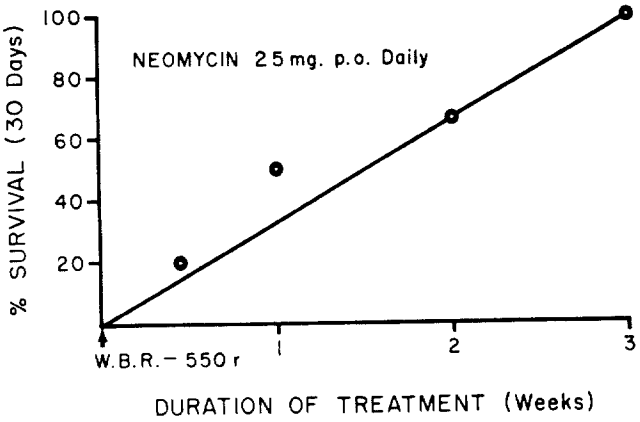

FIG. 5. Survival of rats after exposure to 550 r whole body radiation (W. B. R.) as a function of duration of neomycin treatment. p.o., *per os*.

DISCUSSION

The data presented demonstrate that the death of rats exposed as described to whole body radiation is caused by the activity of Gram-negative bacteria in the gut. This conclusion is based on the following observations:

(*a*) Neomycin and polymyxin, which are poorly absorbed from the gut, when given daily by gavage prior to radiation until cultures of the intestinal flora were free of coliform bacteria and continued for 21 days after radiation, were uniformly successful in preventing death at levels of radiation exposure sufficient to kill all coliform-bearing animals.

(*b*) In every instance in which the antibiotic failed to eliminate the coliform

organisms, no protection occurred. The survival rate was not related to the effect of the antibiotic on the other flora.

(*c*) There was a direct relationship between the duration of the antibiotic therapy and the degree of protection achieved. Survival was maximal when the drug was continued for at least 21 days after radiation.

(*d*) Bacteremia is frequent in the unprotected, lethally radiated animal, but systemic antibiotic therapy does not significantly improve the survival rate even when it prevents bacteremia. On the other hand, poorly absorbed neomycin and polymyxin given orally not only prevent bacteremia, but regularly prevent death.

Since prevention of bacteremia alone does not prevent death, and since survival hinges on the elimination of the intraintestinal Gram-negative bacteria, an essential constituent in the lethal process must be the effect of bacterial activity within the gut. This suggests that the products of such bacterial activity, when absorbed, are not detoxified because of radiation injury to the defense mechanism, and thus cause additional damage to the animal.

Although the nature of this additional damage is obscure, it is clear that it changes a potentially reversible to an irreversible state. In an effort to discover which vital system fails, it may be helpful to describe current studies in which a known product of bacterial growth affects the postradiation state. When *E. coli* endotoxin is given by gavage to the coliform-free, radiated rat, it kills regularly if administered on the 1st or 2nd day after radiation. It is also lethal if given at anytime during the first 10 to 14 days after radiation, but the later in the post-radiation period it is given, the lower the mortality rate. It should be stressed that in each instance, the time interval between administration of the endotoxin and death remains constant; *i.e.*, 8 to 12 days, a period comparable to the survival time of the radiated, coliform-bearing control animals. After 2 weeks, the animals are no longer sensitive to challenge by endotoxin by this route. These results are a striking counterpart to those described above from experiments in which neomycin by gavage was given after, rather than before radiation. When the antibiotic was given within the first few hours after radiation, the greatest protection was achieved. The longer it was withheld, the less it protected, in spite of rendering the animal coliform-free. It appears therefore, that during the immediate postradiation period, products of bacterial activity are being absorbed from the gut, are not detoxified, and are free to inflict lethal damage. From our data, it appears that the system concerned with detoxifying these products recovers normal potency if protected for 2 to 3 weeks.

In the light of these findings, it is not surprising that previous workers have not been able to demonstrate consistent results when antibiotics were used either before, or as treatment after, radiation injury (4–6, 8–10). In these studies, agents were given parenterally to control bacteremia and infection. The limited success of such therapy is more clearly explained in the light of our

data showing that absorbed products of intestinal bacterial activity, rather than invasion and infection, are responsible for the lethal outcome.

Hence, the conclusion is drawn that the vulnerability of the unprotected, radiated animal lies in its reduced capacity to detoxify absorbed products of intestinal bacterial growth, a disorder which is reversible if appropriate protection is provided.

SUMMARY AND CONCLUSION

Non-absorbable antibiotics, neomycin sulfate or polymyxin B, prevent death from an otherwise lethal dose of whole body radiation by suppressing the activity of the Gram-negative bacterial flora of the intestinal tract.

The protective effect of such suppression has been evaluated over a range of radiation exposure from 325 to 675 r. Coliform-free animals uniformly survive exposure to 550 r, a dose which is regularly lethal for coliform bearing animals.

When antibiotic treatment is begun within 1 hour after 550 r whole body radiation, survival is the rule. Delay in starting treatment is critical, for the longer the delay, the higher the mortality, even though the stool cultures meanwhile become coliform-free.

When antibiotic is started prior to or immediately after radiation exposure, it must be continued for at least 3 weeks if maximum effectiveness is to be obtained. The shorter the postradiation period of treatment, the greater the mortality. This suggests that the defense systems involved require protection for at least 3 weeks in order to permit return of maximal function.

The non-absorbable intestinal antibiotics are effective only when cultural data demonstrate successful elimination of the coliform flora in the gut.

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