characters was noted. A blurring of the non-albumin components of the plasma was noted in certain adult females of 4 species, a probable association with egg production.

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Response of Guinea Pig to Sublethal X-Irradiation and Live Tularemia Vaccine. (31972)

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Animals exposed to ionizing irradiation are usually more susceptible to bacterial infection than nonirradiated animals. Also, irradiated animals have less capacity both for antibody formation and for the development of resistance to challenge following vaccination (1-3).

The anticipated increased utilization of live vaccines for immunization of man poses a problem with respect to the potential risk incurred by vaccinees if exposed to irradiation. This report concerns a study designed to determine the response of guinea pigs administered sublethal irradiation before or after administration of live tularemia vaccine.

Materials and methods. Experimental animals. Adult, male guinea pigs (Hartley strain) weighing 325- to 375-g, were caged in groups of 5 and fed water and Rockland guinea pig pellets *ad libitum*.

X-irradiation. A 1,000 KVP Maxitron apparatus was used as the source of irradiation. Guinea pigs were irradiated in groups of 10 at a distance of 100 cm; dose rates ranged from 56- to 73-r/minute.

Bacterial cultures. Pasteurella tularensis vaccine strain LVS(4) and highly virulent strain SCHU S4(5) were grown in modified casein partial hydrolyzate medium, MCPH (6).

Agglutination tests. A formalinized suspension of strain SCHU S4 was used as antigen and tests were performed and read according to the National Institutes of Health method(7).

Aerogenic vaccination. Organisms were nebulized from a 50% MCPH menstruum and dispersed as a small-particle aerosol with a Chicago-type atomizer in a 4,800-liter exposure chamber similar to that described by Wolfe(8). Guinea pigs inhaled approximately 10^5 viable cells of strain LVS.

Assessment of viable P. tularensis. Appropriate dilutions of homogenized tissues were prepared in gel-saline (0.1% gelatin in 0.85% NaCl), plated on glucose cysteine blood agar(9), and incubated 4 days at 37°C.

Results. Mortality resulting from irradiation. The whole-body X-irradiation $LD_{50/30}$ for the guinea pig was established at 290 r. The maximum sublethal dose was approximately 140 r. To ensure maximal irradiation effect with minimal irradiation-induced mortality, a dose of 140 r of whole-body irradiation was selected.

Combined irradiation-P tularensis *LVS vaccination.* The times between irradiation and later vaccination of guinea pigs were 12, 6, 3, or 1 day. The times between aerogenic vaccination and later irradiation were 2 to 4 hours, 1 day, or 3 days. Animals irradiated 3 days prior to vaccination showed the highest mortality (25%) (Table I). One of 40 irradiated controls died; all nonirradiated vaccinees survived.

Deaths in irradiated vaccinated guinea pigs

TABLE I. Guinea Pig Mortality After X-Irradiation and Subsequent or Preceding Aerogenic Vaccination with *Pasteurella tularensis* LVS.

Time animals were vaccinated	% dead 30 days after vaccination*
Vaccinated after irradiation	
12 days	0
6 "	10
3 "	25
1 "	17.5
Vaccinated before irradiation	
2 to 4 hr	0
1 day	10
3 "	0
Vaccinated nonirradiated controls	0
Nonvaccinated irradiated controls	2.5

* Based on 40 animals per group.

occurred 3 to 17 days after vaccination and most of the animals died during the second week after vaccination. Strain LVS was cultured from 60% of these animals. Animals dying within 10 days exhibited multiple subcutaneous, intestinal, and peritoneal hemorrhages in addition to petechial hemorrhages in the lungs. Diffuse hemrrhage of the lungs was the only gross pathologic finding in animals dying later than the tenth day. Animals irradiated before or after aerogenic vaccination could not be differentiated on the basis of gross pathology.

Hematological observations. Animals subjected to both X-irradiation and vaccination exhibited essentially the same pattern of leukocyte response as did unvaccinated irradiated controls. A marked reduction in lymphocytes-monocytes occurred within 24 hours after irradiation; the population did not return to preirradiation levels during the investigation. Maximum granulocytopenia was observed 13 to 18 days after irradiation, but prior to termination of the study the number of granulocytes had returned to preirradiation levels.

This pattern was evident for all irradiated animals regardless of the relative time of radiological and biological exposures. In contrast, nonirradiated vaccinated animals demonstrated a gradual mild increase in both granulocytes and lymphocytes-monocytes.

Bacterial growth. To determine possible differences in the growth of strain LVS in irradiated and nonirradiated guinea pigs, animals irradiated 3 days prior to the planned vaccination and nonirradiated controls were administered 10^5 cells of viable strain LVS *via* the respiratory route. At 8 intervals thereafter, animals from each group were sacrificed and the strain LVS population was assessed. The growth patterns are shown in Fig. 1. Maximum growth in these organs occurred on the third day. With the possible exception of data obtained 8 days after vaccination, no appreciable difference in viable strain LVS population was observed between irradiated and nonirradiated animals.

Serology. The agglutinin response of irradiated and nonirradiated guinea pigs is presented in Table II. In general, the titers of irradiated animals were lower than those of nonirradiated animals but the suppression was significant ($P>.05$) at only 2 intervals (8

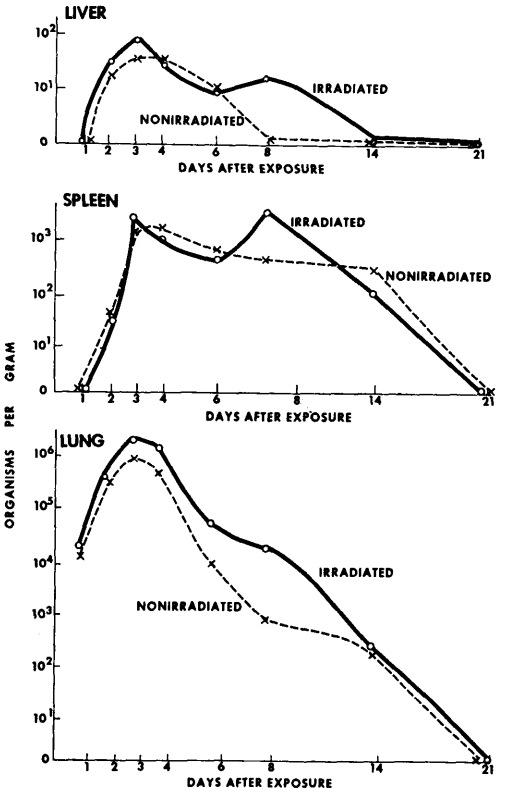

FIG. 1. Growth of *Pasteurella tularensis* LVS in organs of irradiated and nonirradiated guinea pigs.

TABLE II. Average Agglutinin Titers of Irradiated and Nonirradiated Guinea Pigs Vaccinated with *Pasteurella tularensis* LVS.

Day after vaccination	Irradiated	Nonirradiated
1, 2, 3, 4, 6	<1:10	<1:10
8	1:22	1:60
14	1:240	1:160
21	1:256	1:320
30	1:176	1:352

and 30 days). Data on other groups indicated that all irradiated vaccinated animals developed agglutinin titers regardless of when irradiated.

Resistance to challenge with highly virulent P. tularensis *SCHU S4.* To determine the effect of irradiation on the immunity afforded by aerogenic vaccination with strain LVS, survivors of combined irradiation and vaccination were challenged subcutaneously (sc) with 10^3 cells of strain SCHU S4 30 days after vaccination. Irradiated vaccinees exhibited greater resistance than nonirradiated and nonvaccinated controls when challenged with strain SCHU S4 and the degree of immunity developed by these animals was only slightly lower than that observed in nonirradiated animals vaccinated in the same manner (Table III).

Discussion. These studies have demonstrated that an acute radiation syndrome can decrease the resistance of the guinea pig to normally innocuous *P. tularensis* LVS. The demonstration of a time relationship between irradiation and vaccination for the production of maximum mortality indicates the transient nature of sublethal irradiation damage. The normally self-limiting "vaccination-infection" was not changed to a fulminating course under the influence of X-irradiation. These studies, therefore, do not indicate the mechanism responsible for the mortality in the vaccinated irradiated animals but it is evident that X-irradiation did disturb one or more subtle defense mechanisms of the guinea pig. The mortality rate may have been influenced by the route of vaccination. In other studies with irradiated guinea pigs vaccinated by the subcutaneous route(10), the overall mortality attributable to the combined irradiation vaccination was 4-fold less than that observed during the present study.

TABLE III. Immunogenicity of Live Tularemia Vaccine for the Irradiated Guinea Pig.

Time animals were vaccinated	No. of animals challenged sc with 10^3 cells of *Pasteurella tularensis* SCHU S4*	% survival 30 days after challenge
Vaccinated after irradiation		
12 days	20	60
6 "	17	59
3 "	15	53
1 "	17	59
Vaccinated before irradiation		
2 to 4 hr	20	45
Nonirradiated vaccinated	20	70
Nonirradiated nonvaccinated	20	0

* Animals challenged 30 days after vaccination.

The observation that neither agglutinin production nor the development of resistance to challenge was markedly altered by combined sublethal X-irradiation and exposure to strain LVS is in contrast to results reported when other bacterial preparations were used (1-3); however, our data are comparable to those obtained when live *P. tularensis* vaccines were administered by other routes(10-12).

Extrapolation of these results to man would entail consideration of differences in resistance to vaccination and irradiation as well as the dose and extent of body irradiation. It is conceivable that a risk might be involved if irradiated individuals were to receive live tularemia vaccine by the respiratory route, particularly if the irradiation levels were near the maximum sublethal dose and had been administered over a large body area.

Summary. Sublethal whole-body X-irradiation of the guinea pig before or after respiratory vaccination with normally innocuous *P. tularensis* LVS may result in death. No evidence of a change from a self-limiting to a fulminating type of infection was obtained. Irradiated vaccinated animals produced agglutinin titers only slightly lower than nonirradiated controls. Only a small decrease in resistance to tularemia was observed between

surviving irradiated vaccinees and nonirradiated vaccinees.

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Effect of a Low Sodium Diet on Aldosterone-Stimulating Activity Of Angiotensin II in Dogs. (31973)

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In dogs(1) and humans(2) fed a low sodium diet, ACTH produces a greater increase in aldosterone secretion than it does in control subjects. Since angiotensin II also stimulates aldosterone secretion we have studied the effect of a low sodium diet on the adrenocortical response to angiotensin II.

Methods. Male mongrel dogs weighing approximately 12 kg were fed a diet providing less than 1 mEq of sodium per day for 14 days. They were then anesthetized with pentobarbital, subjected to cannulation of the right lumboadrenal vein, bilateral nephrectomy and hypophysectomy *via* the transbuccal route. Using methods described in detail previously(1,3), the increases in 17-hydroxycorticoid and aldosterone secretion produced in these dogs by angiotensin II and ACTH were determined. The doses of angiotensin II infused were 0.017, 0.042, 0.167 and 1.67 μg/min, and the doses of ACTH 10 and 50 mU. Seventeen-hydroxycorticoids in adrenal venous plasma were measured by the method of Silber and Porter (4), and aldosterone by the method of Kliman and Peterson(5). Control dogs fed a diet containing about 40 mEq of sodium and about the same amount of potassium as the low sodium diet were also studied. The results obtained in these control dogs have been published(3).

Results and discussion. The increments in aldosterone and 17-hydroxycorticoid output produced by angiotensin II and ACTH are summarized in Tables I and II, and the pressor response to angiotensin II in Table III. Angiotensin II in doses of 0.017 and 0.04 μg/min produced increases in aldosterone secretion in the dogs fed the low sodium diet, but 0.04 μg/min failed to produce any increase in the control dogs. Larger doses of angiotensin II also produced greater increases in aldosterone secretion in dogs fed the low sodium diet, although the differences between these and the increments in the control dogs were not statistically significant. ACTH produced a greater increase in aldosterone secretion in the dogs fed the low sodium diet, confirming results previously reported from this laboratory(1). On the other hand, 17-hydrocycorticoid responses to angiotensin II and ACTH were unaffected by restricting dietary sodium intake.

The pressor activity of angiotensin II in