Comparative Localizing Response of Inflammatory and Irradiated Tissues to Injected Protein.* (31688)

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A characteristic of the tissues in immunity is their capacity to localize specific antigen (1). This capacity is slight in the absence of immunity and becomes increased within limits as immunity is increased. Inflammatory tissue apparently has a marked localizing capacity without regard to immunity. Opie (2) injected streptococci in an inflammatory peritoneal cavity of rabbits. The inflammation was caused by a previous injection of aleuronat in the cavity. The streptococci became localized and did not reach the blood stream. When he injected the organisms in a normal peritoneal cavity, they reached the blood stream. Menkin(3) found that various substances, including dyes, injected in inflammatory tissue became localized *in situ,* but escaped when injected in normal tissue. In this laboratory it was observed(4,5) that protein or organisms injected in focally irradiated tissue became localized to a lesser degree than when injected in normal tissues. The above findings suggested to compare the localizing capacities for injected protein of irradiated, inflammatory and normal tissues by means of the same method, using albino rabbits.

Methods and materials. The subcutaneous tissue of the right thigh of all rabbits used was chosen for this study. In one group, an area 8 cm in diameter was x-irradiated. The physical factors were 1000R, 80 kVp at 20 cm focal distance, with no added filtration. In a corresponding area of a second group of rabbits, an inflammatory reaction was produced by injections of concentrated beef infusion broth (Difco). Five 0.1 ml amounts of the broth were injected intracutaneously in a skin area of 2 cm in diameter, followed 6 hours later by a subcutaneous injection of 1 ml in the same area. A marked local inflammatory reaction resulted. The corresponding area of a third, control group was employed in a normal state.

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The antitoxin-localizing method(6) was used to determine the comparative localizing capacities of the subcutaneous tissue of the right thigh of the 3 groups of rabbits. This method is based on the finding that the degree of localization by a tissue of a restricted dose of injected horse serum diphtheria antitoxin is a determining factor in the *in vivo* neutralization of a lethal dose of homologous toxin. If the localization is slight, the antitoxin will escape from the injected area and neutralize the toxin. If the localization is marked, the antitoxin will fail to reach the toxin in time to save the animal.

To illulstrate(7), 10 units of a given antitoxin injected intramuscularly protected rabbits against 25 minimal lethal doses (MLD) of toxin standardized for guinea pigs. But 10 or 15 units of antitoxin injected subcutaneously did not save these animals from the toxin; 20 units were required to protect them. Apparently, the localizing capacity of the subcutaneous tissue retained the antitoxin to such an extent as to prevent it from reaching and neutralizing the toxin, necessitating a larger dose for protection.

For this study, the antitoxin was obtained from Eli Lilly Co. and the toxin from the Michigan Department of Health. The toxin dose used was 25 MLD and the antitoxin dose was 7 units. This number of units of 3 lots of antitoxin showed a protein content of 0.43, 0.47 and 0.50 mg, respectively. The use of these antitoxin and toxin doses was based on recent localization studies of this laboratory (8). Dilutions were made so that the final toxin or antitoxin dose was contained in 0.5 ml normal saline. All injections were given subcutaneously 24 hours after the irradiation or after the initial injections of the broth.

Ten rabbits were used in the irradiation experiments. Each was injected with the antitoxin in the irradiated area of the right hip and simultaneously with the toxin in the left hip. At the same time, the normal controls

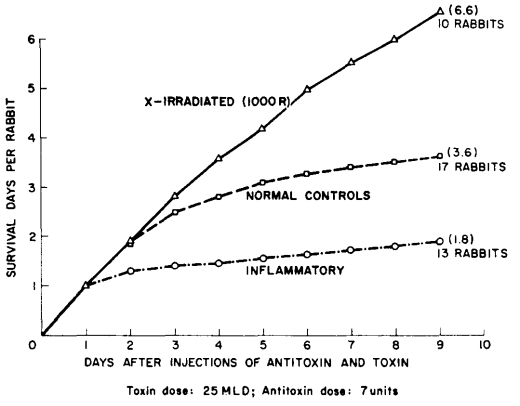

FIG. 1. Fewer survival days from diphtheria toxin when antitoxin is injected in flammatory area and more survival days when injected in irradiated area; apparently the antitoxin becomes localized in inflammatory area and escapes from irradiated area.

(17 rabbits) were similarly injected with the antitoxin in the right hip and the toxin in the left hip. In a parallel experiment, 13 rabbits which showed an inflammatory area in the right hip, due to the injection of concentrated broth, were injected with antitoxin in that area and with toxin in the left hip.

Results. Fig. 1 illustrates the results obtained. The fewest number of survival days per rabbit from the toxin (1.8) resulted when the antitoxin was injected in the inflammatory area, indicating that the antitoxin became localized in the area and was unable to reach the toxin fast enough for neutralization. The largest number of survival days per rabbit from the toxin (6.6) resulted when the antitoxin was injected subcutaneously in the irradiated area, indicating that the antitoxin rapidly escaped from the area and neutralized the toxin. The number of survival days per rabbit of the controls (3.6) was approximately half-way between those shown by the irradiated and inflammatory areas.

The statistical significance for the difference between the means of the irradiated and control groups is $p = <0.001$; between the means of the irradiated and inflammatory groups is $p = <0.001$; between the means of the control and inflammatory groups is $p = <0.025$.

Discussion. The results emphasize the wide difference in the localizing capacities of inflammatory and irradiated tissues. Irradiation is known to cause injury and inflammation. It therefore might have been expected that the localizing response of inflammatory tissue caused by irradiation injury would be similar to that caused by nonirradiation injury. The question whether leukopenia accompanying irradiation is a factor in the antilocalizing response of irradiation is under investigation.

Summary. A restricted dose of diphtheria antitoxin was injected subcutaneously in corresponding areas in 3 groups of rabbits for the neutralization of a lethal dose of toxin. In one group, the antitoxin was injected in an x-irradiated area; in another, in an inflammatory area; in a third, in a normal area. It was found that the rabbits in which the antitoxin was injected in the inflammatory area showed the least protection from the toxin, indicating that the antitoxin became localized *in situ* and was not able to reach the toxin fast enough for neutralization. The rabbits in which the antitoxin was injected in the irradiated area showed the greatest protection from the toxin, indicating that the antitoxin escaped from the injected area, reached the toxin and neutralized it. These differences in localization between the irradiated and inflammatory areas compared with the controls were found to be statistically significant.

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