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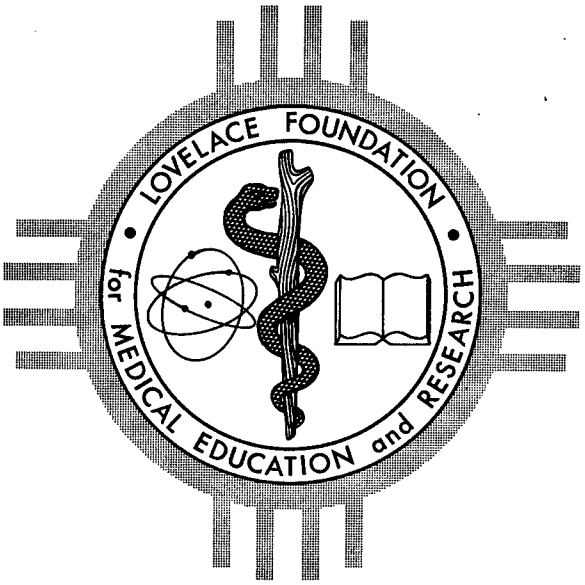

Albuquerque, New Mexico

AEC RESEARCH AND DEVELOPMENT REPORT

UNCLASSIFIED

IMMUNE RESPONSE TO A SECONDARY STIMULUS WITH LEPTOSPIRA CANICOLA AND INFECTIOUS CANINE HEPATITIS IN BEAGLES EXPOSED TO SR^{90}

by

W. E. CLAPPER, A. SANCHEZ and J. LEVY

June 1966

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W. E. Clapper, A. Sanchez and J. Levy

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ABSTRACT

The secondary immune response to L. canicola and ICH virus was depressed in Beagle dogs which had inhaled particles of Sr^{90} 1-2 days and 7 days before the immune stimulus. The initial body burden was 31-46 microcuries per kilogram. The peak titre was reached for both antigens within eleven days in both control and exposed dogs. This amount of exposure did not accelerate the rate of decline of the antibodies, and the animals appeared to have recovered their ability to produce antibodies after five months.

ACKNOWLEDGMENTS

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IMMUNE RESPONSE TO A SECONDARY STIMULUS WITH LEPTOSPIRA CANICOLA AND INFECTIOUS CANINE HEPATITIS IN BEAGLES EXPOSED TO SR^{90}

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INTRODUCTION

The repressive effect on the primary antibody response in animals irradiated before an antigenic stimulus is well documented [1-3]. These changes are related to the time and amount of irradiation, the antigen and the species. Until recently, however, relatively few studies have been made of the effect of radiation on the secondary or "booster" response. Taliaferro, et al[4] have reviewed the work that has been done and have concluded that the secondary response is less radiosensitive than the primary one. If true, this could be important in relation to reimmunization of individuals who have been exposed to radioactive fission products. They point out however, that for several reasons, the chief one being insufficient data, this can only be tentatively accepted.

As part of a continuous evaluation of the health of Beagles exposed by inhalation to various fission products, the effect on the immune response was included. The dogs were routinely vaccinated with Leptospira canicola and infectious canine hepatitis virus, and re-immunized at regular intervals. This made it possible to measure the response to both a bacterial and viral antigen. The various isotopes involved localize in different parts of the body. Continuous radiation from some of these will occur with a greater effect on certain organs because of proximity, i. e., Sr^{90} on bone marrow.

Under such conditions the exact amount of radiation that a given organ will receive at a given time after the exposure cannot be determined. It therefore is of interest to measure the effect on antibody production since it may be different from that which would be predicted from previous observations made after single, multiple or continuous doses of X-rays or even after intravenous injection of the isotope.

In this paper some effects of Sr^{90} as related to the time of injection of the "booster" dose, the time the peak titre is reached, and the recovery from irradiation are reported.

METHODS

Sixteen Beagles between the ages of 11 and 18 months were used. Eight served as controls, and the remaining 8 were allowed to inhale aerosolized particles of Sr^{90}. They were exposed in groups of 2, on different days. Initial total body counts indicated body burdens of between 31-46 microcuries per kilogram. All dogs had been immunized previously with commercially prepared vaccines of Leptospira canicola, infectious canine hepatitis (ICH), distemper, and rabies viruses.* Three of the Beagles were irradiated one day, two at 2 days, and three at 7 days before the secondary (booster) immunization with L. canicola and ICH. Another booster dose of Leptospira was administered at approximately 5 months after the first, and of ICH at about 7 months.

Blood was drawn at rather frequent intervals for the first 3 months, then every month for seven times. The serum obtained was inactivated at 56°C for 30 minutes and complement fixation titres were determined by the tube method described by Lennette[5]. The L. canicola agglutinin titres were determined by a micro-technique adapted for this purpose. Two-fold dilutions from 1:2 to 1:128 were made in micro panels using 0.05 ml serum. To each dilution and a control well with no serum was added 0.05 ml of a 1-10 dilution of L. canicola antigen (Difco). The plates were sealed with plastic tape and left overnight at room temperature. Agglutination was observed by placing a drop from each well on a glass slide and viewing it with a dark field microscope at 100 X magnification. Any definite agglutination greater than that observed in the control was read as positive. This method was found to give comparable titres with a known positive anti-serum when live L. canicola was used. All specimens from

* Fromm Laboratories, Inc., Grafton, Wisconsin.

one experimental and one control animal were titred simultaneously.

RESULTS

1. Leptospira Agglutinins

Table 1 shows the serum agglutinin titres obtained on each animal. Serums were titred before the first secondary immune stimulus but values are not shown since no change was observed from that measured 2-4 days after. Three of the 5 controls showed 4-fold or greater rises in antibody titre during the first 11 days, while only one of six of those irradiated before the booster dose showed a 4-fold rise.

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, given in percent, is shown in the column at the right of the actual values. The titres determined just before the administration of the tertiary stimulus (2nd booster) were used as the initial value for the last five months since preceding titres were not given from some dogs. Examining these mean values, it appears that the inhalation of Sr^{90} 1-2 days prior to inoculation of Leptospira, inhibited the secondary response, although this inhibition is only partial. There was no further increase after the initial rise within the 11 day period in the irradiated dogs as there was in the controls. There were only 2 animals exposed 7 days before immunization but these showed no increase in antibodies. The response after the tertiary stimulus showed little increase in the controls with the exception of dog number 20. There was somewhat greater response in the first group of irradiated animals (1-2 days before secondary stimulus) than was seen immediately after the exposure. No increase was found in 3 Beagles in the 2nd group (irradiated 7 days before).

TABLE 1

EFFECT OF INHALATION OF Sr^{90} ON THE SECONDARY IMMUNE RESPONSE TO LEPTOSPIRA CANICOLA IN BEAGLES

AGGLUTININ TITRES

Days After 1st Booster	Controls									Irrad. Before Secondary Stimulus									
										1-2 Days						7 Days			
	20[1]	18	24	1E	1D	88	11	84	Mean[3]	82	13	17	1C	14	Mean	19	23	83	Mean
2-4				1	1	1	8	8	100	8	8	8	1		100		16	4	
9-11				8	4	4	8	16	303	8	16	64	1		200		16	4	
16-17				8	4	8	8	8	303	8	16	32	4		238		8	--	
24-31				4	8	8	8	8	303	16	8	16	4		200		8	4	
38-45				16	16	8	8	8	459	8	8	16	4		168		8	4	
52-59				8	2	8	8	8	264	8	8	16	8		200		8	4	
3 mo.				16	16	16	8	8	528	8	8	8	4		141		8	4	
4 mo.				8	32	8	8	8	459	8	8	8	2		119		8	2	
5 mo.	8[2]	8	4	8	16	16	8	8	100	8	8	8	2	8	100	4	8	4	100
									Tertiary Stimulus										
6 mo.	128	16	4	16	32	16	8	8	183	8	64	16	16	4	230	8	8	4	126
7 mo.	16	16	4	8	16	8	8	8	109	8	32	16	8	4	174	4	8	4	100
8 mo.	16	8	2	8	16	8	8	8	92	16	32	16	8	--	283	2	4	0	39
9 mo.	16	8	2	16	16	8	8	16	109	8	16	8	8	4	132	2	8	4	79
10 mo.	16	4	2	16	16	16	8	16	109	16	16	8	8	--	200	2	8	4	79

1. Dog Number
2. Reciprocal of the highest dilution showing agglutination. Dilutions began at 1:2.
3. Geometric mean of normalized values.

Dogs 20, 18, 24, 14, and 19 had a stimulating dose of Leptospira 2 weeks before the first one shown here so initial values have been omitted.

2. Complement Fixation Titres (CF) for Infectious Canine Hepatitis.

The results of the CF titres are shown in Table 2. Dilutions were started at 1:16 since higher concentrations of serum were often anticomplimentary. A negative titre was therefore recorded as 1:8. Values for 7 of the 8 controls are included. The serum of control dog number 84 was omitted because all specimens were anticomplimentary. Four of the seven showed a 4-fold or greater rise in titre in the 9-11 day period and one additional Beagle had a 4-fold rise by the 16th day. The average increase was greatest for the controls in the interval between 2-4 days to 9-11 days. There was no significant (4-fold) increase in titre in any of the 8 irradiated animals. These results are shown graphically in Fig. 1.

When the next booster dose was given seven months later, there was no immediate significant rise in any of the controls but one. This dog was the only one which had a low titre at the time the immune stimulus was given. Titres then rose, until by the 3rd month, 4 of the 7 controls had 4-fold or more increases. The irradiated dogs showed very similar reactions to those of the controls except that the 3 dogs receiving their first booster at 7 days after irradiation, all had low titres when the 7 month immunization was given and, although no immediate response was shown by them, their CF titres were all significantly higher two months later.

DISCUSSION

The response to a secondary immune stimulus of both L. canicola and ICH virus occurred within a 5 to 11 day period in the Beagles. This is similar to the time observed for the anamnestic response in rabbits[6] and in mice[1,7].

The inhalation of sufficient Sr^{90} to result in a body burden of 31-46 microcuries per kilogram apparently produced an effect similar to that of whole body exposure to X-irradiation near the LD_{50} 30 day dose. This is between 300 and 400r for dogs. Taliaferro et al[4] states that to depress hemolysin titres in rabbits 100r is ineffective, 200-400r increasingly effective, and 400-500r would be most satisfactory. Four-hundred to 600r

TABLE 2

EFFECT OF INHALATION OF Sr^{90} ON THE SECONDARY IMMUNE RESPONSE TO INFECTIOUS CANINE HEPATITIS VIRUS IN BEAGLES

COMPLEMENT FIXATION TITRES

Days After 1st Booster	Controls								Irrad. Before Secondary Stimulus								
									1-2 Days						7 Days		
	20[1]	88	1E	24	1D	18	11	Mean[3]	82	13	17	1C	14	Mean	19	83	23	Mean
2-4	16[2]	32	32	64	32	64	32	100	32	64	32	64	128	100	16	16	32	100
9-11	64	64	64	256	256	64	128	297	64	128	64	128	256	200	32	32	32	159
16-17	--	128	64	128	128	--	128	303	32	64	32	64	256	115	--	--	32	100
24-31	64	64	32	256	128	64	128	242	16	64	32	32	256	87	16	32	16	100
38-45	32	64	64	64	64	128	64	181	64	64	32	64	256	132	16	32	16	100
52-59	64	64	256	128	64	128	64	269	32	64	32	32	256	100	16	16	32	100
3 mo.	32	64	32	64	64	128	64	164	64	64	32	16	256	100	8	8	8	40
4 mo.	32	64	128	32	64	128	64	181	32	64	32	32	256	100	16	16	16	80
5 mo.	32	32	32	64	64	64	64	135	64	64	32	32	256	115	8	8	16	50
6 mo.	32	64	128	64	64	64	128	200	32	64	64	16	256	100	16	8	8	50
7 mo.	32	32	8	128	32	128	64	122	32	64	32	16	256	87	8	16	8	50
Tertiary Stimulus - 3 Days After 7 Month Specimen																	
8 mo.	32	64	128	64	64	64	64	149	32	64	64	32	16	76	8	16	16	63
9 mo.	128	128	128	128	128	128	128	362	64	64	128	64	128	152	64	256	64	504
10 mo.	128	128	128	128	256	128	128	400	64	256	128	128	128	230	64	128	128	504

1. Dog Number
2. Reciprocal of the highest dilution showing inhibition of lysis.
3. Geometric mean of normalized values.

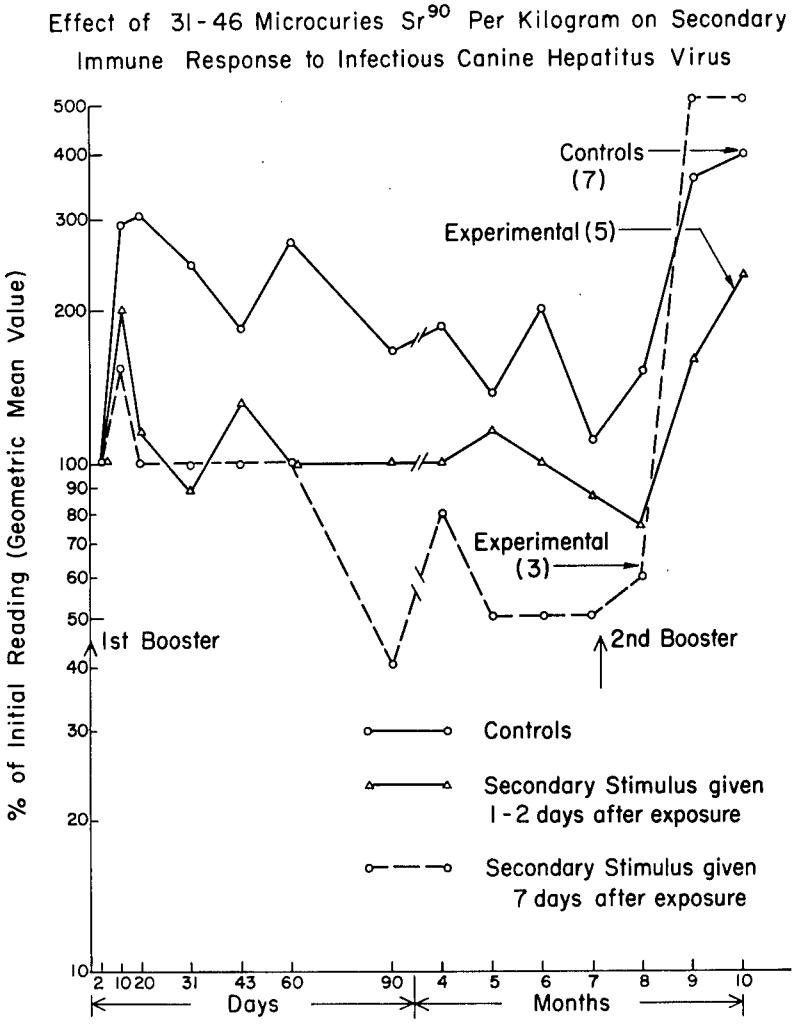
Effect of 31-46 Microcuries Sr^{90} Per Kilogram on Secondary Immune Response to Infectious Canine Hepatitus Virus
% of Initial Reading (Geometric Mean Value)
500
400
300
200
100
90
80
70
60
50
40
30
20
10
Controls (7)
Experimental (5)
Experimental (3)
1st Booster
2nd Booster
Controls
Secondary Stimulus given 1-2 days after exposure
Secondary Stimulus given 7 days after exposure
2 10 20 31 43 60 90 4 5 6 7 8 9 10
Days
Months

Figure 1

were required to repress the secondary response to fluid toxoid in mice[1]. It is likely that most of the exposure to radiation of the antibody producing cells occurs during the first hours after inhalation since much of the isotope is eliminated by the digestive tract and the remainder is accumulated in the bone. This dosage is not sufficient, however, to kill any of the animals in 30 days. All are still alive 2 years after exposure.

The period of greatest sensitivity for the secondary response has been shown to be from 6 hours to 2 days before the antigenic stimulus when a single exposure is given[1, 4].

In this study the cause of the complete suppression of antibody production in the dogs exposed 7 days before the secondary stimulus may have been the continuing radiation from the Sr^{90} in the bone. Those exposed 1-2 days before immunization would not have had the radiosensitive antibody forming cells subjected to as long a period of continuous radiation, and therefore showed inhibition of antibody production but not complete suppression. This exposure did not appear to suppress the antibody production which maintains the balance between production and decay since initial titres did not decline more rapidly in the irradiated dogs than in controls. Claman[8] has reported that X-irradiation has no effect on the rate of decline in antibodies following the secondary immune response because those antibody forming cells which maintain the balance between decay and regeneration are mature radioresistant cells.

When the tertiary stimulus was given 5 months following the Sr^{90} exposure there was a significant response to Leptospira antigen in only one of 8 controls and in 2 of 8 irradiated Beagles. These two were in the group irradiated 1-2 days before the booster. The rises in titre occurred within the month, as would be expected for a secondary immune response. A sample was not taken within the 10 day interval during which the secondary response characteristically occurs. The tertiary stimulus of ICH given 7 months following exposure brought about an immediate response in only one of 7 controls, and in none of 8 irradiated dogs. Significant increases in titre were observed by the 2nd month after this stimulus in seven of 7

controls, two of 5 of the 1-2 day group and all of the three in the 7 day post exposure group. This suggests that the ability to produce antibodies has been recovered in a period of from 5 to 7 months after the initial exposure. The cause of the delay in the response to the later immunizing stimulus is not known. Perhaps the appreciable titres existing at the time the antigen was administered could explain this.

In the controls and the 1-2 day group all but one of the titres were comparatively high. A combination of antigen with antibody could then result in even lower titres. These are seen in 3 cases. Where the titre was low (Dog 1E) a substantial rise occurred. In the 7 day group the delayed response may be due to the fact that there was no response to the secondary stimulus and therefore the tertiary acted like a primary stimulus.

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