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Studies on the Serum Proteins of the
X-Irradiated Rat¹

By Marie A. Fischer, Margaret Z. Magee and Ellen Purvis Coulter
From the Biochemistry Department, School of Medicine, University
of Pittsburgh, Pittsburgh, Pennsylvania

Introduction

Total body x-irradiation variously has been shown to cause a decrease in albumin and γ -globulin and an increase in the α - and β -globulins of plasma or serum (1-3). In the dog (1) the rise in α_3 -globulin and β -globulin was attributed to terminal infection. In the present study the effects of inanition, L-cysteine administration, and antibiotic therapy on x-ray induce changes in the serum proteins of the rat were considered.

Methods

The female rats (Carworth Farms) used in these experiments weighed 140-160 grams and were housed in individual cages. Food (Purina chow) and water were available at all times except when the effects of inanition were investigated. Then, food was withheld but water was always provided.

The irradiated animals received a 800 r total-body dose which was administered in a single exposure at a rate of 18.5 r

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per minute (200kv, 20 m.a. and 5 mm Cu and 1 mm Al filters). During the 30 days following exposure groups of the surviving animals were sacrificed periodically. A sufficient number of animals were treated so that each group contained four survivors. These were decapitated, their blood was collected into a single beaker for preparation of the serum. After dilution with 2 parts of buffer, the serum was dialyzed (4) and the protein fractions were separated in the Klett electrophoresis apparatus. The analyses were made in veronal buffer, pH 8.6, at a field strength of 8.0 volts cm^{-1} for 180 minutes. The scanning technique was used to record the patterns. Because the albumin and α_1 -globulin fractions were more sharply defined on the ascending than on the descending side, and because the β -globulin fraction separates into two distinct peaks only in the rising arm, all measurements of area were made on the ascending patterns. The area (arbitrary planimeter units) of each fraction rather than percentage composition was recorded because it is proportional to concentration and because the area of a single component is essentially independent of changes in the areas of other fractions.

Those rats which were given L-cysteine received an intraperitoneal injection containing 875 mg./Kg/ body weight 20-30 minutes before the animals were exposed to the x-rays. For this purpose a 15 per cent solution of L-cysteine . HCl was prepared, neutralized to pH 7.2 with NaOH and used immediately.

Results

The effects of total body x-irradiation on the serum proteins of the rat are shown in Fig. 1. Within 24 to 48 hours

after treatment both albumin and γ -globulin areas were found to be lower than normal. The concentrations continued to decrease until about the 8th day. In those animals which survived, albumin and γ -globulin returned to the normal range by the 15th day and, in a few of the groups, the γ -globulin area exceeded normal near the end of the experiment. In spite of the fact that significant protection against the lethal effects of x-irradiation was attained by the prior administration of L-cysteine (Table I), this substance had no effect on the rate or extent of the loss of albumin and γ -globulin from the serum nor did it accelerate their return to the normal range of concentration (Fig. 1).

Between the 20th and 30th days of the experiment the α_1 -globulin area appeared to be lower in x-ray treated than in normal animals. Otherwise, no significant changes were observed in either the α_1 or α_2 -fractions. In the dog (1) the striking increase in α_3 -globulin and the increase in β -globulin which were noted prior to the death of the animal coincided with the onset of fever. That neither α -fraction increased in the present experiments was due probably to the fact that the animals remained free of infection. This conclusion is substantiated by the observation that neither streptomycin and/or penicillin administration had any effect on the survival of rats which were exposed to x-rays under identical conditions (Table II). Presumably the animals were not infected with any of the organisms which are susceptible to either antibiotic.

The data of Fig. 1 suggest that the β -globulins are not altered following exposure to a lethal dose of x-rays, for all of

the experimental points fall within the normal range (ave. $\pm$ 2 S. D.). Nevertheless, visual inspection of the Tiselius patterns indicates that an abnormal relationship exists between the β_1 - and β_2 -peaks of sera from x-rayed animals (Fig. 2) for the β -fractions seem to be of equal height and area. Normally the β_1 -peak is the minor fraction of the total β -globulin area. Therefore, the ratios of the β_1 -area to β_2 -area were compared. It was evident (Fig. 3) that the ratios were above normal in x-ray treated animals during the time in which most of them succumbed to the exposure (6th-12th days) and, from the values for the β -globulins given in Fig. 1, it was concluded that the increased ratios resulted from lowered β_2 -areas rather than elevated β_1 -areas. An initial decrease in β_2 -globulin also occurred in x-rayed animals which had been protected by L-cysteine injection, but in this case the area returned to normal on the 6th day and the ratio of β_1 - to β_2 -globulin remained normal (Fig. 3). Since the decrease in β_2 -globulin takes place when most of the deaths from x-irradiation occur, and since a rapid return of β_2 -globulin to normal concentration takes place in cysteine-protected animals, the β_2 -globulin of serum may assume some importance in elucidating the mechanism by which L-cysteine exerts its protective effect.

Effect of Inanition - Malnutrition, due to faulty digestion or absorption or decreased food consumption, results in severe weight loss in x-irradiated animals (5). To determine whether malnutrition is the cause of the low serum albumin levels found in irradiated rats, the effects of starvation on the serum proteins of normal and treated animals were investigated. The results are shown in Fig. 4. When rats were starved, weight loss was continuous, of course, but albumin concentration increased for a brief period

before it started to fall. During the first 6 experimental days identical changes in albumin concentration took place in the sera of x-irradiated and nonirradiated animals both of which were starved. But on the 7th day there was a further decline in albumin in the exposed animals, none of which survived beyond this day. In the starved controls albumin levels rose on the 7th and 8th days but a terminal decrease followed.

A decrease in serum albumin and loss in weight also occurred in animals which were fed ad libitum after exposure to x-rays (Fig. 3). Even though the immediate rise in albumin which was caused by starvation was not evoked, there was essentially no difference in the concentration of serum albumin or in the weight loss of irradiated animals, regardless of whether food was available or not. In the fed groups albumin declined less rapidly when weight loss was arrested and returned to normal when body weight was regained. Therefore, it was concluded that decreased serum albumin in x-irradiated rats is caused by inanition.

X-irradiation induced a continuous fall in serum γ -globulin concentration which was independent of the nutritional status of the animal (Fig. 3). When uncomplicated by x-ray injury, starvation caused a net decrease in γ -globulin area but the reaction to starvation was not immediate as it was following exposure to a lethal dose of x-rays. This confirms other reports that γ -globulin synthesis is depressed as a result of x-ray exposure (2,3) and also demonstrates that decreased synthesis is not caused by the lack of food materials.

On the 7th day after exposure of the animals to x-rays the sera from fed and starved rats contain practically normal

quantities of α - and β -globulins but the sera from the starved, nonirradiated controls contain reduced quantities of these proteins (Fig. 5). Each of the x-irradiated groups shows the typical x-ray-induced inversion of the proportion of β_1 - and β_2 -globulin, whereas the β -components appear as one peak when food is withheld from the normal controls. Since the Tiselius patterns of x-irradiated-starved animals resemble those of the irradiated controls much more than those of the starvation controls it should be suggested that the α - and β -globulins of irradiated animals may not be normal even though the areas of the fractions seem to be so.

Discussion

Dogs can be protected to some degree from the effects of whole body x-irradiation by transfusion with normal, compatible whole blood (6) or by cross-transfusion with a nonirradiated animal (7). Administered intravenously prior to exposure, dog plasma (8) and certain serum fractions (9) give partial protection to mice. The protective agent is neither albumin nor immune gamma globulin but may be another of the globulins. The findings of the present experiments suggest that the β_2 -globulin fraction of rat serum is related to the survival of x-irradiated rats for its concentration decreased after treatment, was lowest during the period in which the animals succumbed to the irradiation, and did not decrease in those animals which were protected from the exposure to radiation by prior treatment with L-cysteine. Although albumin and γ -globulin concentrations are diminished markedly in both unprotected and cysteine-protected animals, the decreases in these fractions are not critical determinants of the lethal effects of

exposure to ionizing radiation.

After exposure of rats to x-rays, the electrophoretic areas of the serum α - and β -globulins do not differ significantly from normal but there is an increase in the ratio of β_1 to β_2 -globulin. When starvation is superimposed on x-ray treatment, the α - and β -globulin regions of the electrophoretic patterns resemble those of the x-irradiated-fed controls much more than those of the starved controls. In the latter, the α -globulin areas are decreased and β -globulin appears as a single rather than a double peak. Either of two mechanisms can explain these findings. A decrease in the serum α - and β -globulins due to inanition or starvation may be masked by a simultaneous transfer of normal or abnormal α - and β -globulins from the tissues into the blood of x-irradiated rats. Otherwise, it must be assumed that irradiation prevents the loss of α - and β -globulin from the circulation when the animal is subjected to the stress of starvation. The first explanation is supported by observations of HShne et al (3). They found increased α - and β -globulin percentages in sera of animals that were exposed to 50 r - a much lower dose than was used in the present experiments. But at this lower dose, the inanition effects may not have masked the rises in α - and β -globulin which resulted from exposure to ionizing radiations.

Summary

Total body irradiation of the rat caused decreases in the albumin and γ -globulin areas of serum and an increase in the ratio of β_1 to β_2 -globulin. Administration of the protective agent, L-cysteine, had no effect on the change in albumin and γ -globulin but it did prevent the increase of the β_1 - to β_2 -globulin ratio. The decrease in albumin is probably due to inanition but the change in γ -globulin is not caused primarily by the lack of food materials. Starvation caused a decrease in the α - and β -globulin areas of normal rats and since a similar decrease was not observed in x-irradiated-starved animals, it was concluded that the α - and β -globulin fractions of the sera of irradiated rats are present in abnormal quantities even though their electrophoretic areas fall into the range for normal, untreated animals.

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TABLE I

Effect of L-Cysteine Injection on the Survival of Rats
Exposed to 800 r Total-Body X-Irradiation

Treatment	No. of Rats	% Survival after Irradiation			
		1st	2nd	3rd	4th
None	22	73	23	23	23
L-Cysteine* (before irradiation)	22	95	82	77	73
L-Cysteine** (after irradiation)	10	50	20	20	10

* 875 mg./Kg. of neutral L-cysteine injected intraperitoneally
20-30 minutes before exposure to x-rays

** 875 mg./Kg. of neutral L-cysteine injected intraperitoneally
5 minutes after irradiation

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TABLE II

Effect of Antibiotic Therapy on the Survival of Rats
Exposed to 800 r Total-Body X-Radiation

Treatment ^a	No. of Rats	% Survival (30 days)
0.5 ml. 0.9% saline daily	12	58
15,000 µg. streptomycin daily	12	50
30,000 µg. " "	12	42
60,000 µg. " "	12	50
15,000 µg. " twice a day	12	50
0.5 ml. saline daily	10	30
30,000 µg. streptomycin daily	10	30
50,000 units penicillin	10	20
30,000 µg. streptomycin + 50,000 units penicillin daily	10	20

* The daily dose of each drug was administered by subcutaneous injection of 0.5 ml. of a solution of the required concentration. Fresh solutions of dihydrostreptomycin sulfate and crystalline penicillin were made in sterile non-pyrogenic saline each day.

Legends for Figures

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Figure 1. The effect of 800 r total-body x-irradiation on the electrophoretic areas (arbitrary planimeter units) of the serum proteins of the rat. The values ($\pm$ 2 standard deviation) for untreated animals are represented by the shaded areas.

Figure 2. The effect of an intraperitoneal injection of L-cysteine on the electrophoretic patterns of serum from normal and x-irradiated rats. 875 mg./Kg. of neutral L-cysteine was injected into 4 normal rats and into 4 x-irradiated rats (20 minutes prior to exposure). These animals and 4 irradiated and 4 normal controls were sacrificed 10 days after treatment and the sera from the 4 animals of each group were pooled for the analyses.

Figure 3. Effect of L-cysteine injection on the ratio of the electrophoretic areas of the β_1 - and β_2 -globulins in the sera of x-irradiated rats. The x-irradiated animals (e) received an 800 r total-body dose. The injected rats (o) were given an intraperitoneal injection of 875 mg./Kg. of L-cysteine 20-30 minutes prior to exposure to 800 r of x-rays. The average value and range of values for untreated animals are shown by the solid lines.

Figure 4. The effects of x-irradiation and/or starvation on body weight, serum albumin and γ -globulin areas (planimeter units) of the rat. After exposure to 800 r total-body x-irradiation some animals were fed Purina chow ad libitum (e); others were starved (Δ). Starved normal animals (o) served as controls.

Figure 5. Electrophoresis patterns of the serum of rats taken 7 days after the animals were x-irradiated and/or after food was withheld.

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AREA

EXPERIMENTAL DAYS

14

UNTREATED
CONTROL

L-CYSTEINE
TREATED
CONTROL

X-IRRADIATED

L-CYSTEINE
TREATED
X-IRRADIATED

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24

Fig. 2

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BODY WEIGHT LOSS

SERUM ALBUMIN

SERUM γ -GLOBULIN

EXPERIMENTAL DAYS

17

X-IRRADIATED
FED

X-IRRADIATED
STARVED

STARVED

TEB 17

Fig. 5

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