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THE GASTROINTESTINAL MICROFLORA OF IRRADIATED MICE
II. EFFECT OF ORAL ANTIBIOTIC ADMINISTRATION ON THE
COLONIC FLORA AND SURVIVAL OF ADULT MICE‡

Postirradiation survival has been related to the suppression of intestinal coliform bacteria.¹ Germ-free animals routinely exhibit less diarrhea, melena, and weight loss and survive radiation doses that are lethal for conventional animals.²⁻⁴ Oral antibiotics, especially those which reach high concentration in the lumen of the gut, prevent bacteremia and in some instances have been reported to prolong life or prevent death.^{1,5} Presumably this is accomplished by limiting proliferation of susceptible intestinal organisms. In this paper, we report the effect of oral antibiotics on colonic bacteria in lethally irradiated adult mice with special reference to mean survival time and survival rates.

MATERIALS AND METHODS

One hundred adult 90 day old mice of the ICR strain (Charles River Breeding Labs, Wilmington, Mass.) were studied. Forty were used for *in situ* bacterial culture of colonic feces. Of these animals, ten were untreated and served as controls, ten were given 1200 roentgens of whole-body X-irradiation in the manner previously described,⁶ ten were given antibiotics and irradiated, and ten were given antibiotics only.

The mice were housed ten per cage in plastic wiretop cages and were fed a diet of unrestricted quantities of tap water and Purina Laboratory Chow. For the antibiotic-fed animals, neomycin sulfate (Mycifradin sulfate, sterile powder 70% as neomycin base, Upjohn, Kalamazoo, Michigan) at a concentration of 1 gm/100 ml. and Potassium Penicillin G (E. R. Squibb and Sons, New York) at a concentration of 0.24 gm(400,000 units)/100 ml. were dissolved in the drinking water four days prior to the day of irradiation and were maintained throughout the experiment. At three day intervals, these solutions were changed to maintain antibiotic activity. Consuming an estimated average of 5 ml. of water per day, each mouse received 50 mg. of neomycin and 20,000 units of penicillin per day.

Two mice from each group were sacrificed 1, 3, 5, 7, and 10 days after the day of irradiation. With the previously described technique,⁶ a segment of mid-colon was re-

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moved for histological study, and 0.1 gm. of colonic feces was serially diluted and cultured. Bacteriologic techniques employed were described previously.⁶ In this study, greater attention was paid to identification of organisms. Gram stains were made from each colony type observed on the selective C, E, G, and Enterococcal media. In addition to their characteristic microscopic appearance, *Clostridia* and *Bacteroides species* were identified by growth on anaerobic blood agar subculture. Lactobacilli appeared as large, pleomorphic, or thick short Gram positive rods on smears from the G medium. As these colonies aged, they lost their ability to retain the crystal violet stain; they were seen as Gram positive, mottled, or Gram negative rods. In these instances, characteristic morphology was observed on fresh smears from blood agar subcultures. In the antibiotic fed animals, Gram stained smears revealed both Gram positive and Gram negative yeast forms and hyphae; no attempt was made to identify fungi more precisely.

Histological preparations and bacterial counts were subjected to the same analysis described previously.⁶

Sixty of the mice were employed in a survival study. Ten were untreated, 20 were irradiated, 20 were irradiated and given antibiotics, and 10 were given antibiotics only. The mice were housed 10 per cage, fed, and given drugs as described above. At eight-hour intervals for 30 days, the cages were checked and dead animals were removed. The time of death for each mouse was recorded as well as the daily count of living and dead animals.

RESULTS

Control mice (see Table 1). In the feces of unirradiated adult mice,

TABLE 1. MEAN MICROBIAL COUNTS IN THE COLONIC FECES OF NORMALLY FED MICE*

Organism	Time of sacrifice					Mean
	1 day	3 days	5 days	7 days	10 days	
Total aerobes	5.50	5.95	6.24	6.15	7.08	6.18
Total anaerobes	8.02	6.50	8.42	7.57	8.30	7.76
Bacteroides	7.06	7.07	7.48	6.80	2.00	6.08
Coliforms	5.00	4.87	5.92	4.90	5.45	5.23
Lactobacilli	8.31	7.62	8.08	8.15	8.08	8.05
Aerobic fungi†	1.00**	1.00	1.00	1.00	1.00	1.00
Anaerobic fungi‡	1.00	1.00	1.00	1.00	1.00	1.00

NOTE: The explanations below apply to this and subsequent tables.

* All numbers represent the Log_{10} organisms/gm. feces. Although the logarithm of the bacterial count was computed for statistical purposes, for clarity, exponential numbers are used in the text. For example, 5.00 in the above table will be discussed as 10^5 in the text. Numbers represent average of bacterial counts obtained from feces of two animals sacrificed at specified time.

** 1.00 indicates no recovery of organisms. To avoid the meaningless mathematical expression $\text{log}_{10} 0$, a small constant (10^1) was introduced when a culture plate showed no growth. Accordingly, the logarithm of this constant is 1.00.

† Isolated from the A medium incubated aerobically.

‡ Isolated from the A medium incubated anaerobically.

TABLE 2. MEAN MICROBIAL COUNTS IN THE COLONIC FECES OF IRRADIATED, NORMALLY FED ADULT MICE*

Organism	Time of sacrifice					Mean
	1 day	3 days	5 days	7 days	10 days	
Total aerobes	4.00	6.32	7.45	7.05	10.05	6.97
Total anaerobes	7.44	8.75	8.50	9.02	10.14	8.78
Bacteroides	7.44	8.78	8.26	7.42	8.96	8.27
Coliforms	3.15	5.98	7.48	6.50	10.23	6.67
Lactobacilli	8.17	8.69	8.47	8.76	9.48	8.72
Aerobic fungi†	1.00**	1.00	1.00	1.00	1.00	1.00
Anaerobic fungi‡	1.00	1.00	1.00	1.00	1.00	1.00

See Table 1 for footnote explanations.

anaerobic bacteria (10^7 - 10^8 /gm.) outnumbered aerobes (10^6 /gm.). The most numerous species of organisms were the anaerobic lactobacilli (10^8 /gm.). *Bacteroides* were present in moderate (10^{6-7} /gm.) numbers and coliforms in comparatively small numbers (10^5 /gm.).

Irradiated mice (see Table 2). In the feces of irradiated adult mice there was a significant ($p < .01$) increase of coliforms (10^{10} /gm. versus 10^5 /gm. for controls) and total aerobes (10^{10} /gm. versus 10^7 /gm. for controls) on the tenth day after irradiation. This agreed with the previously reported findings in weanling mice.⁶ In addition, there was an equally significant ($p < .01$), but less impressive increase in total anaerobes on the seventh (10^9 /gm. versus $10^{7.5}$ /gm. for controls) and tenth (10^{10} /gm. versus 10^8 /gm. for controls) days after irradiation; this finding was not observed in weanling mice.⁶

Antibiotic-fed mice (see Tables 3 and 4). In antibiotic-fed animals, no bacteria were recovered. Anaerobic and aerobic fungi were the only organ-

TABLE 3. MEAN MICROBIAL COUNTS IN THE COLONIC FECES OF IRRADIATED, ANTIBIOTIC-FED ADULT MICE*

Organism	Time of sacrifice					Mean
	1 day	3 days	5 days	7 days	10 days	
Total aerobes	1.00**	1.00	1.00	1.00	1.00	1.00
Total anaerobes	1.00	1.00	1.00	1.00	1.00	1.00
Bacteroides	1.00	1.00	1.00	1.00	1.00	1.00
Coliforms	1.00	1.00	1.00	1.00	1.00	1.00
Lactobacilli	1.00	1.00	1.00	1.00	1.00	1.00
Aerobic fungi†	1.00	6.00	7.52	7.45	5.68	5.53
Anaerobic fungi‡	1.00	5.87	9.00	7.84	5.75	5.89

See Table 1 for footnote explanations.

TABLE 4. MEAN MICROBIAL COUNTS IN THE COLONIC FECES OF UNIRRADIATED, ANTIBIOTIC-FED ADULT MICE*

Organism	Time of sacrifice					
	1 day	3 days	5 days	7 days	10 days	Mean
Total aerobes	1.00**	1.00	1.00	1.00	1.00	1.00
Total anaerobes	1.00	1.00	1.00	1.00	1.00	1.00
Bacteroides	1.00	1.00	1.00	1.00	1.00	1.00
Coliforms	1.00	1.00	1.00	1.00	1.00	1.00
Lactobacilli	1.00	1.00	1.00	1.00	1.00	1.00
Aerobic fungi†	1.00	6.31	7.35	6.00	7.75	5.68
Anaerobic fungi‡	1.00	5.91	7.33	6.25	7.54	5.61

See Table 1 for footnote explanations.

isms recovered on all media, and no significant increases were found in irradiated animals. No fungi were recovered from animals that had not received antibiotics.

Histologic observations. On microscopic examination of the colon, the mucosal surface appeared unbroken for all animals. Radiation changes of

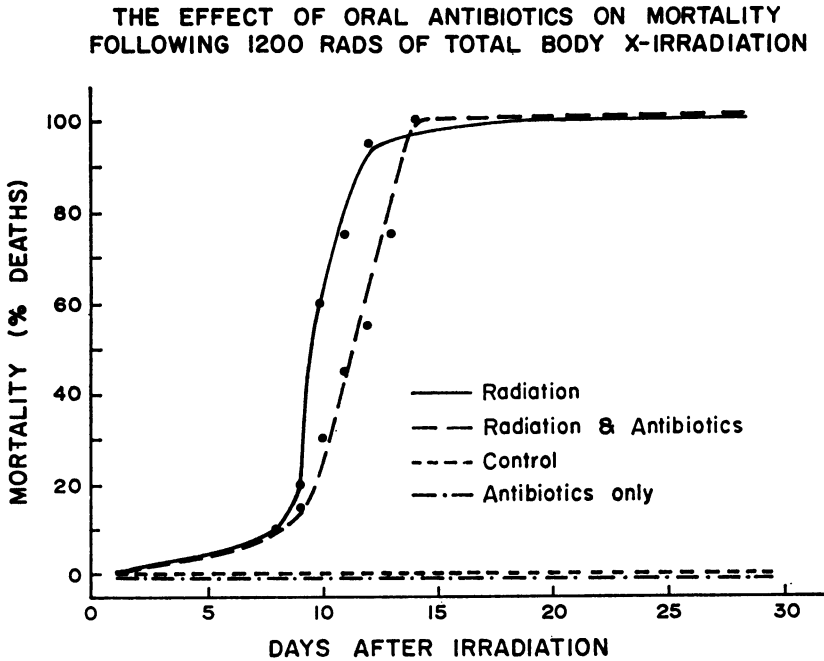


FIG. 1.

the colonic wall were similar to those described previously⁶ with no major cytological differences for mice which had also received antibiotics. Fusiform bacteria, small Gram negative bacilli, and Gram positive rods and cocci were found in the luminal debris of all mice that had not been given antibiotics. In antibiotic-fed mice, a few bacteria were seen in the lumen of the colon even though none was recovered by culture techniques; Gram positive and Gram negative fungi were also seen. No animal demonstrated fungal or bacterial invasion of the colon wall.

Survival study. Irradiated mice, whether fed plain water or antibiotics, began to die on the eighth postirradiation day (Fig. 1). The mean survival time for irradiated mice was 10.7 days and for antibiotic-fed irradiated mice, 11.7 days. By the Kolmogorov-Smernon two-sample test, antibiotics did not produce a significant ($p < \text{or equal to } .05$) prolongation of life.⁷ All irradiated animals died. Among unirradiated animals, there were no deaths for 30 days, and the study was terminated at that time.

DISCUSSION

The mice employed in this study appeared to be more resistant to x-radiation than those animals described by earlier investigators. Previously 1200 roentgens was followed by death in 4-5 days from the acute intestinal radiation syndrome.^{2,8-10} Our mice survived, on the average, until 11 days after irradiation and demonstrated few, if any, gastrointestinal symptoms. When death occurred in animals fed plain tap water, it was associated with the proliferation of fecal bacteria at a time and in a manner consistent with the post-irradiation bone marrow syndrome. This radio resistance is explained probably by our technique of radiation and the differential absorption of radiation by different tissues of the mouse. Although we delivered an average of 1200 roentgens of total body radiation, the estimated absorption was 800 rads in the gut and 1500 rads in the bone marrow and lymphoid organs.

These results of bacteriologic and histologic studies on irradiated adult mice colon agree with those obtained in weanling mice. Although age affects the flora it appears to have no effect on the sequence of events following this dose of irradiation, nor irradiation plus antibiotic administration.

In irradiated animals, earlier studies of antibiotic therapy produced variable and frequently conflicting results. Gonschery, Marston, and Smith inoculated irradiated mice with a variety of exogenous bacteria and tested the therapeutic effect of different antibiotic agents.^{5,11,12} The incidence of bacteremia was reduced; in some cases, mean survival time was prolonged but without any increase in the ultimate percentage of animals surviving. The elimination of recoverable intestinal bacteria with neomycin and peni-

cillin has been described in another study from this laboratory.¹⁸ Our elimination of recoverable fecal bacteria explains the prevention of bacteremia; however, it does not explain the nearly identical mortality curves (Fig. 1) of normally fed and antibiotic fed irradiated mice.

One important determinant of antibiotic effect is the dose of radiation. Antibiotics have a greater protective effect after moderate exposure to radiation (700 rads); after higher doses, mice die before septicemia takes place.¹⁴ Thus, antibiotic therapy is relatively useless in the acute intestinal radiation syndrome compared to its protective effect during the bone marrow syndrome.^{9,10} In the post-irradiation bone marrow syndrome, the host animal is immunologically incompetent and unable to contain or regulate any of the micro-organisms which it harbors. It is susceptible to small numbers of bacteria or to relatively avirulent organisms.^{15,16} Our studies indicate that the complete elimination of recoverable bacteria from the colon and the substitution of non-invasive fungi does not necessarily prolong life or reduce mortality after a bone-marrow syndrome dose. The experiments indicate that it is not the radiation-induced changes of the gastrointestinal flora but rather the amount of damage to the host animal's protoplasm that ultimately determines survival.

SUMMARY

Adult white ICR mice were irradiated with 1200 r and given oral neomycin and penicillin. Control groups were also studied. Segments of colon were obtained 1, 3, 5, 7, and 10 days after the day of irradiation and were studied by light microscopy; at the same times, specimens of colonic feces were obtained for quantitative and qualitative aerobic and anaerobic bacterial cultures. Survivors and nonsurvivors from each group were counted for 30 days.

Irradiated adult mice demonstrated a significant increase of total aerobic, total anaerobic, and coliform bacteria. The antibiotic regimen eliminated all recoverable bacteria from the fecal samples and permitted the subsequent proliferation of aerobic and anaerobic fungi. The suppression of bacteria in the colonic feces was not associated with prolongation of life nor reduction of mortality in these irradiated mice.

Accordingly, we have concluded that tissue damage is more important than bacterial proliferation for the ultimate survival of the host animal.

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