Response of Mice to Certain Avirulent Bacteria after Exposure to Sublethal Total Body X-Irradiation.* (18684)

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It has been found previously(1,2) that whole body X-irradiation markedly lowers the resistance of mice to infection. The appearance of bacteremia following this treatment has been demonstrated by several investigators(2-6). Recently, in a careful and systematic study, Miller and associates(7) stated that during the period of greatest mortality from X-irradiation in mice "bacteria from the lower intestinal tract invaded the blood stream and produced bacteremia often of great severity." Howland and associates(8) and Miller and associates(9) have observed that some degree of protection is afforded animals against the effects of total body irradiation by means of antibiotics. Graham and Graham(10) have reported that groups of mice inoculated with .1 ml of horse serum immediately after exposure to 400 r total body irradiation evidenced greater mortality than did control animals. In the course of current investigations of the relationship of irradiation to immunity(11), it was observed that mice subjected to sublethal doses of irradiation succumbed to subcutaneous inoculation of live and heat-killed avirulent bacteria. This paper describes the initial studies dealing with this phenomenon.

Experimental procedure. Five to 6 weeks old NAMRU strain mice(12) from the animal colony of the Naval Medical Research Unit No. 1 were used in the experiments. This colony was established in 1943 and to date has been entirely free of any detectable infectious disease. The animals were irradiated by means of a constant-potential Westinghouse X-ray unit. The radiation factors were as follows: 250 KV; 15 ma; filter, 0.6 mm copper; H.V.L. 1.3 mm copper; distance from target to skin 100 cm; output in air approximately 25 r per minute. All irradiated mice were given a single dose of 350 r to the entire body. This dose was uniformly sublethal in a 30-day period. The X-ray LD_{50} for this strain of mice under the conditions employed is approximately 500 r. Control mice were

* This work was made possible, in part, through funds provided by the Bureau of Medicine and Surgery, U. S. Navy Department, by the Office of Naval Research and by the Atomic Energy Commission, under ONR contract with the University of California, Berkeley.

The opinions and assertions contained in this report are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large (Article 1252, U. S. Navy Regulations 1948).

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1. Gowen, J. W. and Zelle, M. R., *J. Inf. Dis.*, 1945, v77, 85.

2. Chrom, S. A., *Acta Radiologica*, 1935, v16, 641.

3. Lawrence, J. H. and Tennant, R., *J. Exp. Med*, 1937, v66, 667.

4. Mottram, J. C. and Kingsbury, N., *Brit. J. Exp. Path.*, 1924, v5, 220.

5. Warren, S. L. and Whipple, G. H., *J. Exp. Med.*, 1923, v38, 713.

6. Bennett, L., Rekers, P., Kresge, M., and Howland, J., *Univ. of Rochester Report* UR-76, August, 1949.

7. Miller, C. P., Hammond, C. W., and Tompkins, M., *Science*, 1950, v111, 540.

8. Howland, J., Furth, F., Bennett, L., Coulter, M., McDonnel, G., *Univ. of Rochester Report* UR-94, October, 1949.

9. Miller, C. P., Hammond, C. W., and Tompkins, M., *Science*, 1950, v111, 719.

10. Graham, J. B., and Graham, R. M., *Proc. Nat. Acad. Sci.*, 1949, v35, 102.

11. Shechmeister, I. L. and Bond, V. P., in preparation.

12. Garber, E. D. and Hauth, F. C., *J. Heredity*, 1950, v41, 122.

TABLE I. Response of Normal and Irradiated Mice to Subcutaneous Inoculation with Certain Preparations.

Inoculated preparations	Mortality: Irradiated Dead/Total	Irradiated %	Normal Dead/Total	Normal %
I. *P. pestis*, Strain A-1122				
Administered 6 days after irradiation				
1 × 10^9/ml	15/15	100	7/15	47
6 × 10^8/ml	27/30	90	0/30	0
5.6 × 10^8/ml	91/100	91	17/100	17
5 × 10^8/ml	27/40	68	4/40	10
5 × 10^8/ml	37/50	74	7/50	14
1 × 10^4/ml*	4/15	27	0/15	0
5.1 × 10^1/ml†	32/50	64	5/50	10
Heat-killed‡	16/30	53	3/30	10
II. *P. pestis*, Strain K-3				
1 × 10^8/ml	20/30	67	1/26	4
Seitz filtrate of above culture	10/30	33	0/27	0
III. *E. coli*				
5 × 10^8/ml	8/15	53	0/15	0
5 × 10^5/ml	14/15	93	1/15	7
IV. Other substances				
Physiological saline	1/15	7	No data	No data
Heart infusion broth	0/15	0	" "	" "
Bovine albumin, 1%	0/15	0	" "	" "

* Obtained by diluting 5 × 10^8/ml bacterial suspension.
† Obtained by heating 5 × 10^8/ml bacterial suspension, but not killing all organisms.
‡ Obtained by heating 5 × 10^8/ml bacterial suspension, and killing all organisms.

subjected to the same manipulations as were the experimental animals except for exposure to X-rays. Avirulent *Pasteurella pestis* cultures were grown for 24 hours at room temperature in heart infusion broth (Difco) which was aerated by continuous shaking. Static cultures of *Escherichia coli* were grown in nutrient broth for 24 hours at 37°C. Heat-killed organisms were obtained by exposure of a live culture to 56°C for 2 hours. All cultures were resuspended in physiological saline after centrifugation at 2700 rpm. In the initial experiment, irradiated mice were inoculated subcutaneously with 0.2 ml of the preparations listed in Table I. Mortality over a 30-day period was used as the criterion of response. In the second experiment cultures were taken of inoculated animals as indicated below, in an attempt to elucidate the mechanism of increased susceptibility of the irradiated mice to avirulent or dead bacteria. Two series of female mice were used; the first primarily for serial sacrifice and culture, the second for mortality control animals (see Table II). In the first series, 5 mice of each of 4 groups described below, as well as one normal animal and one animal subjected only to X-irradiation were sacrificed seriatim daily for 9 days by means of nembutal, and were autopsied using aseptic technic. The 4 groups were:

(1) Sixty-five mice inoculated subcutaneously with about 6 x 10^8 live avirulent *P. pestis* strain A-1122, 6 days after irradiation.

(2) Sixty-five irradiated mice treated as in (1) except that the *P. pestis* culture used was heat-killed.

(3) 65 unirradiated mice treated as described in (1).

(4) 65 unirradiated mice treated as described in (2).

Cultures of liver, spleen, bone marrow, and heart blood (cardiac puncture) were made on horse-blood agar and in thioglycollate medium. Generic identification of *Pasteurella* cultures was made by means of pestiphage. Phage negative cultures were studid further for lactose fermentation reaction on eosin methylene blue agar.

The mortality control series was intended to indicate the 30-day lethality response of the mice to the inoculated bacteria used in series

TABLE II. Results of Cultures of Bone Marrow, Spleen, Blood, and Liver of Mice.

	Mice inoculated subcutaneously with Live *P. pestis*		Heat-killed *P. pestis*		Controls	
	Irradiated	Normal	Irradiated	Normal	Irradiated	Normal
Series 1						
No. of mice	65	65	65	65	9	9
No. died during experiment	26	3	18	3	0	0
No. cultured	39	45	40	45	9	9
Gram negative rods isolated*						
lactose +, phage —	10	4	25	0	2	0
lactose —, phage +	16	12	0	0	0	0
not tested	4	5	4	4	0	0
Gram positive cocci or rods	4	5	5	1	0	0
Negative cultures	5	19	6	40	7	0
Series 2 (Mortality controls)						
No. of mice	30	30	30	30	None	None
No. died within 30 days after inoculation	27	0	16	0	"	"

* When one or more organs cultured was positive for organism in question, the animal is positive for that organism.

1. Four groups of 30 mice each were treated in a manner identical to that of series 1, except that no animals were sacrificed.

Results and discussion. In the initial experiments, mortality in groups of irradiated mice inoculated with either live or heat-killed avirulent *P. pestis* and live *E. coli* was considerably greater than in groups of control animals receiving the same inoculum (Table I). Similar results were obtained by the inoculation of a Seitz filtrate of avirulent *P. pestis*. Physiological saline, nutrient broth or 1% bovine albumin did not cause death in irradiated mice.

Table II summarizes the results of the serial sacrifice experiments. No Gram-negative, lactose fermenting rods were isolated from normal mice inoculated with heat-killed cultures of avirulent *P. pestis*. However, of the 40 sacrificed irradiated mice inoculated with the same culture, 25 yielded Gram-negative, lactose fermenting rods that were not lysed by pestiphage.

Cultures of Gram-negative non-lactose-fermenting rods that were lysed by pestiphage were isolated from 12 of the 45 normal control mice,§ and from 16 of the irradiated mice, inoculated with live avirulent *P. pestis*. Four normal animals, and 10 irradiated animals, inoculated with the same culture of *P. pestis*, showed not only the presence of phage positive, Pasteurella-like organisms but also phage negative, lactose-fermenting forms. No bacteria were isolated from normal uninoculated controls, but two out of nine irradiated uninoculated mice showed the presence of Gram-negative, lactose-fermenting organisms.

Of the 65 normal mice inoculated with either live or heat-killed *P. pestis*, 3 died within the duration of the serial sacrifice experiment (9 days). During the same period, 18 of 65 irradiated mice given heat-killed *P. pestis*, and 26 of 65 irradiated animals administered live avirulent *P. pestis* succumbed to the inoculations. Since these groups served as the source of animals for daily sacrifices, the above statistics represent minimal numbers of deaths. More representative lethality data are indicated by the results of the control mortality experiment (series 2). No deaths were observed among normal mice inoculated with the bacteria, while of the mice irradiated 6 days prior to inoculation, 27 out of 30 died after injection of live *P. pestis*, and 16 out of 30 died in response to heat-killed organisms from the same culture.

Conclusions. In these experiments, death

§ Jawetz and Meyer(13) have reported recovery of avirulent *P. pestis* from inoculated mice.

13. Jawetz, E., and Meyer, K. F., *J. Infect. Dis.*, 1944, v74, 1.

was associated with bacteremia.‖ When avirulent live bacteria were injected, the recovered organisms were either of the strain inoculated, or were apparently autogenous bacteria. However, when heat-killed bacteria were introduced, the recovered organisms were of autogenous origin only. It is known that bacteremia, identified with normal bowel inhabitants, is often an integral part of the syndrome observed in animals dying from whole body irradiation. In this instance, however, bacteremia and death were induced in animals exposed to sublethal doses of irradiation and subsequently inoculated with live avirulent, or even dead organisms generically different from the bacteria normally present in the intestines of these animals. This observation may be of importance under conditions where irradiation is a factor and vaccination is indicated.

‖ Complete identification of isolated lactose fermenting cultures was not made.

Received April 16, 1951. P.S.E.B.M., 1951, v77.

Autoradiograms of Irregular Surfaces.* (18685)

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In studies of shell formation using Ca^{45} and P^{32} it became necessary to follow isotope distribution on the curved inner surfaces of mollusc shells. For accurate localization of radioactivity on such surfaces by means of photographic emulsions close contact between the emulsion and the surface is necessary. Photographic plates and cut film which are satisfactory for flat surfaces will not give sufficiently close contact with curved and irregular surfaces. Liquid emulsion, which might be used, has not proved suitable in our experience in providing a layer of uniform thickness; and stripping film is not sufficiently flexible to conform to irregular surfaces. We have found that emulsion sheets removed from x-ray film will serve very satisfactorily. The emulsion layer is uniform in thickness and will faithfully follow and adhere to all contours. This material is also useful for making autoradiograms of bones as well as shells. Details of the method are illustrated below with bones of the guinea pig.

Eastman Industrial X-ray Film, Type A, in strips 1⅞ x 16 inches was used. This film is more sensitive than Type M and less sensitive than No-Screen and Type K. Because of its smaller grain size the resolution is better than the latter two(1,2). All operations are carried out in a darkroom using a Wratten safelight, Series OA. After cutting the film into pieces of appropriate size they are placed in distilled water at 30°C for about 5 minutes to soften the emulsion. By means of a piece of plastic or glass with a polished edge the emulsion can now be completely removed in a sheet by beginning at one end of the film strip and pushing the emulsion ahead of the instrument. With a satisfactory edge the sheet of emulsion is removed without defect and with none remaining on the backing. The emulsion is then placed in distilled water at 20°C where it will toughen and wrinkles will disappear. The emulsion is applied by floating it onto the surface to be studied. As the object is lifted from the water the sheet of emulsion will become closely applied to all contours (Fig. 1 and 2). Any wrinkles can be smoothed with a camel's hair brush. Leaching of radioactive material from shell and bone during the exposure to distilled water and to the wet emulsion will presumably be minimal.

After the emulsion is dry the preparations are stored in light-tight boxes for a suitable

* This work was supported by a grant from the Atomic Energy Commission.

1. Boyd, George A., Personal Communication, 1951.

2. Cobb, J., and Soloman, A. K., *Rev. Sci. Instr.*, 1948, v19, 441.