Influence of Sublethal X-Irradiation on Immunity of Guinea Pigs Administered Live Tularemia Vaccine.* (32473)

JOHN E. NUTTER AND MAURICE L. GUSS (Introduced by Henry T. Eigelsbach)
Biological Sciences Laboratory, Fort Detrick, Frederick, Md. 21701

This laboratory previously reported that sublethal whole-body X-irradiation (140 r) before or after respiratory vaccination of guinea pigs with normally innocuous *Pasteurella tularensis* LVS may result in death(1). 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. The present study concerns the effect of sublethal X-irradiation on the responses of guinea pigs vaccinated by the subcutaneous (sc) route.

Materials and methods. The care and selection of guinea pigs as well as the conditions of X-irradiation, bacterial strains employed, and serological tests have been previously described(1).

Vaccination. Live vaccine organisms, *P. tularensis* LVS, were administered sc in the inguinal region at a dosage of 1×10^5 cells in 0.2 ml gel-saline diluent.

Irradiation. The times between irradiation and later vaccination of the animals were: 12 days, 6 days, 3 days, 1 day, 12 hours, or 1 hour. The times between vaccination and later irradiation were 4 hours, 12 hours, 1 day, or 3 days. Control groups included nonirradiated vaccinated, irradiated nonvaccinated, and untreated normal animals.

Serology. At nine intervals, from 1 to 25 days post-vaccination, 5 animals of each group were bled by cardiac puncture and the serum used for qualitative slide or quantitative tube agglutination tests.

Aerosol challenge. Twenty-three days after vaccination, 20 animals of each test and control group were exposed to an aerosol challenge with the highly virulent SCHU S4 strain of *P. tularensis*. The inhaled dose per animal ranged from 18 to 97 cells contained in a small-particle aerosol generated by a modified Henderson apparatus(2).

Results. Combined irradiation-P. tularensis *LVS vaccination.* During the 23-day observation period following vaccination, 8 of 400 irradiated vaccinated (test) animals died. Seven and one-half per cent of the guinea pigs vaccinated 6 days or 12 hours after irradiation and 2.5% of the groups vaccinated 1 day after or 4 hours before irradiation died (Table I). LVS organisms were not cultured from the lung, liver, or spleen of these animals. Necropsy revealed hemorrhagic involvement of the lung and subcutaneous tissue of 5 of the 8 animals. This pathology is not typical for tularemia.

TABLE I. Guinea Pig Mortality After Sublethal X-Irradiation and Subcutaneous Vaccination with *Pasteurella tularensis* LVS.

Time animals were vaccinated	% dead 30 days after vaccination*
Vaccinated after irradiation	
12 days	0
6 "	7.5
3 "	0
1 "	2.5
12 hr	7.5
1 "	0
Vaccinated before irradiation	
4 hr	2.5
12 "	0
1 day	0
3 "	0
Nonirradiated vaccinated controls	0
Irradiated nonvaccinated controls	0

* Based on 40 animals per group.

Serology. Table II presents the results of the serological study of animals irradiated before vaccination. Listed in the Table are

* In conducting the research reported herein, the investigator adhered to "Guide for Laboratory Animal Facilities and Care" established by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources NAS-NRC.

TABLE II. Agglutinin Response of Guinea Pigs Exposed to Sublethal X-Irradiation and Later Vaccinated Subcutaneously with *Pasteurella tularensis* LVS.

Time animals were vaccinated after irradiation	Days after vaccination 1	3	6	9	12	15	18	21	25
12 days	Neg	Neg	1:20	1:40	1:160	1:320	1:160	1:320	1:160
6 "	"	"	Neg	1:20	1:80	1:320	1:320	1:160	1:160
3 "	"	"	"	1:10	1:80	1:80	1:640	1:160	1:160
1 day	"	"	"	1:10	1:40	1:160	1:320	1:320	1:320
12 hr	"	"	"	1:20	1:40	1:80	1:320	1:320	1:320
1 "	"	"	"	1:10	1:80	1:80	1:320	1:320	1:640
Nonirradiated vaccinated controls	"	"	"	1:20	1:40	1:160	1:320	1:160	1:320

the agglutinin titers obtained from the serum pools of each group at the nine sampling intervals. No appreciable differences in titer between test animals and nonirradiated vaccinated controls were observed.

The results of the serological study of animals irradiated following vaccination showed similar responses. Serum samples were negative for agglutinins through the sixth day following vaccination and no appreciable differences in titer between test animals and nonirradiated vaccinated controls were observed.

Resistance to challenge. Table III presents data on mortality subsequent to the challenge of animals irradiated before vaccination. There was no marked difference between the mortality of irradiated vaccinated and nonirradiated vaccinated guinea pigs. Analysis of the average day of death data from animals of the test groups did not show any marked alteration of the time of death in comparison with the nonirradiated vaccinated group.

TABLE III. Mortality of Guinea Pigs Exposed to Sublethal X-Irradiation and Later Vaccinated Subcutaneously with *Pasteurella tularensis* LVS Following Aerosol Challenge with *Pasteurella tularensis* SCHU S4.

Time animals were vaccinated after irradiation	% Dead after challenge with strain SCHU S4 15 days	30 days	Avg day of death
12 days	20	45	23.6
6 "	35	50	21.8
3 "	60	80	17.5
1 day	30	60	20.8
12 hr	50	70	20.1
1 "	45	60	20.4
Nonirradiated vaccinated controls	45	60	20.4
Irradiated nonvaccinated controls	95	95	7.6
Normal controls	90	90	7.4

A study of mortality subsequent to the challenge of animals irradiated after vaccination revealed no marked differences in mortality of animals vaccinated and irradiated and of the nonirradiated vaccinated controls. In addition, the average time of death among animals of both the test and vaccinated control groups demonstrated a homogeneous response regardless of the experimental procedure prior to the challenge was the highly virulent SCHU S4 organisms.

Discussion. This study has demonstrated that sublethal whole-body X-irradiation of guinea pigs at ten intervals from 12 days before to 3 days after sc vaccination with *P. tularensis* LVS did not alter the agglutinin response or the development of immunity. These findings are comparable to those reported on the effect of sublethal X-irradiation on the course of agglutinin production and the development of immunity when LVS was administered to guinea pigs by the respiratory route(1). Our results in irradiated vaccinated animals, challenged by the respiratory route, are comparable to those previously reported for mice and guinea pigs when other routes of challenge were used(3-5).

A comparison of results of the aerosol and sc vaccination studies in guinea pigs revealed one significant difference. A maximum mortality of 7.5% in any group following irradiation and sc vaccination contrasts sharply with a mortality of 25% when the animals were vaccinated aerogenically 3 days

after sublethal X-irradiation. Total mortality was four-fold less among guinea pigs vaccinated by the sc route when the same treatment intervals were compared.

Summary. Sublethal X-irradiation of guinea pigs 12 days before to 3 days after sc vaccination with viable *P. tularensis* LVS resulted in a maximum mortality of 7.5% at 2 intervals. Surviving animals, however, displayed an agglutinin response and resistance to respiratory challenge similar to that of nonirradiated vaccinated animals.

We wish to acknowledge the collaboration of Lt. Colonel Joshua Henderson in the X-irradiation procedures.

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Teratogenic Compounds of *Veratrum californicum* (Durand). III Malformations of the Veratramine-Induced Type from Ingestion of Plant Or Roots. (32474)

RICHARD F. KEELER AND WAYNE BINNS
Poisonous Plant Research Laboratory, Animal Disease & Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Logan, Utah

We have previously reported that the alkaloid veratramine, a parent alkamine of the steroidal class of veratrum alkaloids, produced congenital abnormalities in lambs born to ewes that ingested the alkaloid on the 14th day of gestation(1). The malformation was characterized by slight lateral or medial bowing of the front legs, slight to marked flexure of the knee joint, looseness (hypermobility) of the hock or stifle joints, or a

TABLE I. Non-Cyclopian Teratogenic Effects in Offspring from Ewes Dosed with *V. californicum* on the 14th Day of Gestation.

	14th day dose		
Ewe No.	Amt (g)	Doses (No.)	Effect on offspring
			Root
528	45	2	(Normal)
220	45	2	(Abnormal) single male lamb with looseness (hypermobility) in the hock and stifle joints.
561	45	2	(Normal)
522	55	a.m. only	(Abnormal) twins—male very weak; female no muscular control in rear limbs.
662	55	a.m. only	(Normal)
376	45	2	(Normal)
777	45	2	(Abnormal) twins—female with looseness (hypermobility) in hock and stifle joints; female dead at birth.
293	45 25	a.m. p.m.	(Abnormal) single female lamb with right front leg twisted.
			Tops
515	225	1	(Normal)
743	225	1	(Normal)
641	200	1	(Abnormal) single female lamb with one front leg bowed laterally, the other medially.
813	200	1	(Abnormal) single female lamb with rear limbs somewhat twisted and poor muscular control in rear limbs.