Sublethal Total Body X-Radiation and Susceptibility of Mice to *Salmonella enteritidis* and *Escherichia coli.** (20308)

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(Introduced by C. G. Harford.)

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The results of a study dealing with the susceptibility of irradiated mice to infection with airborne *Streptococcus zooepidemicus* as a function of post-irradiation time(1) demonstrated a linear increase in susceptibility over the first 15 days, reaching a peak 5 times that of controls, followed by an exponential drop approaching the control level shortly after 30 days. Mice subjected to sublethal doses of radiation exhibited a time pattern of change in susceptibility to airborne infection with this organism, which was different from the time pattern reported for various other post-irradiation changes (peripheral blood counts, weight of certain organs, etc.). It was considered desirable to determine whether the established relationship between the two factors a) the degree to which radiation affects susceptibility to infection and b) the post-irradiation time interval required for such susceptibility to reach a maximum, would also apply to different microorganisms introduced by other than the natural airborne route. Certain results of such a study conducted with two contrasting organisms, the pathogenic *Salmonella enteritidis* and the usually innocuous *Escherichia coli,* are presented in this communication.

Materials and methods. Experimental design. Irradiated mice and control animals were inoculated intraperitoneally at the same time with graded concentrations of *S. enteritidis* at intervals over the 30-day period, and bacterial LD_{50} values for both the irradiated and control animals were calculated from the resulting mortality data and compared. In another series of experiments only a single bacterial concentration of *E. coli* (5×10^7 to 2×10^8 organisms) was introduced intraperitoneally at indicated time intervals to both normal and irradiated animals over the 60-day period following irradiation with different doses of X-rays. Bacterial LD_{50} value was not determined, since tenfold dilution of the administered bacterial suspension failed to kill any irradiated animals. *Microbial agents. S. enteritidis* 1891 (IX, XII . . . . g,m) was obtained from Dr. F. L. Adler, Harvard University School of Medicine, and was identified as culture 64 of the Agricultural Experimental Station of the University of Kentucky. In order to reduce variation, a lyophilized stock of the agent was prepared in individual ampoules. The entire content of a single ampoule was added to 10 ml of broth which, after static incubation for 10-12 hours at 37°C, constituted the first passage. The second passage was prepared by seeding 1 ml of the first passage per 50 ml of nutrient broth and incubating at 37°C for 18 hours. This second serial passage was used in all experiments. Prior to use, each culture was tested for purity and its concentration was determined. The *strain of E. coli* used in these experiments was isolated from the large intestine of a normal mouse. Lyophilized stock cultures and liquid cultures were prepared in the manner described for *S. enteritidis.* The *concentration* of bacterial cultures was determined at the time of inoculation of the organism by dropping a known quantity (about 0.02 ml) of bacterial suspension on E.M.B. agar (Difco) and counting colonies resulting after 18-24 hours of incubation at 37°C(2). *Experimental animals.* Six to 8 weeks old male and female Namru strain mice(3) from our own

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TABLE I. Response of Mice to *S. enteritidis* as a Function of (a) Radiation Dose and (b) Post-Irradiation Time.

Total body radiation, r	Bacterial LD_{50} at stated post-irradiation periods						
	8 hr	1 day	5 days	12 days	15 days	20 days	30 days
350	6×10^1	8.8×10^1	3.3×10^1	3.6×10^2	6.7×10^2	3.7×10^4	3.8×10^5
250	5×10^2		4.7×10^4		1.1×10^6		4.5×10^5
50	8.4×10^5		4.5×10^5				
0	1×10^6	1.3×10^6	1.7×10^6	1.3×10^6	1.4×10^6	1×10^6	4.3×10^5

TABLE II. Total and Differential White Blood Cell Counts of Normal and Irradiated Mice.

Total body radiation, r	Post-irradiation period	WBC/mm³, mean (10 mice) and stand. error of mean		
		Heterophils	Lymphocytes	Total
350	3 hr	1830 ± 140	2230 ± 240	4170 ± 280
	8 "	3920 ± 340	1180 ± 160	5200 ± 380
	1 day	1480 ± 80	700 ± 110	2200 ± 140
200	3 hr	1890 ± 250	2040 ± 140	3990 ± 330
	8 "	2880 ± 440	1250 ± 110	4230 ± 440
	2 days	1690 ± 170	1170 ± 100	2830 ± 160
0 (controls)		1170 ± 90	4140 ± 450	5960 ± 440
	Sham irradiated	1040 ± 90	7700 ± 610	8740 ± 670

breeding colony were used in the experiments. The colony was established in 1950 and, to date, has been free of any detectable infectious disease. The animals were irradiated by means of a Keleket deep therapy X-ray machine. The radiation factors were as follows: 200 KVP, 20 ma, filter 0.25 mm Cu and 1.0 mm Al, HVL 0.75 mm Cu, distance from target to skin 75 cm; output approximately 21 r per minute. All X-ray doses were measured in air. The results of the *X-ray mortality studies,* carried out in the manner previously described(1), confirmed the established observation that the maximal dose that could be given without causing deaths from radiation alone within 60 days was of the order of 350 r. Groups of animals were exposed to doses of X-ray ranging from a) 50 to 350 r, prior to challenge with *S. enteritidis,* and b) 50 to 450 r, prior to inoculation with *E. coli.* Five to six animals were used per dilution in determination of LD_{50} values for *S. enteritidis.* However, when the mortality end point for intraperitoneal inoculation of *E. coli* was investigated, at least 10 and usually 20-30 or more animals were used for a dose of X-ray. All the LD_{50} values were calculated according to the method of Reed and Muench(4). Representative number of mice dying after challenge with *S. enteritidis* were autopsied and cultured for this organism. Total and differential white blood cell counts were made on tail-vein blood of groups of 10-12 animals: a) Exposed to 350 r or 250 r at different post-irradiation periods; b) sham-irradiated mice, and c) normal controls.

Results. Exposure to 350 r increased susceptibility of mice to infection with *S. enteritidis* (Table I). The results were most marked 8 hours to 5 days after irradiation, when bacterial LD_{50} was of the order of 30-100 organisms for the irradiated as opposed to the LD_{50} value of about 1.0×10^6 to 1.7×10^6 organisms for the normal animals. In the course of time this effect gradually decreased until, some 30 days after irradiation, the susceptibility was the same as for non-irradiated animals. Animals exposed to 250 r also showed a marked increase in susceptibility to this agent when challenged 8 hours after irradiation. However, a pronounced rise in the bacterial LD_{50}, evident within 5 days, approached that for normal mice in 15 days. Exposure to 50 r total body radiation prior to inoculation did not seem to influence the susceptibility to this infection. Post-mortem cultures of representative number of mice from each of the groups indicated in Table I revealed the presence of Salmonella in the spleens of all of the tested animals. Although no significant

difference in total leucocyte count was noted within 8 hours after exposure to 250 r or to 350 r, a significant lymphopenia and a concomitant granulocytosis were present at this time (Table II).

Since bacteremia primarily due to *E. coli* was positively correlated with mortality after exposure to lethal doses of X-rays(5), it appeared desirable to evaluate the effect of midlethal and sublethal radiation on the susceptibility of mice to this agent administered intraperitoneally, at different times after irradiation. The results of representative experiments are indicated in Table III. Of the several groups of animals used as controls, it was only in the 30-day post-irradiation group that some mice died following bacterial inoculation. As is seen from Table III, exposure of mice to 450 r and to 350 r total body radiation resulted in death of a certain number of animals. This was considered in calculation of the per cent mortality of groups of animals subjected to the respective dose of radiation. Practically all deaths from radiation alone occurred within 8-20 days following irradiation; however, the average survival period of all irradiated animals inoculated with *E. coli* was of the order of 2 to 4 days, occasionally 6 days.

Increase in susceptibility to *E. coli* was observed as early as one day after irradiation. In the group exposed to 450 r there was a gradual decline in susceptibility until, when tested 60 days after irradiation, mortality rate of 37% was observed. Previous exposure to 350 r resulted in a marked susceptibility to infection, demonstrated within one day and sustained through 11 days, and declining to 20% 24 days after irradiation. Exposure to 200 r brought about an initial increase in the susceptibility, which returned to normal in animals tested 24-30 days after irradiation. After exposure to 50 r, increase in susceptibility to *E. coli* was observed only when challenged 11 days after irradiation.

Discussion. Granulocytosis and lymphopenia were observed in mice 3-8 hours after exposure to 500 r whole body radiation(6). In our study granulocytosis was also observed in mice 8 hours after exposure to 250 r or

TABLE III. Response of Mice to *E. coli* as Function of (a) Radiation Dose and (b) Post-Irradiation Time.

Post-irradiation period, days	450 r							350 r					200 r†			50 r†		
	A Inoculated		B (controls) Radiation		Bacterial		A–B, final % mortality	A Inoculated		B‡ (controls) Radiation		A–B, final % mortality	Inoculated		Final % mortality‡	Inoculated		Final % mortality‡
	Dead/ total	%	*Dead/ total	%	Dead/ total	%		Dead/ total	%	*Dead/ total	%		Dead/ total	%		Dead/ total	%	
1	15/15	100	9/341	3		(0)§	97	10/10	100	†		100	7/10	70	70	2/10	20	20
4	19/20	95	113/450	25	0/10	0	70	19/20	95	1/117	1	94	5/10	50	50	0/10	0	0
7	15/15	100	125/446	28		(0)§	72	10/10	100	7/117	6	94	6/10	60	60	1/10	10	10
11					0/10	0		9/11	82	16/116	14	68	20/25	80	80	14/23	61	61
24	5/9	56	5/222	2		(0)§	54	4/19	21	1/91	1	20	3/18	17	17	4/23	17	17
30	26/28	93	4/213	2	17/44	39	52	22/41	54	†		15	15/37	40	1	9/40	23	0
60	11/25	44	†		1/14	7	37	3/14	21	†		14	0/22	0	0	1/27	4	0

* Cumulative results of several experiments.
† No deaths due to radiation observed.
‡ Same bacterial controls as used for 450 r group.
§ Evidence from overall experience supports the conclusion that, in general, occurrence of deaths in bacterial controls is very unlikely, and that the 39% mortality for the 30-day period is exceptional.

to 350 r. It is, therefore, apparent that the increased susceptibility to the experimental (intraperitoneal) infection at this post-irradiation period cannot be accounted for by granulocytopenia at the time of injection. The pattern of total, and differential white blood cell counts for certain other periods following irradiation also indicates no consistent correlation between granulocyte count and susceptibility to infection. The present data may possibly be explained either by a damage to some system involved in the defense against this experimental infection other than that provided by the granulocytes, or by an alteration in functional ability of granulocytes. However, no implication regarding the possible role of granulocytopenia in natural infections of irradiated animals is intended by this statement.

Inoculation of *E. coli* at different times after exposure to 50 r reveals an increase in susceptibility to this microorganism only in the group of mice that were challenged 11 days after irradiation. This peak is of interest in the light of the high mortality and bacteremia reported initially at that particular post-irradiation period by Miller *et al.*(7) in mice subjected to 450 r total body radiation, an observation frequently confirmed in this laboratory. The 11-day peak in increased susceptibility to infection was only suggested after exposure to 200 r, but was not apparent at higher doses, probably because it formed a part of the "post-irradiation susceptibility range" initially evident one day after irradiation.

Exposure to sublethal radiation increases the susceptibility of certain animals to experimental infection with bacterial(8), fungal(9), viral(10) and rickettsial agents(11,12), as well as bacterial toxins(13). The increased susceptibility to infection by mice challenged with airborne *Str. zooepidemicus* was shown to be a function of the post-irradiation time, with the peak slowly reaching a maximum 15 days after irradiation. However, a very pronounced increase in susceptibility to the enteric organisms was noted 8-24 hours after irradiation. While inhalation of *Str. zooepidemicus* stimulated a natural portal of entry of the organisms, intraperitoneal administration of *S. enteritidis* and *E. coli* represented an artificial situation with bacteria by-passing the "primary line of defense", exemplified in the case of *Str. zooepidemicus* by the intact respiratory mucosa. Apparently radiation rapidly brought about alteration of "the secondary defense mechanisms", to the extent that the extremely pronounced increase in susceptibility to *S. enteritidis* was observed 8 hours after irradiation, and to *E. coli* in one day.

The importance of the time of challenge in relation to radiation (*i.e.*, post-irradiation period) very evident here, has also been pointed up by variation in susceptibility of a) mice exposed to a single total-body irradiation in the LD_{50} range followed by intramuscular administration of a beta hemolytic streptococcus at different post-irradiation periods(13), and b) sublethally irradiated mice to toxin of *Clostridium septicum*(14) and influenza virus(15). Similarly, the importance of the route of infection was also demonstrated in experiments with *Corynebacterium pseudotuberculosis murium*(16).

Summary. 1. Mice were challenged intraperitoneally with *S. enteritidis* over a 30-day period following exposure to 50-350 r whole body X-radiation. A very marked increase in susceptibility to infection was observed as early as 8 hours after irradiation with 250-350 r, but not after 50 r. This increase in susceptibility cannot be explained by granulocytopenia at the time of injection, since granulocytosis was observed in peripheral blood of mice at this post-irradiation period. 2. A very marked increase in susceptibility to intraperitoneal inoculation with *E. coli* was observed one day following irradiation with 200-450 r. This gradually declined but was still evident 60 days after exposure to 450 r, and 11 days after exposure to 200-350 r. After exposure to 50 r, the single peak of susceptibility to *E. coli* occurred in 11 days. 3. The relationship of the route of inoculation and the post-irradiation time to the degree of susceptibility to infection is discussed.

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Aortic Albuminoid Titration and Swelling Curves from Persons Differing in Age and Degree of Atherosclerosis. (20309)

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The present investigation is concerned with one phase of a study on the chemical changes associated with atherosclerosis and increasing age of the human aorta. Titration and swelling curves of the human aortic albuminoid (collagen, reticulin, and elastin) have been studied with reference to possible changes in protein constitution associated with atherosclerotic processes. Previously, the existence, in the human aortic albuminoid, of a carbohydrate fraction not quantitatively altered by pathological or aging changes has been demonstrated(1). However, albuminoids from aged and atherosclerotic aortae have been shown to combine with larger quantities of hydrochloric acid than do relatively normal or younger samples, although combination with sodium hydroxide remained constant in all cases(2). The mean percentage of elastin in this aortic albuminoid showed no significant change with age or atheromatous condition. However, a statistically significant increase in albuminoid elastin was apparent in samples showing visible calcification(3).

Materials and methods. The albuminoid samples were prepared from 23 human aortae by methods previously described(2). Following desiccation, each sample was ground in a Wiley mill (40 mesh) and replaced in the desiccator for a minimum of 24 hours. The volume swelling and titration curves were determined by the method of Highberger(4), which was slightly modified according to the special needs of the present problem. Eleven 100 mg samples from each aorta were weighed out on an analytical balance and placed in an equal number of graduated 15 ml centrifuge tubes. Ten ml of the appropriate standard solution was added to each tube and the contents shaken. Five samples were incubated in hydrochloric acid standards (0.0005N-0.05N), 5 in sodium hydroxide standards (0.00055N-0.055N), and one in distilled water. All samples were stoppered and incubated for 48 hours at room temperature. Following the incubation period, the tubes were centrifuged for 10 minutes at about 400 g. The volume of solid was estimated imme-