Mechanism of Protective Action of Glutathione Against Whole Body Irradiation.* (18502)

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The ability to modify the effects of *whole body* radiation was first proposed by Hektoen (1) who discovered an apparent protection by injection of foreign protein 10 days prior to radiation. Treadwell *et al.*(2) demonstrated protection by injection of estradiol 10 days before irradiation in mice. Ellinger(3) reported increased survival by post radiation treatment with desoxycorticosterone acetate. Patt *et al.*(4) and Cronkite *et al.*(5) have shown that pre-irradiation administration of sulfhydryl compounds will protect against radiation injury within certain defined limits. Dowdy *et al.*(6) have shown that anoxia during irradiation will afford significant protection. Miller(7) has shown that post radiation administration of antibiotics will increase the survival of mice exposed to a little less than an LD_{100}.

Upon conclusive proof that glutathione could protect against radiation injury(8) it was decided to make a systematic study of the comparative organ weights, complete blood counts, and histologic appearance of the tissues of the treated and untreated mice. These studies with technics will be reported in detail in a subsequent communication(9).

White Swiss inbred male mice weighing 20-24 g were irradiated with the radial beam of a 2.0 Mev. GE industrial X-ray tube. All

TABLE I. Splenic-thymic Weights in mg of Glutathione Treated and Untreated Male White Swiss Mice Exposed to 820 r (LD_{85}) Whole Body X-ray. Mean weight and range of 6 animals sacrificed on each day are tabulated. Testicular weights are from the 750 r experiment.

		Days after irradiation						
		0	1	3	5	7	12	14	21
Thymus	Treated	19.8 14-28	15 12.0-19.2	7 5.4-9.6	7 4.0-11.8	8.1 3.4-13.2	27 7.0-39.8	35.7 5.2-120	14.6 4.8-35.4
	Untreated	19.8 14-28	16.8 11.8-22.6	8.6 6.8-10.0	6.2 4.0-10.0	7.2 2.4-12.0	10.1 4.0-15.8	6.6 3.2-12.8	8.6 2.6-12.4
Spleen	Treated	192 126-298	58.1 50.0-66.4	48.6 37.0-67.6	48.6 26.0-76.8	32.8 25.8-42.4	120.0 54.6-201	80.5 49.8-147	217.1 138.0-271.8
	Untreated	192 126-298	66.8 55.2-79.0	47.9 30.8-94.0	44.5 19.0-67.8	48.9 23.2-66.4	48.0 38.0-57.4	86.4 44.2-197	206.5 87.8-210.6
Testicle	Treated	182 151-202			159 139-199	145 119-189	109 82-149	111 84-148	94 57-123
	Untreated	182 151-202			163 117-204	142 120-156	132 106-151	109 87-132	83 71-97

* Opinions are those of the authors and are not to be construed as reflecting those of the U. S. Naval Service.

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TABLE II. Mean Blood Counts and Range of Glutathione Treated and Untreated Mice Sacrificed at Stated Intervals After Irradiation with 820 r. Mean and range is tabulated.

		Days after irradiation							
		0	3 hr	1	3	5	7	12	14	21
Leukocytes × 10³/mm³		7 4.1-14.7	9.2 4.0-23.4	3 1.0- 5.9	1.2 .7- 1.8	.7 .4- .9	7. .2- 1.6	.5 .1- .9	.5 .4- .6	5.5 2.0 -11.8
	Treated	7	4	2.4	1.3	.4	.2	.3	.9	5.3
	Untreated	4.1-14.7	2.3- 6.4	.9- 5.3	.5- .2	.2- .7	.1- .4	.1- .5	.4-2.7	1.8 -11.8
Granulocytes × 10³/mm³		2.3 .9- 4.7	5.9 2.9-11.2	1.8 .4- 5.2	.5 .3- .7	.3 .2- .5	.3 .06- .6	.2 .01- .4	.3 .1- .6	3.8 1.3 - 9.8
	Treated	2.3	2.3	1.8	.7	.03	.07	.06	.3	2.9
	Untreated	.9- 4.7	1.9- 3.7	.7- 4.4	.2- 1.2	—	.01- .1	.02- .16	.04-1.5	.99- 6.6
Lymphocytes × 10³/mm³		4.7 2.4-10	3.2 .4- 5.5	1.1 .5- 3.1	.4 .3- .7	.4 .2- .6	.3 .04- 1.1	.3 .09- .7	.2 .1- .4	1.6 .7 - 3
	Treated	4.7	1.6	.7	.6	.3	.11	.2	.6	2.3
	Untreated	2.4-10	.6- 3	.2- 1	.3- .8	.01- .6	.09- .16	.08- .4	.3-1.2	.8 - 5.2
Red blood cells × 10⁶/mm³		9.8 7.9-11	10.7 8.8-12.2	8.8 7.7- 9.3	9.9 8.0-11.5	8.6 7.8-10.2	8.2 7.4-10.2	5.9 4.2 -7.6	5.5 2.8-7.5	7.7 5.7- 9.1
	Treated	9.8	10.3	9.6	9.4	8.6	8.0	5.2	4.6	6.1
	Untreated	7.9-11	9.0-12.2	8.4-12.1	8.1-10.6	7.5-10.1	5.7- 9.1	2.6-7.1	2.4-7.4	2.0 - 7.9

mice were given 820 r with the exception of the mice in which the testicles were studied. The latter received 750 r. Before irradiation the mice received either 4 mg of glutathione per gram of mouse subcutaneously as a 10% solution at pH 6.5 or isotonic saline in the same volume.

In Table I are shown the comparative changes in the weights of radiosensitive organs of the treated and the untreated mice after exposure to 820 r whole body X-ray.

In Table II are seen the mean blood counts of treated and untreated mice. Sections of bone marrow, thymus, spleen and lymph nodes showed no definite differences in the rate and extent of destruction of hemopoietic tissues of treated and untreated animals killed 1 and 3 days after irradiation. The subsequent regeneration of myelopoiesis in the bone marrow and in the pulp of the spleen was markedly accelerated in the treated animals. Differences in the regeneration of the lymphoid tissues were only slight, but appeared compatible with the concept of more rapid regeneration in the treated animals which was suggested by the greater organ weight gains in the treated animals.

Discussion. The similarity of the glutathione treated and untreated irradiated mice in respect to their radiosensitive organ weights, blood counts and histologic picture during the first 3-5 days is highly suggestive that cellular destruction of radiosensitive tissues (bone marrow, thymus, spleen, and testis) is of equal degree in the two groups. It is impossible to prove this conclusively by histologic technics because a few blasts might survive and be undetected.

In contrast to the similarity in the destructive phase the bone marrow, spleen, and thymus regenerated much more rapidly in the glutathione treated animals as judged by the criteria used.

The leukocytes in the peripheral blood show very little difference between the treated and the untreated mice, but this is not surprising because it has been shown(10) that the levels of leukocytes in peripheral blood do not

10. Brecher, G., Endicott, K. M., Gump, H., *Blood J. Hematol.*, 1948, v3, 1259.

necessarily reflect the histologic activity of the hemopoietic tissues. The decrease in the red blood counts during the first 7 days is similar. The Rbc's of the glutathione treated mice do not fall as low thereafter and recover more rapidly.

The testicular injury by histologic criteria and testicular weight is identical in both groups over a 21 day period during which there is no evidence of regeneration in either.

It appears that glutathione by an unknown manner protects the mechanisms which accelerate the regeneration of the hemopoietic tissues but does not prevent the cellular destruction. This concept is not proved. Evidence collected elsewhere lends support. Trowall(11) has shown that cysteine does not protect lymph node cultures. Hennessey (12) by a precise technic has shown that neither glutathione nor cysteine protect against the radiation inhibition of iron uptake by the erythropoietic tissue.

Conclusions. 1. By the criteria used there is no significant difference in the destructive effects of irradiation on normal mice or mice treated with large doses of glutathione before irradiation. 2. A striking difference is seen in the rate of regeneration of the hemopoietic tissues.

11. Trowall, O., personal communication.
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Acid-Binding Capacity of Dialyzed Mucin Fractions from Human Gastric Juice.* (18503)

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(Introduced by D. P. Barr.)

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The mechanisms responsible for variations in gastric acidity are still incompletely understood. Hollander(1) has shown that hydrochloric acid is secreted in constant concentration at 0.17 normal, but at a variable rate. It has generally been assumed that variations in gastric acidity are attributable not only to dilution but to the partial neutralization of the hydrochloric acid by the constituents of the gastric mucus, although full data on the buffering power of human gastric mucin are not available. By the method of Glass and Boyd(2) the chief constituents of the gastric mucin can be determined separately. These authors found that a mucous substance which they called mucoprotein is secreted by the glandular cells, and that another constituent of the dissolved mucin, which they called mucoproteose, is derived from the visible mucus fraction which in turn is secreted by the lining columnar epithelium. Which of the mucous substances or their degradation products is chiefly concerned with neutralizing hydrochloric acid is still not known and there is little agreement to be found in the literature concerning the buffering role of gastric mucus. Some workers have attributed a marked buffering effect to the mucous substances(3), others, a moderate effect(4), and

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