THE EFFECT OF X-RAYS ON THE SECONDARY ANTIBODY RESPONSE.

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MANY investigators have shown that a sublethal dose of X-rays interferes with a primary antibody response if the antigen is given after the X-rays; the subject has been reviewed by Taliaferro and Taliaferro (1951). Rather fewer investigations have been made on the effect of X-rays on the secondary response and opinion on this matter seems divided (Taliaferro and Taliaferro, 1954; Silverman and Chin, 1953; Dixon, Talmage and Maurer, 1952).

The Army Pathology Advisory Committee recommended that wounded soldiers who have been actively immunised should be given a dose of tetanus toxoid and not tetanus antitoxin as had been the practice previously, and this recommendation was accepted (Sachs, 1952). The question later arose whether, after exposure to X-rays, re-immunisation was still the best treatment for those at risk from tetanus.

With these facts in mind, the present work describes two experiments on rabbits given X-rays at varied times and doses before a second dose of tetanus toxoid.

MATERIALS AND METHODS.

Each of the two experiments was performed on comparable groups of 3 rabbits inoculated intramuscularly with the same batch of formol tetanus toxoid, strength 8 Lf/ml., on day 0 and day 45. Irradiation when given was from a 240 kV Westinghouse constant potential machine, the distance between target and entry surface being 1 m. The area of uniform dosage was 35 × 28 cm. using a ½ value layer of 1·2 mm. of copper. Bilateral irradiation to the whole body at a rate of 23 R/min. was used throughout as suggested by Chapman and Barnes (1953).

In the first experiment 400 R were given either 10 days, 2 days or 6 hr. before the second dose of toxoid and in the second experiment either 100 R or 25 R were given 10 days before the second dose of toxoid. One group in each experiment was not irradiated and served as a control.

The serum antitoxin was titrated at 10–20 per cent intervals using the mouse method described by Glenny and Stevens (1938). Bleedings for this purpose were taken on day 45 immediately before the second dose of toxoid and subsequently as follows:

	Day of bleeding for antitoxin titre.											
Exp. I . .	47	—	49	51	53	55	58	61	65	70	90	120
Exp. II . .	47	48	49	51	53	56	—	60	65	72	—	—

The secondary response for each group was judged by the following criteria:

1. The time of maximum rate of rise/day in antitoxin titre.
2. The time of maximum serum antitoxin concentration (peak titre).
3. The maximum overall logarithmic rise in titre from day 45.

As the number of animals used is small, the just significant difference ($P = 0{\cdot}05$) is calculated from the pooled variations within the groups for both experiments combined.

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RESULTS.

The effect of a second injection of tetanus toxoid 45 days after the first injection and its modification by X-rays is shown in Tables I and II and will be considered under three headings.

TABLE I.—*Exp. I: Effect on Antitoxin Production of* 400 R *given at Various Intervals before Day* 45 *(Second Dose of Toxoid).*

Interval between X-rays and toxoid.	Time in days for: Max. rate antitoxin rise/day.	Mean.	Max. titre.	Mean.	Overall rise in titre (log).	Mean.
10 days	9		20		0·41	
„ „	7	8·3	20	16·7	0·26	0·32
„ „	9		10		0·30	
2 „	5		16		0·97	
2 „	9	6·3	45	27	0·58	1·09
2 „	5		20		1·71	
6 hours	9		16		1·28	
6 „	3	5·7	16	16	2·12	1·45
6 „	5		16		0·95	
Controls	3		6		1·51	
„	3	3·0	8	6·7	1·51	1·54
„	3		6		1·61	
Just significant difference $P = 0·05$		2·7 days		5·8 days		0·92

TABLE II.—*Exp. II: Effect on Antitoxin Production of Various Doses of X-rays given* 10 *Days before Second Dose of Toxoid.*

Dose of X-rays.	Time in days for: Max. rate antitoxin rise/day.	Mean.	Max. titre.	Mean.	Overall rise in titre (log).	Mean.
100 R	5		11		1·07	
„ „	3·5	4·0	15	12·3	1·05	1·09
„ „	3·5		11		1·16	
25 R	3·5		11		0·77	
„ „	3·5	3·5	6	9·7	1·18	1·02
„ „	3·5		11		1·12	
Controls	2·5		8		2·90	
„	2·5	2·5	8	10·3	0·84	1·62
„	2·5		15		1·08	
Just significant difference $P = 0·05$		2·7 days		5·8 days		0·92

1. *The time of maximum rate of rise in antitoxin/day.*

In all the control rabbits there was a period of 2 days before the antitoxin titre started to rise significantly and the rise, when it occurred, was at first rapid and then lessened. The maximum rise per day in the controls took place between the 2nd and 3rd day in experiment II, but in the first experiment, as the titre was only done on day 49, the maximum rise could not be timed with such certainty.

In all the irradiated animals the maximum rise was postponed. The overall difference is highly significant ($P < 0{\cdot}01$) and it will be seen that as the interval between X-rays and toxoid increased up to 10 days so also the average time before the maximum antitoxin rise per day increased.

2. *Time of peak titre.*

The interval before peak titre in both experiments was variable, 5 out of 6 control rabbits achieving peak titre within 8 days. In all the rabbits irradiated with 400 R this interval was prolonged ($P < 0{\cdot}05$). With smaller doses of X-rays the delay in reaching peak titre was less significant.

3. *The total secondary rise in antitoxin titre.*

The overall rise varied considerably within different experimental groups. Only when 400 R were given 10 days before the second dose of toxoid was the peak titre depressed significantly in all animals.

DISCUSSION.

As in the primary response the action of X-rays is twofold. There is the delaying action on the commencement of antitoxin rise and peak titre, and there is reduction in the ability to form antitoxin. The former may occur without the latter and these effects will be called " delay " and " reduction ".

Dixon *et al.* (1952), observing the antigen disappearance rate of ^{131}I-labelled bovine gamma globulin as well as precipitin formation in response to it, found delay only in the secondary response when the antigen was given two days after 400 R. Taliaferro and Taliaferro (1954), studying the formation of sheep cell haemolysin in rabbits, found delay and reduction in the secondary response when the antigen was given 2 days after 500 R but delay only when given 1–2 hours before. Silverman and Chin (1953), measuring egg albumen precipitin, found no reduction in peak titres in the secondary response when the antigen was given 24 hours after 400 R. Their figures suggest, however, that there was some delay. In the present experiment there was delay when the antigen was given 6 hours, 2 days or 10 days after 400 R, reduction in all rabbits when the interval was 10 days, and probably reduction in one of the three rabbits at the 2 days' interval.

The inference from these results is that the effect of X-rays in causing diminution of peak titre takes two days or more to develop. If, for example, it took only one day, then Silverman and Chin would have found reduced peak titres.

Taliaferro and Taliaferro (1954) showed in the primary response that the ability to form antibody is lost very rapidly when the antigen is given at the time of irradiation, or up to one day later. In the secondary response this sharp break in antibody-forming ability, if it occurs, is probably two days later, *i.e.*, when the antigen is given 2 days after the X-rays. The sharpness of the break explains the different results of Dixon *et al.* and Taliaferro. The start of the antibody rise is nearly 2 days earlier in the secondary response studied by the Taliaferros and perhaps this is the radio-sensitive period. If so, then the " reduction " damage by X-rays takes 9 days to develop in this experiment; unless the time of antibody rise is 9 days or more after 400 R no reduction in peak titre occurs. The same applies to the Taliaferros' work on both primary and secondary response.

The delay effect occurs more readily; persons exposed to radiation may be

expected to show a delay in antitoxin rise if given a booster dose of tetanus toxoid. The value of giving a stimulating dose of toxoid to those at risk from tetanus depends on a rapid rise in antitoxin titre within the incubation period of the disease. The findings in these experiments suggest that this procedure may be of doubtful value in irradiated persons.

SUMMARY AND CONCLUSIONS.

The action of X-rays on the secondary response in rabbits to tetanus toxoid has been studied. This response is radio-sensitive but differs from the radio-sensitivity of the primary response in that a dose of 400 R has to be given two or more days before the antigen to reduce the peak titre. There is delay in antitoxin rise whether the X-rays are given 6 hours, 2 days or 10 days before the antigen.

Smaller doses of X-rays (100 and 25 R) given 10 days before the antigen may delay the antitoxin response but have very little effect on the peak titre.

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