Lengthened Induction Period or Immunologic Unresponsiveness Depending on Antigen Dose in Adult, X-Irradiated Rabbits.* (28289)

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Previous investigations have shown that whole-body X-irradiation inhibits immunologic capability(1). These studies established X-ray dose and time of antigen administration as critical factors determining the radiation effect. Taliaferro and Taliaferro(2) stressed that dose of antigen was also important. Smaller amounts of antigen resulted in maximal suppression of hemolysin synthesis when sheep red blood cells were injected into X-irradiated rabbits. Although measurements of antigen persistence were not made their data suggest that a long period of antigen availability is assured when a large amount of antigen is employed for primary injection following irradiation.

Stevens(3) found that rabbits injected with small amounts of bovine gamma globulin 40 hours after receiving 500 r of X-ray yielded a primary antibody response when rechallenged with antigen an average of 3.2 months later. Stevens concluded that sufficient active antigen had not persisted beyond the period of radiation recovery so that when exposed to the second antigenic stimulus the rabbits responded as if it were the first. One may speculate that if sufficient antigen had been present as radiation effects disappeared belated sensitization followed by the appearance of antibody would have occurred. If true a lengthened induction period could be produced predictably by varying the amount of antigen administered after a given dose of radiation.

The experiment detailed here followed the elimination of radioiodinated antigen from the blood of X-irradiated rabbits in order to obtain quantitative estimates of remaining antigen at a time when radiation recovery was expected. The amount of antigen persisting could then be related to the appearance or absence of a delayed primary antibody response.

In addition we describe a state of immunologic unresponsiveness produced by a small quantity of soluble protein antigen in adult, irradiated rabbits. This unresponsiveness was small-dose dependent and could not be established if much larger amounts of antigen were employed.

Materials and methods. Bovine plasma albumin (BSA), powdered, from Armour Pharmaceutical Co., was dissolved in 0.9% saline as antigen. Either 10 mg or 100 mg of BSA in 1 ml of saline were injected intravenously into rabbits (adult, male, New Zealand, 2.5-3.8 kg).

Whole-body X-irradiation was with a Picker X-ray machine (400 r generated at 250 kv and 12 ma, filtered by 1.0 mm of aluminum and 0.5 mm of copper; half value layer of the beam 3.0 mm of copper).

Each experimental animal was exposed to X-radiation only once and was given a primary injection of antigen 24 hours later, a time approximately midway in the period of maximal immunological depression(4).

X-rayed rabbits were divided into 2 groups. Individuals in the first group (7 animals) received 10 mg of BSA: those in the second group (4 animals) 100 mg of BSA. Antigen was injected into the marginal vein of the left ear and blood samples were drawn from the vein in the right ear. In addition to pre-irradiation samples, blood was collected just after antigen administration, daily through the 14th day, and again 24 days after injection. On the 27th day all of the animals were reinjected intravenously with 10 mg of BSA and periodic blood samples were again collected beginning immediately

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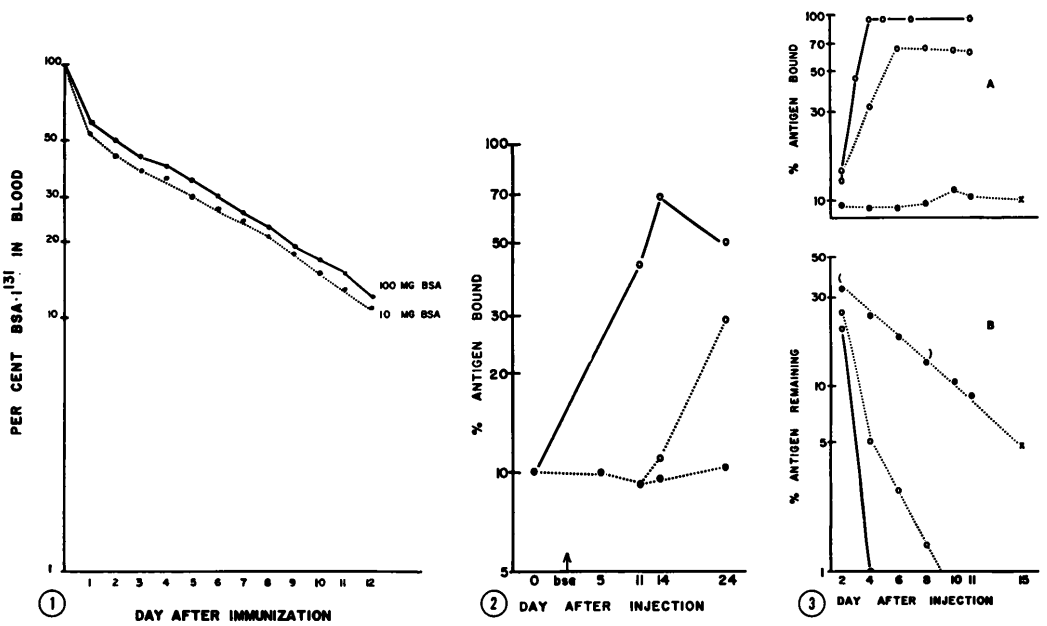

FIG. 1. Semilogarithmic plot of antigen elimination in x-irradiated rabbits. Mean disappearance of circulating antigen expressed as % of initial activity. Bovine albumin injected 24 hr after x-irradiation.

FIG. 2. Semilogarithmic plot of mean antigen binding capacity after primary injection. O———O 4 normal rabbits (10 mg BSA); O--------O 4 x-irradiated rabbits (100 mg BSA); ●-------● 7 x-irradiated rabbits (10 mg BSA). Antigen injected 24 hr after x-irradiation.

FIG. 3. Semilogarithmic plot of secondary response to 10 mg BSA. Symbols as in Fig. 2. A. Mean antigen binding capacity. B. Mean disappearance of circulating antigen expressed as % of initial activity. Parentheses—after third injection of 2 x-irradiated rabbits (10 mg primary injection). ×—six rabbits. Response of only one normal rabbit is shown in B.

after injection. Rabbits in Group 1 received a total of 20 mg of BSA and in Group 2, 110 mg. In addition 4 unirradiated control rabbits were given a total of 20 mg of BSA in 2 injections 27 days apart.

Three criteria were used to determine whether a primary antibody response occurred. 1) Early appearance of antibody *in vivo* as detected by the antigen elimination procedure of Talmage *et al.*(5) employing I^{131} labeled crystalline BSA(6) administered with unlabeled antigen. 2) Antibodies were detected *in vitro* by the ammonium sulfate precipitation method of Farr(7). Antibody content is revealed by its antigen binding capacity expressed as the percent of 0.05 μg of BSA I^{131} bound by a 1:5 dilution of serum. As tested by us pre-immunization rabbit sera diluted 1:5 bind approximately 10% of the antigen. 3) The ability of a rabbit to yield a characteristic secondary response when challenged with 10 mg of antigen 27 days after primary injection also served as an indicator of primary sensitization. Methods 1 and 2 were used to follow the secondary response.

Results. Fig. 1 illustrates mean disappearance rate of BSA I^{131} from the blood of irradiated rabbits through the 12th day after injection. Following the first 24 hour period of equilibration in the tissues antigen was catabolized at similar constant rates in both groups. Neither group showed the phase of rapid elimination indicative of antibody formation. It thus appeared that both groups had failed to respond to primary immunization up to the 12th day.

Fig. 2 shows antibody responses of the 2 groups compared to unirradiated controls. The dose of radiation employed prevented a primary response in those animals injected with 10 mg of antigen (the antigen binding capacity of sera from this group remained at the pre-irradiation level). Sera of irradi-

ated rabbits injected with 100 mg of antigen, however, showed an increase in binding capacity at 24 days. From the data in Fig. 1 and 2 we conclude that a delayed primary antibody response occurred in this group between 14 and 24 days after immunization.

The responses to secondary immunization seen in Fig. 3 reinforce the conclusion that irradiated rabbits receiving the smaller quantity of antigen were not sensitized by the first injection. These animals show neither the early accelerated rate of antigen elimination nor the early increased antigen binding capacity seen both in the group given 100 mg BSA and in unirradiated controls. The conclusion that the 100 mg group exhibited a delayed primary response correlates with the secondary response data in Fig. 3 and emphasizes the importance of antigen dose in measuring the suppressive effects of X-ray on the immune response.

Fig. 1 can be used to obtain an estimate of the amount of circulating antigen in each of the 2 irradiated groups during the first 12 days after irradiation and immunization. Since the days represented are those after primary immunization they are one day late with respect to irradiation. The relationship of a 10-fold difference in antigen dose established at time of injection was maintained throughout the first 13 days after irradiation. Seven days after exposure, a point at which early immunologic recovery may sometimes be seen(2), there were approximately 2 to 3 mg of BSA circulating in those animals which had received 10 mg while those which had received 100 mg had 20 to 30 mg of antigen in the blood. By the 13th day after irradiation while approximately only 1 mg of BSA was still circulating in the 10 mg group there were in the 100 mg group at least 10 mg, a quantity adequate to sensitize these animals at this point and to account for the delayed primary response measured at 24 days.

Not only did the 10 mg group of x-irradiated rabbits not respond anamnestically, but more importantly none of the 7 animals produced a primary response to the second antigenic stimulus even though the effects of x-ray exposure had long since disappeared. The delayed primary and normal secondary responses evident in the 100 mg group indicate that persisting radiation effects were not factors in the inability of the 10 mg group to synthesize antibody subsequent to the second injection.

The course of unresponsiveness persisted through the 15th day after secondary injection. This length of time fits Weigle's(8) recent definition of immunologic unresponsiveness to BSA, (*i.e.*, failure to show accelerated or immune elimination over a 13 day period). By this criterion such an unresponsive condition was established in these rabbits.

Two of the unresponsive animals were again injected intravenously with 10 mg of BSA 27 days after the second injection. Again they showed no evidence of response. The resultant BSA I^{131} elimination curves were identical to those displayed by these rabbits after the second injection. The mean of these curves is represented by the parenthesis in Fig. 3B.

Discussion. The amount of antigen and the time required for the animals to dispose of it appear to have determined whether complete suppression or delayed synthesis of antibodies was to take place in these rabbits. If a delayed response occurred it would seem to have required the persistence of enough unaltered antigen to sensitize the host during the period of x-ray recovery. The possibility of aberrant responses to antigen during the period of radiation suppression discussed elsewhere(9) leads to the suggestion that small amounts of antigen may divert the antibody synthesizing mechanism away from production of reactive antibodies and thereby promote a state of unresponsiveness.

Until recently immunologic unresponsiveness in adult animals had been successfully established only after injection of relatively massive amounts of antigen. Dresser(10) and Battisto and Miller(11) reported that very small doses of bovine gamma globulin can produce unresponsiveness in adult mice and guinea pigs.

Our experiment supports prior data(12) which indicate that immunological unrespon-

siveness does not appear to require persisting antigen to continuously neutralize newly formed antibody. The inverse concentration requirement seems direct evidence that excess antigen is not required. It may be that it is where or how antigen is located which is of importance in unresponsiveness.

The importance of antigen concentration is emphasized since repeated injections of much smaller amounts of protein antigen than those used here have been shown to stimulate specific antibody production when injected along with very large amounts of a second antigen to which unresponsiveness was produced. According to Dixon and Mauer(13) rabbits (including one x-irradiated animal injected 48 hours after irradiation) becoming unresponsive to bovine albumin formed antibody to contaminating bovine gamma globulin present as less than 0.01% of the albumin preparation.

The nature of the antigen may also be important. Both Stevens(3) and Porter(14) found that x-irradiated rabbits injected with bovine gamma globulin during the period of immunologic depression produced primary antibody to a subsequent injection of the same antigen. However it should be noted that in their experiments the second antigenic stimulus was administered later than in the experiment described here.

The limiting concentrations for the kind of unresponsiveness described have not yet been explored; we can only say that approximately 10 mg of BSA is effective 24 hours after x-ray exposure and 100 mg of BSA is not. Five irradiated rabbits treated to exactly the same injection schedule except that the primary injection was made with 15 mg of BSA incorporated into rabbit hemoglobin particle adjuvant(15) showed a similar unresponsiveness through the 16th day after secondary injection as measured by ammonium sulfate precipitation. Further experiments should define more clearly the limits not only of antigen but also of radiation and time.

Summary. Adult rabbits were injected with either 10 mg or 100 mg of bovine albumin 24 hours after 400r x-irradiation. The dose of irradiation was shown to suppress completely the antibody response to the smaller amount of antigen but not to the larger amount. In the latter case the induction period was lengthened and a delayed antibody response was determined to have occurred sometime between the 14th and 24th day after injection. Whether antibody formation was to be suppressed or delayed could be correlated with the disappearance of radioiodinated antigen from the blood and the amount of antigen remaining. It was further shown that the 10 mg dose of antigen but not the 100 mg dose established a state of immunologic unresponsiveness to a subsequent challenge with antigen.

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