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Effect of Irradiation and Test System on Development of Tetanus Antibodies.* (28095)

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The effect of whole-body irradiation on antibody formation has been extensively investigated(1,2,3). Studies using small laboratory animals have generally indicated a marked depressive effect of irradiation on response to a primary stimulus; response of a previously sensitized animal to a booster dose was not significantly affected. Since prophylaxis against tetanus depends on routine immunization with toxoid, followed by a booster dose administered at time of injury to evoke a rapid and high antitoxin response, it was felt necessary to reinvestigate this response should the injury be associated with whole-body irradiation. The dog was used as the experimental animal.

Methods. Young adult, healthy, mongrel dogs weighing 11 and 16 kg, and negative on repeated examination for internal parasites, were used. A modified live virus vaccine to protect the dogs against canine distemper, infectious canine hepatitis, and rabies was used several weeks prior to irradiation. Irradiations were performed with Co-60 pellets in a cylindrical arrangement around the exposure area. Single whole-body doses of 250, 310, 375 and 544 r were administered at a dose rate of approximately 30 r/min as measured in air at the position of the midpoint of the dog.

Three lots of standard commercial com-

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TABLE I. Mortality and Survival Time of Dogs Exposed to CO^{60}.

Dose, r	Mortality	Range, Day of death
250	0/10*	—
310	5/10	8–33
375	7/9	12–19
544	3/3	10–33

* No. dead within 30 days/No. irradiated.

bined tetanus-diphtheria toxoids, adsorbed to potassium alum, were used during these studies. They contained approximately 10 flocculating units (Lf) of tetanus toxoid per 0.5 ml dose. Toxoids were composed from sublots which ranged in purity from approximately 500 to 1500 Lf per mg non-dialyzable nitrogen. These toxoids also contained 2-3 Lf/ml of diphtheria toxoid per ml (purity ranging from 1200 to 2100 Lf/mgn), and 4 mg potassium alum per ml. Eighteen to 24 hours after irradiation the dogs were inoculated subcutaneously with 0.5 ml toxoid. To study the secondary response, the dogs were prepared by a 0.5 ml dose of toxoid administered 30 days before irradiation and a booster dose of 0.5 ml was given 18 to 24 hours after exposure. Non-irradiated and non-vaccinated dogs were included as controls.

Venous blood was obtained at appropriate intervals for hemograms and to furnish serum for antitoxin titration. Standard hematological methods were used. The phase microscope was used for the platelet counts.

Tetanus antitoxin levels in blood were determined by 2 different methods; the *hemagglutination technic* in which tanned type O human red cells sensitized with tetanus toxoid are agglutinated by the positive sera and related to units of antitoxin by comparison with reference antitoxin obtained from the Division of Biologic Standards, Nat. Inst. Health; and by the *toxin neutralization test* in which the serum is titered for its ability to prevent death or paralysis of mice from a standard dose of tetanus toxin, calibrated by titration with reference antitoxin.

Results. The LD 50/30 was estimated as 320 ± 25 r by probit analysis(5) of the radiation mortality data (Table I). The dose response curve indicated that 250 r and 375 r were approximately LD 15/30 and LD 80/30 doses, respectively. The median days of death were 19, 16 and 10 for 310 r, 375 r, and 544 r, respectively. These results, as well as the circulating leukocyte counts, blood platelet, reticulocyte, hematocrit, ESR, clinical course and necropsy findings were consistent with the radiation dose administered and were as reported by others(6,7,8).

If only the response to primary vaccination in those animals surviving irradiation is considered, all dogs had protective levels of antitoxin by the fourth week, as measured by the toxin neutralization technic (Table II). However, as seen in Table III and Fig. 1, the mean antitoxin titers were distinctly lower in the irradiated animals and appearance of the antibody was delayed. When the antitoxin is measured by the hemagglutina-

TABLE II. Development of "Protective" Antitoxin Titers After Primary Vaccination.

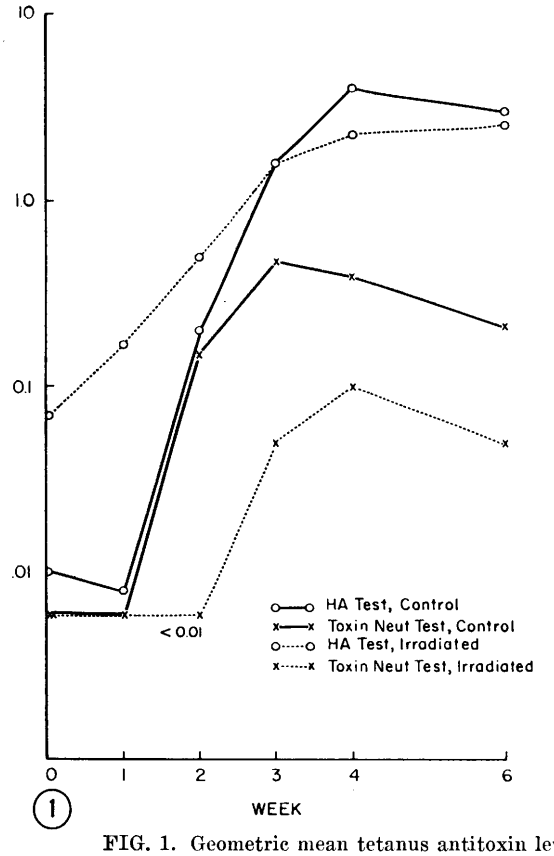
Treatment	Time after vaccination (wk) 1	2	3	4	6	9
Vacc, not irrad	3/16*	14/15	15/15	16/16	4/4	—
" 250 r	0/8	1/8	7/7†	7/7	7/7	3/3
" 310 r	0/6	2/6	2/4‡	5/5	3/4	1/2
" 375 r	0/6	0/4	0/1	1/1	1/1	—
" 544 r	0/3	—	—	—	—	—

* No. with 0.01 or greater units antitoxin (mouse protection)/No. vacc.
† One dog sacrificed for other purposes.
‡ Two dogs not tested.

TABLE III. Mean Tetanus Antitoxin Levels by Week After Primary Immunization Geometric Means (95% Confidence Limits)—International Units.

Weeks	Irradiated*	Control
	By Toxin Neutralization Test.	
0	<.01†	<.01†
1	<.01†	<.01†
2	.01 (<.01 - .01)	.15 (.05- .47)
3	.05 (.01 - .26)	.47 (.26- .85)
4	.1 (.05 - .30)	.40 (.21- .76)
6	.05 (.01 - .19)	.21 (.02-1.21)
	By Hemagglutination Test	
0	.07 (.025- .16)	.01 (0 - .023)
1	.17 (.07 - .4)	.008 (0 - .022)
2	.5 (.17 - 1.4)	.2 (.08- .4)
3	1.6 (.7 - 3.0)	1.6 (.9 -3.0)
4	2.3 (1.3 - 4.8)	4.0 (2.2 -6.0)
6	2.5 (.5 -20.0)	3.0 (1.3 -6.1)

* All levels of irradiation pooled.
† All values at same level.



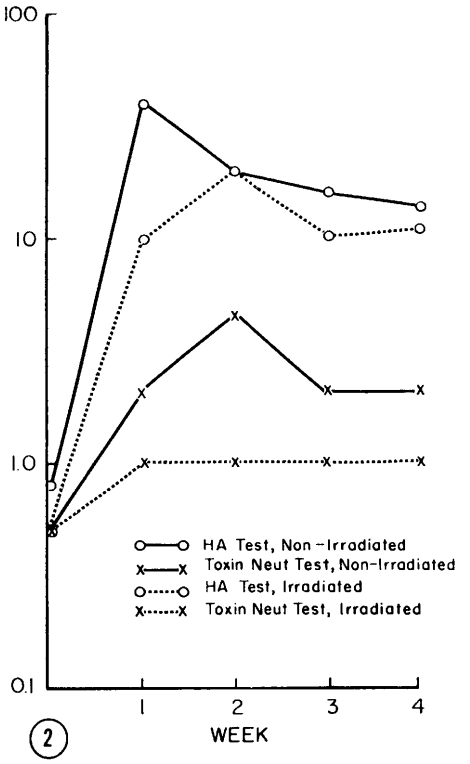


FIG. 1. Geometric mean tetanus antitoxin levels by week after primary immunization.
FIG. 2. Geometric mean tetanus antitoxin levels by week after booster immunization.

tion technic, irradiated animals appear to produce antitoxin earlier and in quantities comparable to control dogs.

The results obtained following booster vaccination differed somewhat from the results of primary vaccination (Table IV and Fig. 2). In general, non-irradiated dogs which received a second dose of tetanus toxoid one month after the primary dose responded to this second stimulus, whether examined by the hemagglutination or toxin neutralization test. Irradiated animals, on the other hand, showed clear cut secondary response to the booster dose only as measured by the hemagglutination method, but showed none or very little antibody response with the toxin neutralization test. In 8 of the 9 dogs, antitoxin level was definitely increased when measured by hemagglutination, but in only 3 was an increase indicated by toxin neutralization. With doses of irradiation of 310 and 375 r, even though the dogs ultimately died, 5 out of 6 showed a marked response as measured by hemagglutination tests, while with the

TABLE IV. Mean Tetanus Antitoxin Levels by Week After Booster Immunization Geometric Means (95% Confidence Limits)—International Units.

Weeks	Irradiated*	Control‡
By Toxin Neutralization Test.		
0	.5 (.20- 1.4)	.5
1	1.0†	2.1
2	1.0†	4.7
3	1.0†	2.1
4	1.0†	2.1
By Hemagglutination Test.		
0	.5 (.24- 1.0)	.8
1	10.0 (5.5 -18.0)	40.0
2	20.0 (5.6 -20.0)	20.0
3	10.0‡	16.0
4	11.0‡	14.0

* All levels of irradiation pooled.
† All values at same level.
‡ Only 3 or 4 animals, confidence limits unrealistic.

toxin neutralization test, poor, if any, antitoxin response was noted.

Discussion. The geometric mean titers obtained by the 2 technics show discrepancies. In the non-irradiated animal responding to first contact with toxoid, antitoxin unit values are in excellent agreement for the first 2 weeks, after which the levels measured by hemagglutination inhibition continue to rise, with only a slight fall between the 4th and 6th weeks. With irradiated animals, the rise in antibodies measured by toxin neutralization in the mouse does not occur until the third week, and then only feebly, while the response according to the hemagglutination test does not differ significantly from the response of the non-irradiated animal. Similar results are obtained with a booster dose, except that, as expected, the response occurs earlier.

Conclusions as to the effect of irradiation on either the primary or secondary response to toxoid depend on whether the antibody titers obtained by mouse neutralization test or by the hemagglutination technics are considered.

Measured by the mouse neutralization test, dogs given a primary vaccination 18-24 hours after irradiation showed a definite lowering of the antitoxin titer compared to non-irradiated dogs. This agrees with results reported in other species in which a distinct radiosensitivity of the primary antibody response has been noted by numerous workers. However, when the antibody titers of the same animals were measured by hemagglutination titer, no real effect of radiation on primary response was noted. It is noteworthy that all surviving irradiated animals developed protective levels of antitoxin; however, this may be attributed to the use of an alum precipitated antigen which remained within the body until the dog regained his ability to produce antibody. The delay in antibody formation may be the measure of the period during which the dog was not able to respond to the presence of the antigen. Had this been a soluble antigen no antibody response might have appeared at higher levels of irradiation.

The results obtained following the secondary response were similar, insofar as measurement of antitoxin by mouse neutralization demonstrated a poor to zero antitoxin response. These results are in marked contrast to those of other authors in other species who found but little, if any, effect of irradiation on the secondary antibody response. They are also in marked contrast to the result of the hemagglutination tests in the same dogs which indicate that irradiation had little effect on the secondary response.

The possible reasons for the discrepancies between the titers must be considered. The first possibility is a systematic error in carrying out either the mouse neutralization or the hemagglutination test. This is unlikely since the tests were performed by skilled workers who had repeatedly performed both tests before, and the same results were obtained on retesting the sera. Moreover, this would not explain the fact that in unirradiated animals both primary and secondary response hemagglutination and mouse protection titers in general, although not in every case, paralleled each other and that only following irradiation was this dissociation between the 2 tests manifest. Thus, there seems to be a real discrepancy between the two tests. The mouse neutralization test measures only true antitoxin, while the hemagglutination test may measure other antibodies, in addition to antitoxin. The hemagglutination reaction is not a non-specific phenomenon; sera of dogs to which tetanus toxoid had never been administered and which died after high levels of irradiation did not produce hemagglutination. It is clear that irradiation has altered the quality of the secondary response to this antigen. The increase in titer by the hemagglutination test may mean that either (1) an antibody is being formed which is less avid for the toxin and is unable to neutralize it, or (2) the hemagglutination test detects an antibody against some constituent or fragment of the tetanus toxoid other than the physiologically active prosthetic group. Other data support this latter hypothesis, indicating marked discrepancies in young adult men between results of the toxin-neutralization and hemagglutination tests. These observations are con-

sistent with those of Talmage(9) and others, relating the avidity of an antibody to the phase of immunization which it represents, and suggest that an attempt to quantitate the hemagglutination test is valid only when the reference serum in historically comparable to the test serum. Studies are under way to differentiate the mechanism.

Conclusion. Whole-body irradiation of dogs immunized with an alum precipitated tetanus toxoid results in a delay in appearance of antitoxin if the first toxoid is administered after radiation. Dogs irradiated 30 days after a first dose of toxoid and receiving a booster injection 24 hours after irradiation presented a good antitoxin response when measured by the hemagglutination test. However, a poor antibody response was found if the antitoxin was measured by the toxin neutralization technics.

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Comparative Immunology. Antibody Response in *Dipsosaurus dorsalis* at Different Temperatures.* (28096)

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A lizard from the California desert, *Dipsosaurus dorsalis* has been found to produce antibodies for the H-antigen of *Salmonella typhosa* after a short period of immunization provided that ambient temperature was maintained at 35°C(1). At 25°C antibody response was minimal or absent. This observation suggested that the process could be regulated by changing the ambient temperature. The present report concerns a study of the influence of temperature on antibody response in this group of animals.

Materials and methods. Details of the capture and maintenance of animals, preparation of *S. typhosa* vaccine and titration of antibodies have been described(1). The antigen used in the present experiments differed in that one volume of *S. typhosa* H-antigen (2×10^9 killed cells per ml) was diluted with an equal volume of Pentex rabbit gamma globulin (RGG) dissolved in 0.9% NaCl at a concentration of 20 mg/ml. The RGG was included to study simultaneously the antibody response to a soluble protein. Measurement of antibodies to RGG has presented difficulties due to the small volumes of serum available. These studies will be reported separately.

Daily injections of antigen (0.2 ml) were given subcutaneously into the thigh for the first 3 days of the first week. During the second week, the same dose was given intraperitoneally on each of the first 3 days.

During the 10-day immunization period animals were maintained at constant ambient temperatures of 25°C, 35°C or 40°C. Cloacal temperatures fell within the range: ambient temperature ± 1°C.

During the post-immunization period, various groups were exposed to different com-

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