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THE GASTROINTESTINAL MICROFLORA OF IRRADIATED MICE
I. RELATIONSHIP OF MUCOSA AND MICROFLORA IN WEANLING MICE‡

Little attention has been paid to *in situ* bacterial culture of the gut following irradiation.[1,2] The death of irradiated animals does not occur when intestinal damage is greatest but seven to ten days later when the epithelium has been repaired.[3-7] However, Gram negative bacteremia has been observed at the time of death[8-13] and has been attributed to direct bacterial invasion of the intestinal wall.[8,10,11,14,15] Earlier investigators studied the induction of bacteremia with artificially infected irradiated animals.[4-7] An intestinal portal of entry for Gram negative rods had been inferred from such studies but never clearly demonstrated. In this paper, we report the changes of intestinal bacterial populations occurring in the ten day period following lethal irradiation and correlate them with histopathologic observations.

METHODS AND MATERIALS

Irradiation. Twenty-eight white male weanling mice (three-week old ICR strain, Charles River Breeding Labs., Wilmington, Mass.) were employed in this experiment. Fourteen were given 1,200 roentgens (114 r/minute for 10.53 minutes; 250 kv., 1/2 aluminum-1/2 copper filter, HVL = 1.65 mm. copper, in a 10 section circular mouse container mounted on a turntable with the target 54 cm. from the cone) of whole body x-radiation.

Throughout the course of the experiment, the mice were housed ten per cage in plastic wiretop cages with sawdust litter. The diet consisted of unrestricted quantities of tap water and Purina Laboratory Chow (Purina Labs, St. Louis, Mo.). No attempts were made to prevent fighting, cannibalism, or coprophagy.

Histology. At one hour and 1, 2, 3, 5, 7, and 10 days after irradiation two mice from each group were killed by a quick blow to the head. The fur was sprayed with a germicidal aerosol (Staphene Spray, Vestal Laboratories, St. Louis), the abdomen was entered by sterile dissection, and all specimens for bacteriologic assay were handled with aseptic technique. The entire stomach and 2 cm. segments of mid-jejunum and mid-colon were excised and placed individually in sterile petri dishes.

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For histologic purposes, a specimen of each organ was mounted on a slice of liver, quick-frozen in liquid nitrogen, and stored in a plastic bag at −70°C; at a later time, four micra cryostat sections were stained especially for bacteria,[16] with hematoxylin and eosin, and studied by light microscopy.

Bacteriology. From the remainder of each organ, 0.1 gm. or ml. of lumenal contents was removed, serially diluted, and cultured within 15-30 minutes. At the same time, approximately 0.1 gm. of each organ was sliced longitudinally, placed in a tube of sterile norite A water, and shaken vigorously for five minutes. This amount of washing and shaking was sufficient to lift away the mucus overlying the intestine without damaging the tissue itself. The washed specimen was removed, weighed to three decimal places, homogenized by a motorized pestle in a grinding tube (TRI-R STIR-R, Model S63C, TRI-R Instrument Co.), serially diluted, and cultured. The wash water was also serially diluted and cultured. The qualitative and quantitative bacteriological techniques employed in this experiment have been described in detail previously.[17-19] Serial dilutions were made starting with 0.1 ml. or 0.1 gm. of each specimen in 9.9 ml. of norite A and streaked with a measured loop (0.01 ml.) on five selective media. Medium A was employed for total counts, and two plates were streaked; one was incubated aerobically and the other anaerobically. Medium C was used for the isolation of *Bacteroides species* and *Clostridia species* and was incubated anaerobically. Medium G was used for lactobacilli and Enterococcus M medium for Gram positive cocci; each of these was incubated anaerobically. Medium E was used to isolate coliform organisms and was incubated aerobically. Incubation was performed in the manner previously described.[19] Aerobic plates were incubated for 24 hours and anaerobic plates for 48 hours.

Colony counts for total aerobic and total anaerobic growth were made from medium A and were correlated with counts from the other, more selective media. Bacterial identification for this experiment was accomplished primarily by comparing colony morphology on each medium with that of known laboratory specimens. Gram stains, blood agar subcultures, and sugar slants were employed on a spot-check basis to assure the accuracy of our identifications.

Statistics. Bacterial counts (bacteria/gm. of feces or tissue homogenate, or bacteria/ml. of liquid contents or wash water) were determined for each specimen and converted to a logarithmic equivalent. The logarithmic number of each bacterial count was key-punched onto a separate computer data card along with information such as the presence or absence of irradiation, the time of sacrifice, and the type of specimen (e.g. stomach contents, jejunal wash, colon homogenate, etc.). When no bacteria were found on a given plate, it was necessary to record a small constant (10^1) to avoid the imaginary expression log 0.

A programmed factorial analysis of variance was performed on the IBM 7094 computer. Mean bacterial counts for control and irradiated mice were computed each time two animals were sacrificed. The marginal mean bacterial count for each group of mice was obtained by averaging all the daily bacterial counts for each experimental group. From the behavior of these parameters, significance levels were calculated.

RESULTS

Microflora of control weanling mice. In unirradiated weanling mice, the stomach (see Table 1) contained large numbers (10^8/ml.) of anaerobic lactobacilli in close relationship to the gastric wall; these organisms were

TABLE 1. MARGINAL MEAN BACTERIAL COUNTS IN THE GASTROINTESTINAL TRACT OF WEANLING MICE*

Specimen	*Total aerobes*		*Total anaerobes*		*Bacteroides*		*Coliforms*		*Lacto-bacilli*	
	I	*C*	*I*	*C*	*I*	*C*	*I*	*C*	*I*	*C*
Stomach contents	4.77	5.61	8.01	8.45	2.54	3.49	1.57	1.57	8.35	8.73
Stomach wash	3.80	4.37	7.18	7.30	2.25	2.41	1.00**	1.00	7.60	7.64
Stomach homogenate	3.20	4.24	7.09	7.41	1.77	3.59	1.00	1.00	7.31	8.30
Jejunum contents	5.46	4.10	7.70	6.77	1.27	1.26	1.90	1.51	6.86	7.00
Jejunum wash	3.02	1.00	5.33	5.71	1.00	1.00	1.26	1.67	6.07	6.07
Jejunum homogenate	4.27	2.82	6.13	6.04	1.00	2.14	1.00	1.00	5.81	6.29
Colon contents	7.80	6.54	8.42	8.44	5.63	4.93	3.30	2.84	8.74	8.72
Colon wash	4.55	2.29	5.97	6.08	1.49	2.76	1.84	1.00	5.73	6.13
Colon homogenate	4.68	1.95	6.34	6.19	1.86	2.14	1.74	1.47	6.48	6.07

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

I = irradiated animals.

C = control animals.

* Numbers represent Log_{10} bacteria/gm. of specimen. Although the logarithm of the bacterial count is presented here, for clarity exponential numbers are used in the text. For example, 7.00 in the above table will be discussed as 10^7 in the text.

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

recovered in large numbers (10^7-10^8) from gastric washes and homogenates as well as from gastric contents. In contrast, the jejunum contained large numbers (10^7/ml.) of anaerobic lactobacilli in the lumenal contents, but only a moderate number (10^5-10^6) in the washes and homogenates. *Bacteroides species* and coliforms were recovered only in small numbers from the stomach or jejunum.

The colon contained a heterogenous bacterial population (see Tables 2-4). Anaerobic lactobacilli predominated and were recovered from the stool (10^8-10^9/gm.), wash (10^6-10^7/ml.) and homogenate (10^5-10^6/gm.). The colonic feces contained 10^8/gm. aerobes early in the experiment but only 10^5/gm. near the end. A similar decrease was noted in the colon wash, and practically no aerobes were recovered from the homogenate at any time. Coliforms did not appear in the stool until the fifth sampling period, when they were recovered at 10^7/gm.; they decreased to 10^5/gm. by the end of the experiment. Except for one occasion, no coliforms were recovered from colon washes or homogenates. The number of *Bacteroides* in colonic feces was moderate (10^6-10^7/gm.) but highly variable. They were recovered at 10^3-10^5/ml. in the colon wash and only on sporadic occasions in the colon

TABLE 2. MEAN BACTERIAL COUNTS IN THE COLON CONTENTS OF WEANLING MICE AFTER IRRADIATION*

Time of sacrifice	Total aerobes		Total anaerobes		Bacteroides		Coliforms		Lactobacilli	
	I	C	I	C	I	C	I	C	I	C
1 hour	6.45	7.00	7.22	9.15	7.13	6.92	1.00**	1.00	7.00	8.74
1 day	7.66	8.38	8.63	8.85	6.34	6.35	1.00	1.00	8.66	8.69
2 days	7.89	7.00	8.32	8.00	1.00	1.00	1.00	1.00	8.76	8.78
3 days	8.50	7.00	8.66	8.00	7.92	1.00	5.81	1.00	8.68	8.78
5 days	7.03	5.53	8.02	8.34	5.31	6.86	5.53	7.00	8.69	8.30
7 days	9.07	5.30	8.69	8.28	3.60	4.30	7.75	4.00	9.54	9.00
10 days	8.00	5.60	9.38	8.48	8.08	7.90	1.00	4.90	9.82	8.78
Marginal mean	7.80	6.54	8.42	8.44	5.63	4.93	3.30	2.84	8.74	8.72

See Table 1 for footnote explanations.

homogenate. At no time were *Clostridia* recovered on the C medium nor streptococci on the G or Enterococcus M media.

Microflora following irradiation. In feces (see Table 2) of irradiated weanling mice, there was a significant ($p. < .01$) increase in total aerobes on the fifth (10^7/gm. versus 10^5/gm. for controls), seventh (10^9/gm. versus 10^5/gm. for controls), and tenth (10^8/gm. versus 10^6/gm. for controls) days after irradiation. In addition, coliforms were significantly ($p < .01$) increased, especially on the seventh (10^8/gm. versus 10^4/gm. for controls) day after irradiation; however, this increase did not persist to the end of the experiment. Bacterial counts for the colonic wash water (see

TABLE 3. MEAN BACTERIAL COUNTS IN THE COLONIC WASH OF WEANLING MICE AFTER IRRADIATION*

Time of sacrifice	Total aerobes		Total anaerobes		Bacteroides		Coliforms		Lactobacilli	
	I	C	I	C	I	C	I	C	I	C
1 hour	3.50	1.00	7.32	4.65	4.41	1.00**	1.00	1.00	7.03	6.00
1 day	5.33	1.00	5.85	6.30	1.00	2.59	1.00	1.00	6.48	6.18
2 days	2.96	5.51	5.02	7.70	1.00	1.00	1.00	1.00	5.85	6.70
3 days	6.09	5.51	5.81	7.70	1.00	1.00	2.59	1.00	3.95	6.70
5 days	2.70	1.00	5.24	5.23	1.00	4.93	1.00	1.00	6.09	6.00
7 days	7.24	1.00	6.85	5.70	1.00	3.70	5.63	1.00	5.55	6.40
10 days	4.00	1.00	5.70	5.26	1.00	5.11	1.00	1.00	5.18	4.93
Marginal mean	4.55	2.29	5.97	6.08	1.49	2.76	1.84	1.00	5.73	6.13

See Table 1 for footnote explanations.

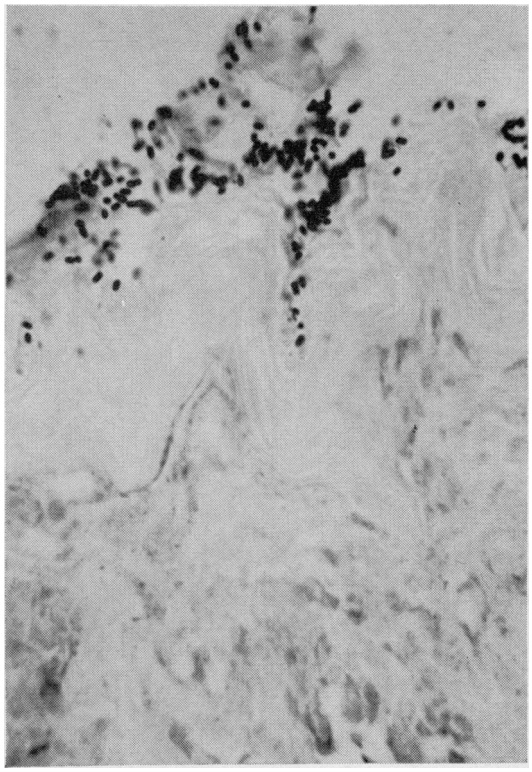

FIG. 1. High power photomicrograph demonstrating Gram positive rods adhering to the mucus coating of the gastric mucosa. (× 400).

TABLE 4. MEAN BACTERIAL COUNTS IN THE COLON HOMOGENATE OF WEANLING MICE AFTER IRRADIATION*

Time of sacrifice	*Total aerobes*		*Total anaerobes*		*Bacteroides*		*Coliforms*		*Lactobacilli*	
	I	*C*	*I*	*C*	*I*	*C*	*I*	*C*	*I*	*C*
1 hour	4.41	4.78	5.99	6.41	1.00**	1.00	1.00	4.30	6.60	6.00
1 day	6.07	1.00	6.78	4.73	2.74	4.60	1.00	1.00	8.78	4.83
2 days	3.12	1.00	6.05	6.72	1.00	1.00	1.00	1.00	5.78	6.26
3 days	1.00	1.00	5.00	6.72	1.00	1.00	1.00	1.00	4.14	6.26
5 days	5.87	1.00	6.87	5.85	1.00	1.00	1.00	1.00	6.26	6.78
7 days	7.89	1.00	6.50	5.99	1.00	1.00	6.21	1.00	7.22	6.23
10 days	4.40	3.88	7.11	6.88	5.28	5.36	1.00	1.00	6.58	6.18
Marginal mean	4.68	1.95	6.34	6.19	1.86	2.14	1.74	1.47	6.48	6.07

See Table 1 for footnote explanations.

Table 3) showed a significant ($p < .01$) increase in total aerobes, especially on the fifth ($10^{2.7}$/gm. versus 10^1/gm. for controls) and seventh (10^7/gm. versus 10^1/gm. for controls) days after irradiation; this may be explained by the significant ($p < .05$) proliferation of aerobic coliforms seen best on the seventh ($10^{5.6}$/gm. versus 10^1/gm. for controls) day after irradiation. Bacterial counts for the colonic homogenate showed a significant ($p < .01$) increase of total aerobes similarly on the fifth ($10^{5.8}$/gm. versus 10^1/gm. for controls) and seventh ($10^{7.8}$/gm. versus 10^1/gm. for controls) days after irradiation. This may be explained on the seventh day by an increase in aerobic coliforms (10^6/gm. versus 10^1/gm. for controls). Thus, bacterial proliferation appeared in the mucus layer and colonic wall as well as within the colonic contents of irradiated mice.

Histologic observations. On the first postirradiation days, observable damage was confined to the crypts of the small intestine. Mitotic figures disappeared, and progressive vacuolation, pyknosis, karyorrhexis, and karyolysis took place. In the colon, histologic changes consisted of nuclear inclusion bodies and mononuclear infiltration of the glandular epithelium and lamina propria. In the stomach, the only observable change was an increased secretion of mucus.

On the second postirradiation day, mitotic figures reappeared in the small bowel, cellular destruction slowed, and the crypts began to regenerate. On the third postirradiation day, damage was limited to the villi of the small bowel. Most of the villi were short, stubby, edematous, and partially denuded. Remaining epithelial cells were large, vacuolated, and misshapen.

By the fifth postirradiation day, the villi were reconstituted with only a few abnormal cells remaining at the tip. By the sixth postirradiation day, the microscopic appearance of the gastrointestinal mucosa was entirely normal.

Gram positive rods and cocci, presumably lactobacilli, were seen in the lumen of the stomach and in the mucus layer overlying both glandular and nonglandular areas of the organ (Figure 1). In the jejunum, lactobacilli were confined to the lumenal debris. In the lumen of the colon small Gram negative rods, larger Gram negative fusiforms, and Gram positive rods could be seen. In both the large and small bowel, bacteria were sometimes seen in the mucus adjacent to the epithelium and, on rare occasions, just beneath the surface of the epithelium. In the latter situation, bacteria were not clearly in the same focal plane as the tissue and appeared to overlie the specimen. No micro-abscesses and no evidence of bacterial invasion could be seen histologically.

DISCUSSION

Dubos and his co-workers have described the normal murine gastrointestinal flora and have shown that it changes with the age of the animal.[17,20] Our results agreed except that our techniques yielded fewer bacterial species. In no animal could we find anaerobic streptococci; however, this may be entirely appropriate for the three week old mouse that we studied.[17] Although our recovery of bacteroides and total anaerobes was slightly less, lactobacilli, total aerobes, total anaerobes, bacteroides, and coliforms appeared in the same locations and proportions described by Dubos.[17,18]

In previous experiments of this type, proliferation of fecal coliforms and aerobes have been described.

The proliferation of fecal coliforms and aerobes is consistent with the oft-demonstrated postirradiation Gram negative bacteremia.[8-14,21,22] An intestinal portal of entry is the most logical source of this bacteremia. The absence of intestinal microabscesses upon histological examination does not exclude the possibility of bacterial invasion of the intestinal wall. Serial blood cultures and complete blood counts were not done in this study; others have demonstrated severe lymphopenia and neutropenia four to eight days after similar doses of radiation.[5,23] Such damage to white blood cells may preclude the formation of microabscesses, and significant numbers of bacteria could penetrate the gut wall without any evidence of it histologically. For this reason, the transient increases seen in the colonic washes and homogenate may hold great significance.

Osborne,[22] Bond,[23] Gordon,[24] and others have suggested that small numbers of bacteria cross the epithelial barrier at the time of greatest villus

damage (three days after irradiation). When immunological defenses fail in the second week after irradiation, bacteria may be found in regional lymph nodes, liver, and spleen.[15] Bacteremia, according to these authors, reflects the immunological incompetence of these organs following the initial infection of intestinal tissues.

Osborne also suggested that bacteria may enter the bloodstream by way of the tonsils and cervical lymphatics following coprophagy.[22] Irradiated mice are more susceptible to endotoxin,[21,25] and others have implicated endotoxemia and vascular collapse as the mechanism of death. Matsuzawa, Wilson, and others[26-28] have suggested that intestinal bacteria enhance the radiosensitivity of intestinal tissues by increasing the mitotic rate of epithelial cells; this subject has been more extensively reviewed in another study from our laboratory.[29]

Whatever the mechanism, increased numbers of Gram negative bacilli occur in the irradiated mouse intestinal tract, and contribute to postirradiation morbidity and mortality. This relationship will be more fully explored in a series of experiments where we studied adult rather than weanling mice.[30]

SUMMARY

Mice were given 1200r of total body radiation. The bacteriology of the lumen and wall of the stomach, small intestine, and colon were studied for ten days after irradiation. Results were subjected to stringent statistical analysis and were correlated with histologic observations. Coliforms and total aerobes but not anaerobes, significantly increased, but not massively, above control levels in the lumenal contents of the colon, in washings of the colonic wall, and in homogenates of colon. No significant increases were noted in the stomach or small bowel flora. All increases in bacterial counts occurred as agonal or pre-agonal events.

On microscopic examination, bacteria were seen in the lumen of all organs studied. Maximal mucosal injury occurs at the first to third day after 1200r radiation. No microabscesses or progressive cellulitis could be seen in damaged gastrointestinal tissue, but this is attributed to the animal's inability to form such abscesses secondary to bone marrow and lymphoid tissue suppression.

It is concluded that bacteria proliferate in the colonic lumen following irradiation, and subsequently may invade the intestinal wall producing bacteremia and contributing to death; however, massive bacterial proliferation does not occur.

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REFERENCES

1. Klainer, A. S., Gorbach, S., and Weinstein, L.: Studies of intestinal microflora. VI. Effect of X-irradiation on the fecal microflora of the rat. *J. Bact.*, 1967, *94*, 378.
2. Fritz, T. E., Brennan, P. C., Giolitto, J. A., and Flynn, R. J.: Interrelations between X-irradiation and the intestinal flora of mice. In *Gastrointestinal Radiation Injury*, ed. by M. F. Sullivan; New York Excerpta Medicine Foundation, 1968, p. 279.
3. Hammond, C. W., Tomkins, M., and Miller, C. P.: The time of onset and duration of the endogenous bacteremias in mice. *J. exp. Med.*, 1954, *99*, 405.
4. Shechmeister, I. L. and Bond, V. P.: Response of mice to certain avirulent bacteria after exposure to sublethal total body X-irradiation. *Proc. Soc. exp. Biol. (N.Y.)*, 1951, *77*, 77.
5. Shechmeister, I. L., Bond, V. P., and Swift, M. N.: The susceptibility of irradiated mice to infection as a function of post-irradiation time. *J. Immunol.*, 1952, *68*, 87.
6. Hammond, C. W., Colling, M., Cooper, D. B., and Miller, C. P.: Studies on susceptibility to infection following ionizing radiation. II. Its estimation by oral inoculation at different times after irradiation. *J. exp. Med.*, 1954, *99*, 411.
7. Hammond, C. W., Ruml, D., Cooper, D. B., and Miller, C. P.: Studies on susceptibility to infection following ionizing radiation. III. Susceptibility of the intestinal tract to oral inoculation with *Pseudomonas aeruginosa*. *J. exp. Med.*, 1955, *102*, 403.
8. Warren, S. L. and Whipple, G. H.: Bacterial invasion of the bloodstream as influenced by x-ray destruction of the mucosal epithelium of the small intestine. *J. exp. Med.*, 1923, *38*, 713.
9. Mottram, J. C. and Kingsbury, A. N.: Some researches into the action of radium and x-rays correlating the production of intestinal changes, thrombocytopenia, and bacterial invasion. *Brit. J. exp. Pathol.*, 1924, *25*, 220.
10. Chrom, S. A.: Studies on the effect of roentgen rays upon the intestinal epithelium and upon reticuloendothelial cells of liver and spleen. *Acta Radiol.*, 1935, *16*, 641.
11. Lawrence, J. H., and Tennant, R.: The comparative effects of neutrons and X-rays on the whole body. *J. exp. Med.*, 1937, *66*, 667.
12. Miller, C. P., Hammond, C. W., and Tompkins, M.: Role of infection in radiation injury. *J. Lab. clin. Med.*, 1951, *38*, 331.
13. Gonshery, L., Marston, R. Q., and Smith, W. W.: Naturally occurring infections in untreated and streptomycin-treated X-irradiated mice. *Amer. J. Physiol.*, 1953, *172*, 359.
14. Mottram, J. C.: Some effects of exposure to radium upon the alimentary canal. *Proc. Roy. Soc. Med.*, 1923, *16*, 41.
15. Congdon, C. C., Williams, F. P., Haberman, R. T., and Lorenz, E.: The histopathology of bacterial infection in irradiated mice. *J. Nat. Canc. Inst.*, 1955, *15*, 855.
16. Brown, J. H. and Brenn, L.: A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections. *Bull. Johns Hopk. Hosp.*, 1931, *48*, 69.
17. Schaedler, R. W., Dubos, R., and Costello, R.: The development of bacterial flora in the gastrointestinal tract of mice. *J. exp. Med.*, 1965, *122*, 59.
18. Dubos, R., Schaedler, R. W., Costello, R., and Hoet, P.: Indigenous normal and autochthonous flora of the gastrointestinal tract. *J. exp. Med.*, 1965, *122*, 67.
19. Floch, M. H., Gershengoren, W., and Freedman, L. R.: Methods of quantitative study of the aerobic and anaerobic bacterial flora of man. *Yale J. Biol. Med.*, 1968, *41*, 50.

20. Savage, D. C., Dubos, R., Schaedler, R. W.: The gastrointestinal epithelium and its autochthonous bacterial flora. *J. exp. Med.*, 1967, *127*, 67.
21. Rosoff, C. B.: The role of intestinal bacteria in the recovery from whole body radiation. *J. exp. Med.*, 1963, *118*, 935.
22. Osborne, J. W., Bryan, H. S., Quostler, H., and Roades, H. E.: X-irradiation and bacteremia. IV. Studies on Roentgen death in mice. *Amer. J. Physiol.*, 1952, *170*, 414.
23. Bond, V. P., Silverman, M. S., and Cronkite, E. P.: Pathogenesis and pathology of post-irradiation infection. *Radiat. Res.* 1954, *1*, 389.
24. Gordon, L. E., Ruml, D., Hahne, H. J., and Miller, C. P.: Studies on the susceptibility to infection following ionizing radiation. IV. The pathogenesis of the endogeneous bacteremias in mice. *J. exp. Med.*, 1955, *102*, 413.
25. Silverman, M. S., Chin, P. H., Greenman, V., and Young, J. C.: The cause of increased susceptibility to avirulent Pasturella pestis after exposure to a sublethal dose of X-radiation. *J. infect. Dis.*, 1954, *94*, 47.
26. Abrams, G. D., Bauer, H., and Sprinz, H.: Influences of the normal flora in mucosal morphology and cellular renewal in the ileum. *Lab. Invest.*, 1963, *12*, 355.
27. Matsuzawa, T. and Wilson, R.: The intestinal mucosa of germ-free mice after whole-body X-irradiation with 3 kiloroentgens. *Radiat. Res.*, 1965, *25*, 15.
28. Wilson, R., Bealmear, P., and Matsuzawa, T.: Acute intestinal radiation death in germfree and conventional mice. In *Gastrointestinal Radiation Injury*, ed. by M. G. Sullivan; New York, Excerpta Medica Foundation, 1968, p. 148.
29. Khoury, K. A., Floch, M. H., and Herskovic, T.: Effects of neomycin and penicillin administration on mucosal proliferation of the mouse small intestine. *J. exp. Med.*, 1969, *129*, 1063.
30. Livstone, E. M., Spiro, H. M., and Floch, M. H.: The gastrointestinal microflora of irradiated mice. II. Effects of oral antibiotics in adult mice. *Yale J. Biol. Med.*, this issue.