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The Effect of Massive Doses of 32 MeV Protons and ^{60}Co Gamma Radiation on Serum Enzyme Levels of Whole Body Irradiated Primates

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Physical measurements of the radiations found in space have shown that protons will present the most significant radiation hazard to man when he penetrates deep space (1, 2). Because man will soon be going into space for extended periods of time, the necessity of studying the effects of protons on mammalian systems has emerged. This communication is concerned with the results of an experiment designed, in part, to investigate the effects of protons on serum levels of Lactic Dehydrogenase (LDH) and Glutamic Oxaloacetic Transaminase (SGOT) of primates, and to compare the results of the proton radiation with data from the more familiar ^{60}Co gamma radiation.

METHODS

Animals. Small primates *(Macaca mulatta)* were used for all studies. Of the 34 animals used in the experiment, 20 were males and 14 were females. They had a mean weight of 3.6 kg with a standard deviation of 0.55 kg. The animals were divided into four basic groups. Groups I (6 animals) and II (9 animals) received 32 MeV protons; following irradiation, the animals of Group II were placed in special holding chairs which allowed considerable freedom of motion but which kept them in such a position that the collection of the necessary blood samples could be easily performed. These chairs greatly reduced the trauma to the animals, since repeated catchings were not necessary. Group III (5 animals) received ^{60}Co gamma radiation; following exposure, these animals were placed in the special holding chairs. Group IV (4 animals) received 2 MeV x-rays; these monkeys were returned to their cages after exposure—they were not placed in the chairs. Four sham irradiated controls were held in the special chairs; 6 animals were sham irradiated but not restrained.

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Protons. The Oak Ridge Isochronous Cyclotron (ORIC) was used for the proton irradiations. During exposure, the animals were held in aluminum mesh cylinders which were rotated at 2 rev/min. Because of inhomogeneity of the beam, it was necessary to irradiate the animals in two portions; the lower half of the animal was first exposed, the beam shut off, the cylinder lowered by a remote controlled device, and then the upper half of the animal was irradiated.

Total doses of either 6500 or 6700 rads were used; the proton dose rate was 1000 rads/min.

The range of 32 MeV protons is approximately 1 cm in soft tissue. Since the monkeys used in this study had an average body radius of about 5 cm, the central portion of the animal received no dose while the peripheral 1 cm received a very high dose.

Comparison of the range of the 32 MeV protons with the cross-sectional anatomy of the *Macaca mulatta* indicated that the skin, musculature, and appendicular skeleton was heavily irradiated while the bone marrow, gastrointestinal tract, kidneys, and axial skeleton received relatively little dosage. Because the head of the monkey is somewhat smaller in cross-sectional diameter than the torso, a relatively greater amount of brain was irradiated as compared with the widest portions of the trunk.

^{60}Co. The USAF School of Aerospace Medicine Cobalt Facility was used for the ^{60}Co irradiations. Each animal was placed in a wood chamber and half of the total dose was delivered to the ventral aspect of the animal. The chamber was then rapidly turned and the remaining portion of the dose given to the dorsal aspect. This technique has been shown to improve the depth dose distribution (3). A midline dose rate of 395 rads/min was used. Depth dose measurements in a masonite phantom and in a specially constructed primate phantom indicated that less than 10 per cent variation of dose rate occurred along the length or through the volume of the animal. Total doses of 6500 rads were given to each animal irradiated with ^{60}Co gamma radiation (Group III).

2 MeV X-rays. A 2 MeV Van De Graaff Accelerator was used for the irradiation. A dose rate of 10.7 rads per minute was employed. The beam had a half value layer (HVL) of 7.5 mm Pb and was delivered from a target-axis of rotation distance of 255 cm; during irradiation, the animals were held in plastic cylinders and rotated at 2 rev/min. Depth dose measurements in the primate phantom indicated that less than 10 percent variation in the dose rate occurred along the length or through the volume of the animal. Total doses of 802 rads were delivered to each animal of Group IV.

Collections of Samples and Enzyme Assays. At intervals following irradiation (See Tables I and II) 2 to 5 ml specimens of blood were removed by femoral venipuncture. Also, preirradiation baseline samples were obtained from 25 of the animals. Immediately after collection the blood samples were allowed to coagulate in the cold. They were then centrifuged at 4000 rpm for 10 min, after which the serum was removed and immediately frozen at $-10°C$; hemolyzed sera were discarded. Prior to assay, the serum samples were allowed to thaw without heating. After a sample had been allowed to thaw, all determinations were immediately performed, which alleviated the necessity for refreezing.

Lactic Dehydrogenase (LDH) was assayed by the method of Plauche and Mosey (4); Glutamic Oxaloacetic Transaminase (SGOT) was determined by the method of Babson *et al* (5).

RESULTS AND DISCUSSION

Figures 1 and 2 summarize the experimental results. The time spacings of the sampling were such that a wide variety of time periods could be evaluated. The extreme elevation of LDH and SGOT following doses of 6500-6700 rads of either ^{60}Co gamma radiation or 32 MeV protons is highly significant, both as compared to unirradiated control animals or with animals that had received 802 rads of 2 MeV x-rays. In several instances in both the ^{60}Co and the proton groups, marked elevations (8 to 10 fold above normal levels) of both LDH and SGOT had occurred by 5 to 12 hours postirradiation. No real difference in response between the proton and ^{60}Co groups is evident, with the exception that the onset of the changes occurs somewhat earlier in the ^{60}Co groups.

During the past several years, changes in serum enzyme levels following irradiation have been investigated in several different animals. The mechanism of elevation seems to be a consequence of cell injury with subsequent leakage of enzyme into the blood stream (6). The possibility of increased enzyme synthesis, however, has not yet been excluded. The degree of elevation of LDH values following 802 rads of 2 MeV x-rays in the current study is compatible with those previously found following doses that are in the range of the $LD_{50/30}$ (7). Parallel changes have been demonstrated in rats, rabbits, and monkeys (8, 9).

Elevation of SGOT levels has been found in animals in a wide variety of injury states, including radiation (8–12). In general, a transient increase of SGOT levels occurs during the first six postirradiation hours, but with normal ranges being reached by 24 hours. The magnitude of these elevations, however, is not as severe as seen in the present study. Two studies not related to radiation injury showed that significant elevation of SGOT levels may be produced in monkeys by vibration, handling, and exercise (13, 14). Therefore, the minimal changes of SGOT levels in the present study following 802 rads could not be considered indicative of radiation injury alone in view of the possibility that the process of catching and handling the animals could cause this degree of abnormality.

The marked postirradiation changes of both LDH and SGOT levels demonstrated by this study are certainly not the result of handling, since LDH did not exceed 1100 units and SGOT was not greater than 100 units in the sham irradiated controls. The LDH levels of 18,000 to 32,000 units and the SGOT levels of 750 to 1400 units are the results of irradiation, and not due to minor trauma from restraint or handling.

There is no completely satisfactory explanation for the absence of the marked rise of enzyme levels in some animals, while others, that were treated in the same manner had extreme changes. It is our impression that the onset of the elevation of serum enzyme levels begins later in some animals than in others, and that early measurements fail to demonstrate these changes in some instances. By 48 hours following irradiation, significant elevations of both LDH and SGOT

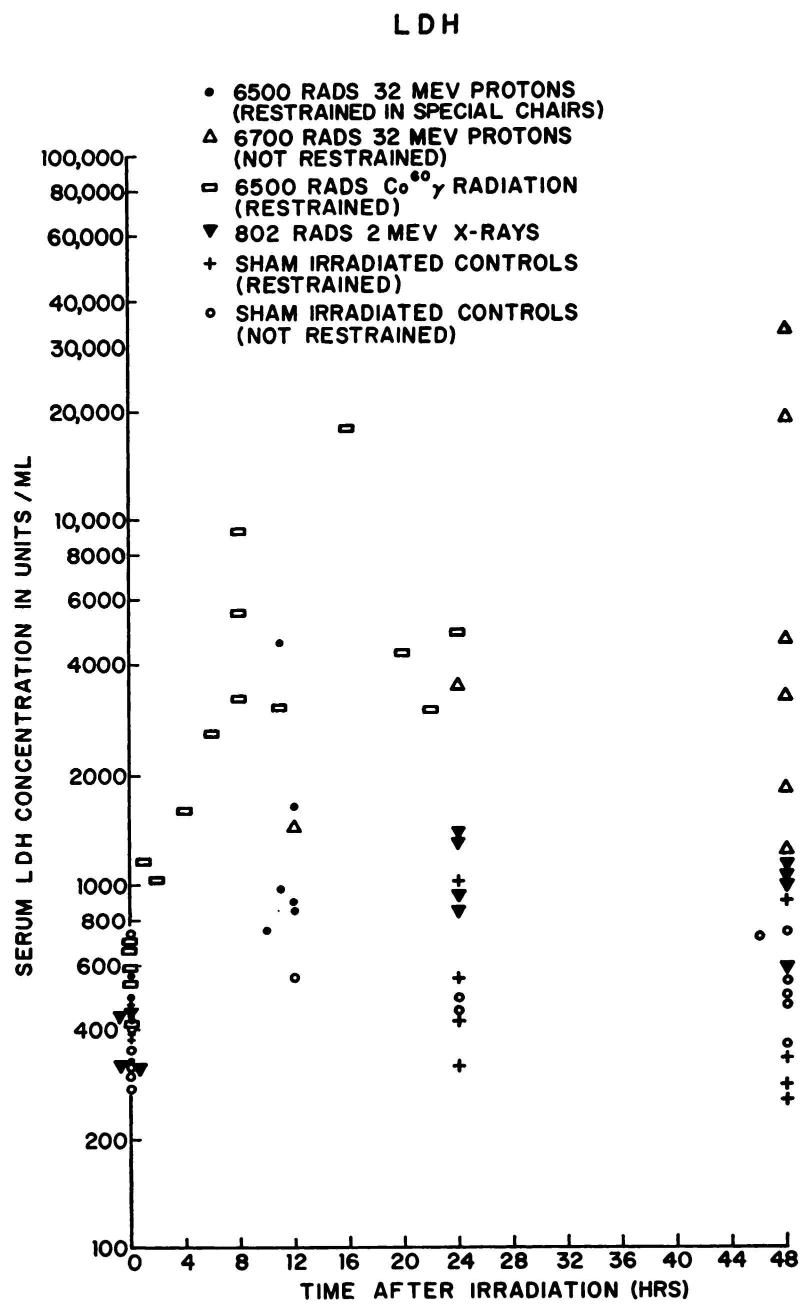

Fig. 1. Serum Lactic Dehydrogenase (LDH) levels after irradiation. The points on the figure represent single measurements. The 0 time points are the preirradiation baseline values.

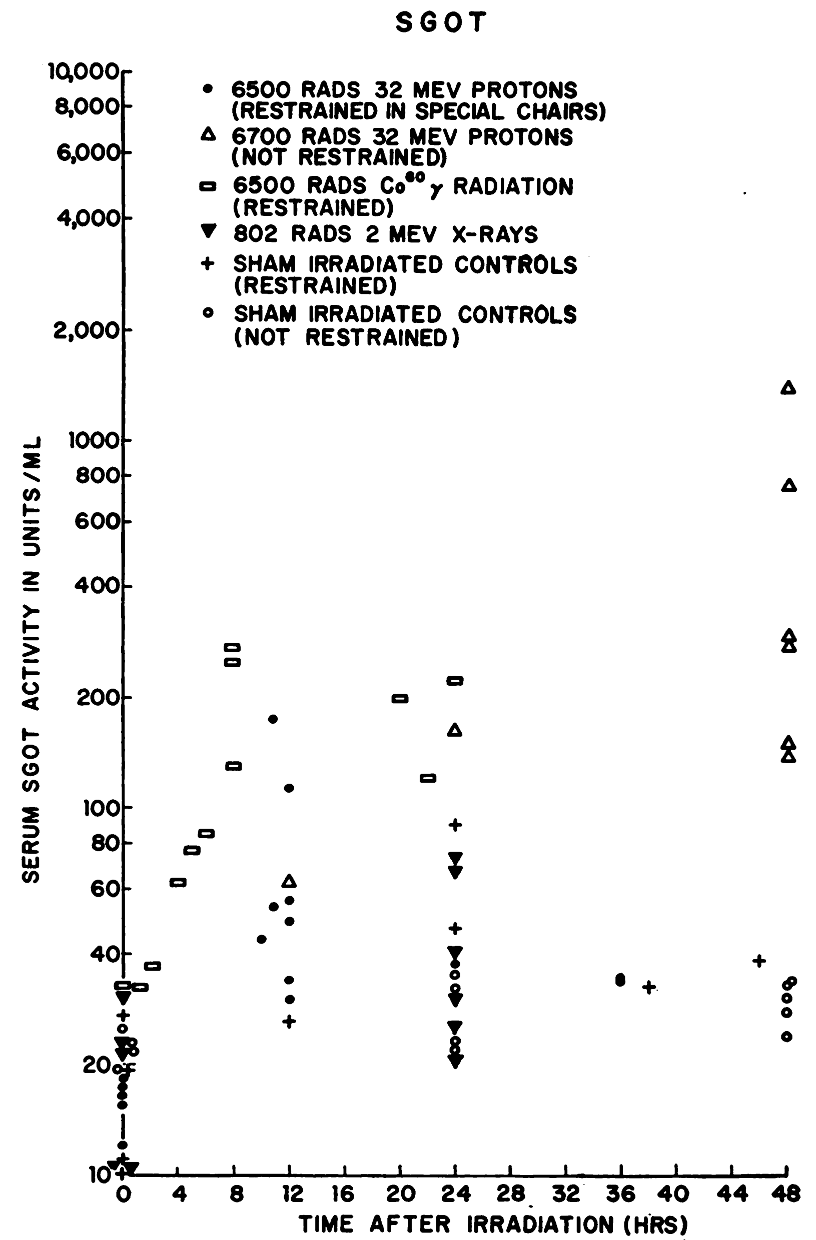

Fig. 2. Serum Glutamic Oxaloacetic Transaminase (SGOT) levels after irradiaton. The 0 time points are the preirradiation baseline values.

levels had occurred in all animals that had received 32 MeV protons. The somewhat inconstant elevations that occurred before 48 hours would support the hypothesis that there is considerable variation as to time of onset of the elevations.

The data presented in this report would suggest that at least part of the elevation of LDH and SGOT levels is the result of cell injury with secondary leakage of the enzymes, since considerably less marked changes occurred after 802 rads of 2 MeV x-rays. The 32 MeV protons and the ^{60}Co gamma radiation would seem to behave in a similar manner. They both appear to produce severe tissue injury as a result of ionization, which is in turn followed by a leakage of LDH and SGOT. This is indeed interesting when one considers that the 32 MeV protons reached only about 30 per cent of the total body volume while the ^{60}Co gamma radiation irradiated the entire body.

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