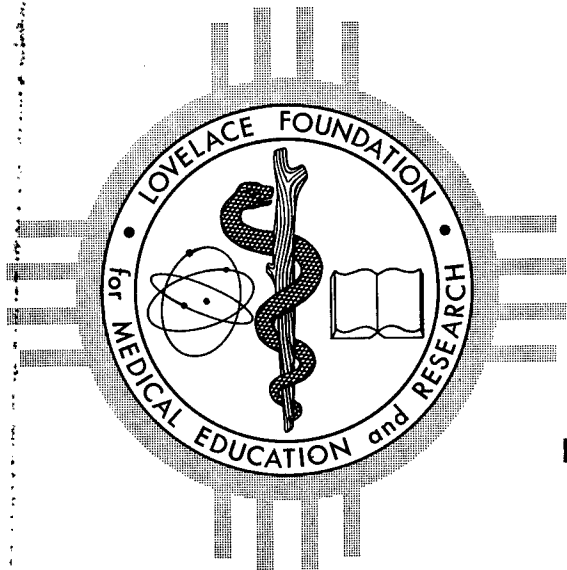


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**RESPONSE TO SECONDARY ANTIGENIC
STIMULUS AFTER WHOLE BODY
X-IRRADIATION IN THE BEAGLE**

Albuquerque, New Mexico

by

**F. F. PINDAK,
J. F. STARA
AND W. E. CLAPPER**

September 1964

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by

F. F. Pindak, J. F. Stara* and W. E. Clapper

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ABSTRACT

The results of the administration to healthy beagles of a secondary antigenic stimulus (booster dose) of Leptospira canicola and infectious canine hepatitis virus (ICH) are reported. Twelve previously vaccinated animals were used. Six were exposed to a 335 r dose of x-irradiation. Three controls and three irradiated dogs received the booster dose two days after the irradiation and the remaining six, seven days after. Agglutinin titres for Leptospira canicola of 1:32 to 1:256 were observed in the 12 beagles before the booster dose. No increases of significance were found in either the controls or the experimental group after the booster dose.

Complement fixing titres for the ICH virus of 1:16 to 1:64 were seen in all animals before revaccination. There was no significant difference in the titres of the control group and the experimental group which were given the ICH booster seven days after irradiation. However, the response observed in the controls given the booster after two days was absent in the irradiated animals.

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INTRODUCTION

Evidence of a repressive effect on the primary antibody response when animals are irradiated before the antigenic stimulus is well documented (1-4). However, Klemparskaya et al.² state that the literature contains little on the efficiency of revaccination of irradiated animals. Reference is made to a study which indicated that rabbits injected with polonium were not protected when revaccinated with diphtheria anti-toxin³. Revaccination of rabbits with tularemia bacteria six to seven months after irradiation did stimulate the formation of agglutinins according to other work cited. The authors then reported that survival rates were increased from 24.2% to 62.4% in irradiated mice that were re-immunized with typhoid vaccine⁴. Stoner and Hale¹ showed that the secondary response to tetanus toxoid in mice is sensitive to irradiation, but the primary is more sensitive than the secondary. The sensitivity is dependent upon the time of irradiation with reference to the second antigenic stimulus and upon the radiation dose.

The importance of the determination of the effect of radiation on the antibody response to revaccination assumes more than academic interest, if it is considered that such immunization may offer some protection against radiation damage.

The Department of Microbiology is participating in a program to make a continuous evaluation of the health of beagles exposed by inhalation to various fission products. Since the immune response may vary somewhat with different species, it was considered advisable to carry out some exploratory work with the beagle before beginning the long-term experiments. The results, using animals immunized with leptospira and infectious canine hepatitis virus (ICH) and exposed to x-irradiation, are reported.

METHODS

Twelve dogs of approximately one year of age were inoculated with 3 ml of the leptospira antigen three times at weekly intervals, followed by 0.5 ml two times at weekly intervals. A booster dose of 1 ml was given two months after the last injection of the primary immunization series, and the test booster dose of 1 ml one month following this. The leptospira antigen consisted of a suspension of Leptospira canicola. This organism was grown in Vervoot's (5) medium containing 10% of heat-inactivated, normal rabbit serum. Fully grown cultures were centrifuged at 12,000 g for one hour at 5°C. The supernate was discarded. The sedimented spirochetes were washed three times in Vervoot's medium without serum and resuspended in this to a volume of one-half that of the original culture. Two-tenths per cent of formalin was added to the vaccine.

The same dogs were also immunized by two 2-ml injections of ICH virus spaced two weeks apart. The secondary response was elicited by injection of 1 ml of the vaccine one month following the last injection. The ICH vaccine was furnished through the courtesy of Dr. L. E. Carmichael of Cornell University and contained 1,500 tissue culture doses per ml.

Six dogs were exposed to a midline air dose of 335 r of x-irradiation. The booster dose of each organism was given to three of these animals two days after exposure and to three seven days after exposure. Blood

was drawn for serological studies before irradiation and before the secondary antigenic stimulus and at intervals as shown in Table 1. The titres for L. canicola were determined by agglutination tests and for ICH by complement fixation.

RESULTS AND DISCUSSION

1. Leptospira Agglutinins

No rise in titre was seen when the "booster" or secondary immunizing stimulus was given, either in the controls or the irradiated animals. The response to the primary vaccination was good, as evidenced by the values shown in Table 1 before the booster dose was given. The agglutinin titres of 1:32 to 1:256 correspond closely to those reported by Menges et al. (6), who measured agglutinins up to 77 days after a single inoculation with living organisms. The values shown in Table 1 were obtained approximately one month after the completion of primary immunization. There was a general decrease thereafter, even though the booster dose consisted of the same vaccine as the original. No explanation can be given for the lack of response in the control groups unless the antibodies were in sufficient concentration to combine with the antigen and so interfere with the antigenic stimulus. It is of some interest to note that low titres were still observed approximately four months after the primary stimulus.

2. Complement Fixing Antibodies for ICH

Table 2 shows the values in the ICH experiments obtained for complement fixing antibodies. There was considerable variation among animals, but none of the dogs in the group receiving the booster dose two days after irradiation showed an increase in titre. In the controls, two dogs (numbers 2 and 3) showed a four-fold rise in titre, three (numbers 1, 4, and 11) a two-fold, and one (number 12) showed no increase, but technical difficulties prevented obtaining a reading from the serum of dog 12 on the day when an increase in titre would have been most likely. In the animals given the booster dose seven days after irradiation, dog 9 showed a two-fold increase in titre at one time, while the other two

TABLE 1

AGGLUTININ TITRES TO LEPTOSPIRA CANICOLA

Booster Dose 2 Days After Irradiation						
Days before or after booster dose	Controls			Irradiated		
	1 ^b	2	3	5	7	8
Before						
1	256 ^a	256	128	64	128	128
After						
5	256	32	64	32	128	32
12	-	32	64	8	128	32
26	64	32	64	<8	64	32
54	64	8	16	<8	64	16
97	64	8	16	<8	died	16

^aValues are given as the reciprocal of the last dilution which gave a positive reaction.

^bNumbers identify the dogs.

Booster Dose 7 Days After Irradiation						
Days before or after booster dose	Controls			Irradiated		
	4	11	12	6	9	10
Before						
8	32	128	64	32	32	128
After						
0	32	128	32	32	32	128
7	32	64	64	32	16	128
21	32	64	-	64	<8	8
49	16	32	16	32	died	<8
92	8	16	16	16		died

TABLE 2

CF ANTIBODIES TO ICH VIRUS

Booster Dose 2 Days After Irradiation								
Days before or after booster dose	Controls				Irradiated			
	1 ^b	2	3	G.M. %	5	7	8	G.M. %
Before								
1	32 ^a	32	64	100	64	64	32	100
After								
5	32	32	64	100	64	32	64	100
12	-	64	256	283	32	64	64	100
26	64	128	256	317	64	32	64	100
54	32	16	64	79.4	32	32	32	63
97	32	16	64	79.4	16	died	32	50

^aValues are given as the reciprocal of the last dilution which gave a positive reaction.

^bNumbers identify the dogs.

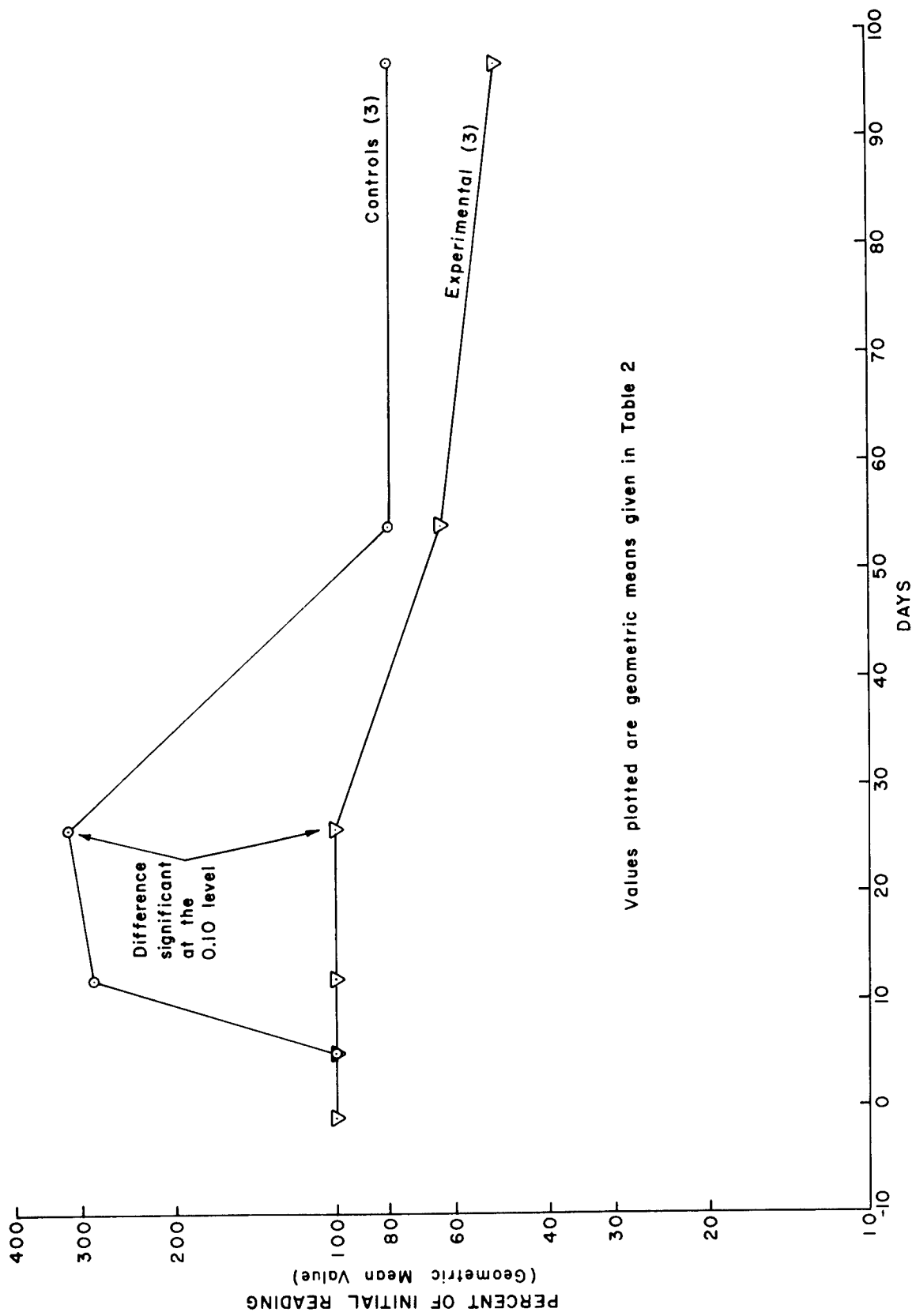
G.M. = Geometric mean of values normalized to the initial reading.

Booster Dose 7 Days After Irradiation								
Days before or after booster dose	Controls				Irradiated			
	4	11	12	G.M. %	6	9	10	G.M. %
Before								
0	32	16	32	100	64	64	32	100
After								
7	32	16	32	100	64	128	32	126
21	64	32	-	200	32	32	16	50
49	32	16	32	100	32	died	16	50
92	32	16	32	100	16		died	25

showed no increase.

Since the relative changes in the measurements for a particular animal are significant rather than the absolute values, the measurements were normalized to the initial measurement. This procedure also allows easy comparison between the measurements for different animals even though their initial values may have been different. The geometric mean of the normalized values, as shown in Table 2, was calculated for the three animals in each group (experimental and control) at each time interval. A graph of these means (Figure 1) shows the difference between the control group and those given a booster dose of ICH two days after exposure to radiation. The greatest difference was at 26 days and this is significant at the 0.10 level. These results indicate that the secondary immune response in beagles is probably affected by irradiation in much the same way as those reported for tetanus antitoxin in mice by Stoner and Hale (1). They found wide variation in individual animals, but when the serum was pooled, the titres of the controls were considerably higher than the titres from the pool of the irradiated animals. As shown in Figure 1, the response noted in the controls was not apparent in the irradiated animals. When the booster dose was given seven days after irradiation, there was a smaller difference between the control and experimental groups. This may be due to the samples having been taken at a time when the peak titres were missed, since the control group showed a doubtful response. The geometric means were again calculated and are shown in Table 2. Statistical significance could not be shown. However, Stoner and Hale (1), using large numbers of mice, found the sensitivity of the response was greater when the stimulus was given several days after exposure than when it was given one to two days after.

The maximum response in the beagles to the secondary stimulus was between the fifth and 26th days. Talmadge et al. (7) found the anamnestic response in rabbits to be higher on the ninth day than on the fifth day and that the response to a stimulus given two days after irradiation was less than that of controls. Makinoden et al. (8) reported that the



Values plotted are geometric means given in Table 2

Fig. 1 Effect of 335 r X-Irradiation on Secondary Immune Response to Infectious Canine Hepatitis Virus. Secondary Stimulus Given 2 Days After Irradiation.

maximum response to a secondary stimulus in mice was at 12 days.

SUMMARY

Twelve beagles were immunized with antigens of Leptospira canicola and ICH virus approximately one month before the test. Six of these dogs were exposed to 335 r (an LD₅₀ dose) of whole body x-irradiation. Two days later a booster dose was administered to three of the exposed animals and three control animals. Seven days after exposure the remaining three irradiated animals and three control animals were given a booster dose.

No secondary response was observed in the control or experimental animals receiving L. canicola, although the antibody levels produced by the primary immunization were adequate.

The secondary response to ICH was eliminated in the dogs irradiated two days before the injection. However, a significant increase of titre was not observed in either the control group or those given the booster dose seven days after irradiation.

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