

Radiation Studies on Mice of an Inbred Tumor-Resistant Strain¹

The Alteration of Endogenous Susceptibility to Amyloidosis by X-Irradiation

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Spontaneous amyloidosis was found to be an inherent characteristic of X/Gf mice, an inbred strain known to resist the carcinogenic or leukemogenic effects of X-irradiation. Amyloid was predominantly deposited in the heart and adrenals, with lesser amounts observed in the spleen, liver, kidneys, and blood vessels. When X/Gf mice were exposed, at 2 to 3 months of age, to 300 R of whole-body X-irradiation in one exposure and sacrificed 2 to 13 months later, it was found that irradiation had an inhibitory effect on the deposition of amyloid. This was demonstrated most rigorously by serial sacrifice of a large number of mice that were included in the experimental colony at the same time and, therefore, shared until their respective deaths identical environmental conditions, except for the experimental variable, irradiation. At 7 months the controls had developed extremely high levels of amyloidosis. The irradiated group had significantly less amyloid at 7 months, and, even though amyloid increased with the age of the irradiated animals, it remained significantly less, even at 15 months, than that already present in the younger, 7-month controls.

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INTRODUCTION

The purpose of this communication is to describe observations concerning the influence of whole-body X-irradiation on the development of spontaneous amyloidosis in a specific strain of mice. Amyloidosis is known to occur in many strains of inbred mice (1-6). The incidence of amyloidosis in these strains is related not only to genetic factors (1) but also to the age of the animals (3, 4, 6), to the presence of incidental infections or infestations (1) and to dietary factors (2). Small doses of daily γ -irradiation enhance the development of spontaneous amyloidosis (7), but we are not aware of studies describing the effects of whole-body X-irradiation on the spontaneous disease, although X-rays have been reported to accelerate or enhance the development of casein-induced amyloidosis in mice (8-10).

The strain of mice utilized in the present study has recently been designated X/Gf (11). It is an albino strain which develops, in very few instances, spontaneous mammary fibroadenomas and which displays a marked resistance to the carcinogenic effects of X-irradiation (12-15) and a mild susceptibility to the carcinogenic and leukemogenic effects of urethan (16, 17). In the search for intrinsic characteristics that might shed light on the resistance of this strain to neoplasia, it was found that X/Gf mice have a high incidence of spontaneous amyloidosis and that irradiated animals did not develop amyloid at an accelerated rate. In fact, our data indicate that whole-body X-irradiation, when given under the conditions of these experiments, inhibits the development of spontaneous amyloidosis in this strain. An account of the incidence and distribution of amyloidosis in irradiated and non-irradiated X/Gf mice forms the basis of this report.

METHODS AND PROCEDURES

The X/Gf mice of both sexes and of various ages were employed in this study. The mice were fed Rockland Laboratory animal diets (occasionally they received bread soaked in milk) and water ad libitum. All animals were housed in the same animal quarters in stainless-steel cages kept at approximately 75°F.

Irradiation Techniques

For X-ray treatments, a General Electric Maximar X-ray machine was used which operated at 200 kv peak and 15 ma. The X-ray beam was filtered through 0.5 mm Cu and 1 mm Al; the HVL equaled 1.1 mm Cu. Measurement of the dose rate was made with a Victoreen ionization chamber placed in the center of the abdominal cavity of a dead mouse situated in one of the compartments of a plastic box (Fig. 1), partitioned into twenty-five compartments; the other twenty-four compartments were occupied by live mice. This arrangement permitted the determination of the absorbed X-ray dose. An average dose rate of 16.2 rads/min ($\pm 5\%$) was obtained at 86.5 cm distance from the X-ray source to the middle of the mouse

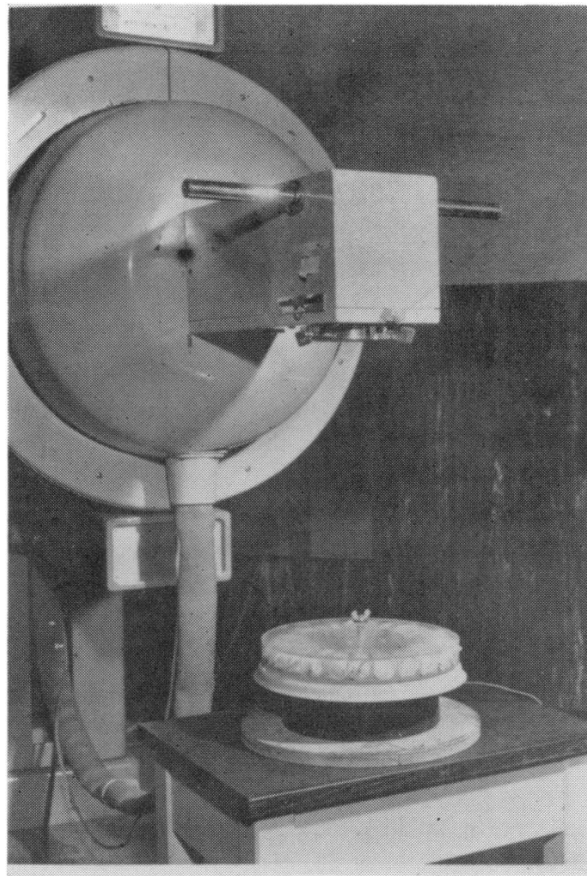


FIG. 1. Plastic box with 25 mice in separate partitions on a rotating table situated below the X-ray tube.

body. At the distance of 86.5 cm the distribution of the X-ray intensity over the plastic box was fairly uniform, as verified by X-ray film. The plastic box with the mice was placed on a rotating table making three turns per minute. This arrangement permitted an even distribution of the same X-ray dose to all the animals in the box. For experimental purposes, each mouse was earmarked and its weight noted. The animals were placed in the circular plastic box described above. Forty males and 40 females of the X/Gf strain between 2 and 3 months of age were exposed whole-body to 300 R in a single exposure. Previous experiences showed that this dose and mode of treatment permitted the majority of the mice to live about 1 year, and some even longer, after X-irradiation.

Histologic Technique

Samples of heart, kidney, lung, spleen, adrenals, and gonads were taken from almost all animals. Liver, pancreas, intestine, skin, marrow, and thymus were taken from lesser numbers of mice. The tissue was fixed in Zenker's solution and embedded in paraffin, and all sections were stained with hematoxylin and eosin (H & E). Amyloid was readily recognized as an amorphous, palely eosinophilic hyalin material with a characteristic location and distribution. However, the identification of the hyalin material as amyloid was confirmed in selected sections by staining with

crystal violet, which demonstrates the metachromasia of the material (18–20). In addition, staining with Congo red (20) was attempted, regardless of reports that Zenker fixation is known to diminish the affinity of amyloid for this dye (21) and although both human (18, 19) and mouse amyloid (22) may be stained weakly or not at all by Congo red. Azo-carmines was used as a connective tissue stain, in selected sections, to confirm that the metachromatic hyalin material found in these studies was not collagen (3).

Quantitation of Observations

An attempt was made to quantitate the production of amyloid in the present study by grading the extent of its deposition in the heart, adrenal, spleen, and kidney on a scale from 0 to 3. These organs were used for grading because they were most consistently involved. The four points on the scale designate either absent, slight, moderate, or severe amyloidosis. A score was obtained for each mouse by adding together the scores for each of the organs. The Mann-Whitney test was used to determine the statistical significance of differences between groups (23).

The study was essentially divided into two parts. First, 32 X/Gf males and 13 females were chosen at random from groups 5 to 15 months of age, and the organs were examined histologically. These mice were used to establish the incidence and distribution of amyloidosis among various groups randomly selected from the same inbred colony (Table I). Among these 45 animals, 13 (6 males and 7 females) were obtained from a larger group of 93 X/Gf mice, born at the same time, and therefore entering the colony under exactly the same environmental conditions. Age-matched animals from this group of 93 lived their entire lives under environmental conditions even more similar than those to which inbred colony controls, born at different times, had been exposed. These 93 animals are referred to as "littermates," although they came from multiple litters produced at the same time. Observations made in this group of 93 mice form the basis of the second part of this study. From these 93 animals, 80 mice (40 males and 40 females) were selected for irradiation. The remaining 13 mice, used in the first part of the study, also served as nonirradiated controls for the second part of the investigation. These 13 nonirradiated mice were examined at 5 and 7 months, and the amount of amyloid was compared with that in age-matched littermates taken from the 80 irradiated animals. Additional irradiated mice were sacrificed at 9, 11, 13, and 15 months. Of the 80 irradiated animals, a total of 46 were sacrificed at 5 to 15 months of age. Of the remaining 34 mice, 26 died spontaneously during the study and were not used; 8 remained alive when the investigation was terminated.

RESULTS

Spontaneous Amyloid

The susceptibility to amyloidosis of the X/Gf mice of both sexes is one of the endogenous properties of this strain uncovered in the present studies. Essentially