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Tissue Nonprotein Sulfhydryl Levels in Rats following Exposure to X Irradiation (40970)[1]

SHERWOOD M. REICHARD* AND MICHAEL J. GALVIN†

*Departments of Radiology and Physiology, Medical College of Georgia, Augusta, Georgia 30912, and †Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709

Abstract. The administration of glutathione and other nonprotein sulfhydryl (NPSH) compounds is known to increase the survival of animals receiving lethal doses of ionizing radiation. Little information is available concerning the effects of radiation on the tissue levels of NPSH compounds. In this study, the tissue nonprotein sulfhydryl concentration was determined in various organs of the rat during the early postirradiation period. Animals were sacrificed at 15 min; 4, 12, and 24 hr; and 5 and 21 days following 650 R of X irradiation. Livers, spleens, and kidneys were rapidly removed and NPSH levels were determined. In the spleen there was a significant increase in NPSH concentration occurring as early as 15 min, after exposure (75 vs 90 mg%). It returned to control levels at 4 hr, but increased again at 24 hr, following exposure to X irradiation. At 24 hr levels decreased sharply and remained depressed throughout the remainder of the observation period. In the liver a significant decrease occurred at 5 days. The data suggest that in addition to their pharmacologic action, glutathione and other naturally occurring SH compounds may be important in the physiologic response to radiation.

The administration of nonprotein sulfhydryl-containing (NPSH) compounds such as cysteine or glutathione, a physiologically important tripeptide containing a molecule of cysteine, prior to total-body X irradiation has been demonstrated to diminish the acute lethal effects of radiation (1–3). The $LD_{50/30}$ of mice and rats injected with these radioprotective agents was dramatically increased, splenic atrophy and anemia were decreased, and the hematopoietic system was protected (4–6). Concentrations of glutathione after administration were found to be high in the livers and spleens of treated rats (7). Shielding of the spleen is also known to increase the survival of X-irradiated animals (7, 8), but an increased survival of glutathione-treated animals was not observed when the spleens of these animals were shielded. In addition, radioprotectors were not effective in animals in which the spleens were removed (9). Thus, radioprotectors may function to preserve the integrity of the cellular and humoral elements of the spleen.

Relatively little information exists, however, on the naturally occurring tissue levels of NPSH compounds during the early postirradiation period. Such knowledge may contribute to a better understanding of the pathophysiologic changes set in motion by this environmental stimulus, and may provide additional insight into the mechanism of radiation protection. In these experiments, the NPSH levels of the spleen, liver, and kidney were monitored during the early postirradiation period following exposure of rats to total-body X irradiation.

Materials and methods. Young adult female rats of the CF-Nelson Strain (160 to 195 g) were used in these experiments. Whole-body irradiation was administered by a therapeutic X-ray unit operated at 200 kVp and 15 mA with a filter of 0.5-mm Cu and 1-mm Al as described earlier (10). The half-value layer was equivalent to 0.9 mm Cu. The skin-to-target distance was 40 cm and the dose delivered to the position of the center of the body, as measured by a Victoreen dosimeter, was 40 R/min in air. Unanesthetized rats were irradiated in a Lucite

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cage on a rotating table. Each animal received a total dose of 650 R of X irradiation.

The concentration of NPSH for liver, spleen, and kidney was determined by the method of Hewitt *et al.* (11) which measures total nonprotein sulfhydryl. At 15 min; 4, 12, and 24 hr; and 5 and 21 days following irradiation, rats were sacrificed and the tissues quickly removed. Duplicate tissue samples (50 to 100 mg) were weighed, and placed in a Kontes glass homogenizer with sufficient 6% tricholoracetic acid to give a 5% homogenate. The homogenate was centrifuged at 4°C for 10 min at 1000*g*. An aliquot of the supernatant (1 ml) was added to 8 ml of phosphate buffer (0.5 *M*, pH 8) and vortexed. A blank tube was prepared by adding 1 ml of 6% TCA to 8 ml of phosphate buffer. To each assay and blank tube, 1 ml of DTNB (0.04% 5,5-dithiobis-2-nitrobenzoic acid in 1% sodium citrate) was added and vortexed. The increase in absorbance which developed after 10 min, was then measured on a Beckman spectrophotometer at 412 mm. The NPSH content was determined by comparison with a standard curve using glutathione as a standard. The curve was determined over the range of 2 to 100 g/ml. Samples whose values were outside of this range were diluted with 6% TCA. The results are expressed in milligrams NPSH per 100 g of tissue (mg).

Statistical comparisons were made using Student's *t* test and Duncan's range test for comparing several means. *P* values less than 0.05 were considered significant.

Results. Changes in the spleen sulfhydryl concentration following radiation are presented in Table I. Tissue NPSH concentration exhibited a biphasic response. It increased in concentration as early as 15 min, following total-body X irradiation from 75 to 90 mg%. NPSH concentrations returned to normal at 4 hr, but increased significantly again at 12 hr. NPSH levels remained elevated throughout the rest of the 21-day observation period averaging 30% above control levels.

Table II presents the kidney NPSH levels following X irradiation. In this tissue the changes were opposite from those observed in the spleen. The levels remained stable for 24 hr at which time there was a 26% decrease in NPSH from 99 to 73 mg%. These values remained significantly below the controls at 5 and 21 days.

In contrast to the changes observed in the spleen and kidney, the liver sulfhydryl concentration showed no apparent trend of activity during the 21 days of observation (Table III). However, there was a decrease at 5 days which was significantly below the control reaching a level of 163 mg%, a decrease of 17% from preradiation levels.

Discussion. These studies show that alterations in the tissue levels of nonprotein sulfhydryl concentration occurs following X irradiation. The variations observed during the postirradiation period indicate a role for NPSH in the tissue response following radiation.

With the doses of total-body X irradiation used in these experiments, the hematopoietic system shows preferential damage (12) and the increased level of NPSH compounds in the spleen, a major hematopoietic organ in the rat, may represent a physiologic adjustment by this tissue

TABLE I. NONPROTEIN SULFHYDRYL CONCENTRATIONS IN THE SPLEEN FOLLOWING X IRRADIATION[a]

Time after X irradiation	Group	No. of rats	Activity	Percentage change
Control	1	12	75 ± 3.6	
15 min	2	10	90 ± 2.8*	20
4 hr	3	10	81 ± 2.7	8
12 hr	4	10	93 ± 2.9*	24
24 hr	5	10	101 ± 5.7*	35
5 days	6	9	100 ± 2.4*	33
21 days	7	6	95 ± 5.6*	27

[a] Mean (±SE) NPSH concentrations in mg/100 g tissue (mg%). Rats received 650 R of X irradiation.
* Groups 2, 4, 5, 6, 7 > 1 ($P < 0.01$).

TABLE II. NONPROTEIN SULFHYDRYL CONCENTRATIONS IN THE KIDNEY FOLLOWING X IRRADIATION[a]

Time after X irradiation	Group	No. of rats	Activity	Percentage change
Control	1	11	99 ± 7.5	
15 min	2	10	85 ± 2.2	14
4 hr	3	10	85 ± 3.5	14
12 hr	4	10	92 ± 3.8	7
24 hr	5	10	73 ± 3.0*	26
5 days	6	9	76 ± 3.7*	23
21 days	7	6	65 ± 5.5*	34

[a] Mean (±SE) NPSH concentration in mg/100 g tissue (mg%). Rats received 650 R of X irradiation.
* Groups 5, 6, 7 < 1 ($P < 0.02$).

in response to radiation. It is known that the presence of sulfhydryl groups before or during the time of irradiation protects sensitive receptor sites from being oxidized by radiation-induced free radicals (1–3). Since the spleen exhibits an elevated NPSH level during the postirradiation period, sulfhydryl compounds may also be important in modulating the latent damaging effects of ionizing radiation or cellular repair processes. Such a role may be possible because of the wide range of functions attributed to NPSH compounds in cellular and metabolic activities (13–15).

Although the pathway by which an increase in NPSH occurs in the spleen is not known, the spleen is thought to have a reservoir of sulfhydryl compounds in the form of mixed disulfides bound to proteins (16, 17). These thiols might act as a physiologic pool of NPSH groups which are released during and following radiation. On the other hand, the liver is the major site for the synthesis and breakdown of glutathione which makes up 90% of the NPSH (13). Glutathione may be released by the liver into the circulation and be preferentially sequestered by hematopoietic tissues perhaps at the expense of other less radiation-sensitive tissues such as the kidney.

The critical role of the spleen in the response of animals to radiation has been frequently reported. The administration of radioprotective sulfhydryl compounds has been shown to be taken up primarily by the spleen and liver (7). Such experiments suggest that protection from radiation results from an accumulation of NPSH at these sensitive sites. Shielding of the spleen has also been shown to increase the survival of X irradiation animals (7, 8); however, the increased survival of glutathione-treated animals was not augmented when the spleens of these animals were shielded. In addition, the removal of the spleen overcame the effect of radioprotectors (9). It is possible that a change in the NPSH levels is part of a homeostatic mechanism by which the body tries to maintain the integrity of the cellular and humoral elements of the spleen.

The decreased NPSH levels observed in

TABLE III. NONPROTEIN SULFHYDRYL CONCENTRATIONS IN THE LIVER FOLLOWING X IRRADIATION[a]

Time after X irradiation	Group	No. of rats	Activity	Percentage change
Control	1	11	196 ± 10.3	
15 min	2	10	181 ± 6.2	8
4 hr	3	9	196 ± 6.2	0
12 hr	4	10	219 ± 13.9	12
24 hr	5	10	215 ± 13.0	10
5 days	6	9	163 ± 7.3*	17
21 days	7	6	174 ± 12.9	11

[a] Mean (±SE) NPSH concentration in mg/100 g tissue (mg%). Rats received 650 R of X irradiation.
* Group 6 < 1 ($P < 0.05$).

kidney during the postirradiation period is correlated with the renal damage which occurs following X irradiation (18). Numerous studies have been reported which relate a decrease in NPSH levels to altered kidney physiology. For example, depletion of NPSH in rat kidney cortex was reported to interfere with the accumulation and transport of amino acids (11, 15). In addition, glutathione was shown to maintain the sulfhydryl groups essential to maintain enzymes in their active form (19, 20) and to maintain cell membranes (14). Consequently, the lowered levels of NPSH following radiation may interfere with kidney metabolism and cellular integrity.

Similar changes in NPSH levels have been observed in the spleen and kidney following exposure to trauma (21, 22). This is of interest because cross-resistance between these two experimental models, both of which severely affect homeostatic mechanisms, has been previously demonstrated (23). Thus, rats which had acquired resistance by exposure to repeated sublethal doses of trauma not only are resistant to traumatic shock, but also exhibit an increased survival to total-body irradiation. Since the same pattern of changes in NPSH levels is seen in both models, these compounds may be involved in the acquisition of resistance.

The results from these experiments indicate that naturally occurring NPSH compounds may be involved in the defense against radiation. Differences in the tissue levels of these compounds or mobilization thereof may account for the different radiosensitivities within a given population.

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