TECHNICAL PAPERS

Cysteine Protection against X Irradiation

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It is generally considered that many of the biological effects of radiation can be attributed to the activated water reactions which result from the irradiation. Inhibition or inactivation of enzymes and other materials by the actions of the decomposition products of water (OH, H_2O_2, O_2H, H) and the prevention or reversal of these effects under certain conditions have been reported by a number of investigators. Barron (*1*) has found that sulfhydryl-containing enzymes are readily oxidized by radiation and that it is possible to reactivate such enzymes after moderate radiation doses (100–200 r) on addition of a reducing agent such as glutathione. Reducing amino acids, glutathione, and ascorbic acid have been reported by Ephrati (*2*) to protect tetanus toxin against the effects of X-rays, whereas oxidizing substances are without effect. The finding by Evans (*3*) that the toxicity of irradiated water to *Arbacia* sperm is reduced by catalase also supports the concept of indirect action by the decomposition products of water. Although other related observations are cited in the literature (*4*), the extent to which oxidizing and reducing substances contribute to radiation damage *in vivo* has received little, if any, consideration. We should like at this time to communicate some of our observations concerning the influence of cysteine, the amino acid-containing –SH, and cystine, its oxidized counterpart, on the radiosensitivity of rats.

Since, in our experience, the product (toxicity, survival time, hematologic change) of radiation dose rate times duration of exposure appears to be a constant over a fairly wide range, we have employed brief exposures to fairly intense radiation on the assumption that a potentially protective material should be present in the blood and body fluids in high titer during the entire exposure. The radiation factors were: 250 kv, 15 ma, 0.5-mm Cu and 3.0-mm Bakelite filters, 27.5 cm target distance, and 210 r/min dose rate. Male and female rats (Sprague-Dawley) weighing 150–250 g received 800 r total-body X radiation in a single exposure. Animals of the same sex were irradiated in pairs, one serving as a control, the other as a treated animal.

Cysteine was injected into a tail vein either 1 hr before, 5 min before, or 5 min after exposure. Neutralized (pH 7) as well as unneutralized (pH 1) cysteine-HCl solutions were compared. Four different doses were employed (175, 350, 575, and 875 mg as cysteine per kg body weight). A 20% cysteine-HCl solution was used to decrease the volume of injected fluid. Since rapid injection of this concentrated solution, especially with the higher doses, may result in acute pulmonary edema and death (also true with 20% NaCl), the cysteine was injected slowly over a period of several minutes. Occasional sloughing of the tail was seen at the injection site. It is of interest that this reaction can be prevented by anesthetizing the rat before injection. Cystine is relatively insoluble at pH 7, and only an acid solution of this material was used (6.66%, pH 1, 280 mg/kg body weight). Irradiated controls for the neutralized cysteine-HCl groups were injected intravenously with an equivalent volume of a 5% solution of NaCl. All other controls received I.V. injections of 0.9% NaCl adjusted to the pH of the test solutions. All rats were fed Derwood Checkers and water ad lib.

TABLE 1

EFFECT OF PRETREATMENT WITH CYSTEINE AND CYSTINE ON SURVIVAL AFTER TOTAL-BODY X IRRADIATION WITH 800 ROENTGENS*

Treatment group	Dose mg/kg	pH of injected material	Number of rats	% Survival after irradiation: 1st week	2nd week	3rd week	4th week	5th week
Control†			20	100	35	25	20	20
Cysteine	175		20	95	80	80	75	75
Control			22	86	32	27	27	27
Cysteine	350	1	19	100	95	89	89	89
Control			22	95	27	10	10	10
Cysteine	575		17	94	82	82	82	82
Control‡			14	57	7	7	7	7
Cysteine	350		15	100	87	47	47	47
Control			14	64	7	0	0	0
Cysteine	575	7	15	93	66	47	47	47
Control			27	70	19	7	7	7
Cysteine	875		25	92	92	84	80	80
Control†			27	52	22	15	15	15
Cystine	280	1	22	68	18	18	18	18

* All injections intravenously 5 min before irradiation.

† Controls received equivalent volume of 0.9% NaCl (pH 1) I.V.

‡ Controls received equivalent volume of 5% NaCl (pH 7) I.V.

Our findings reveal that pretreatment with cysteine markedly reduced toxicity from total-body X irradiation (82% survival, cysteine [pH 1] pretreated; 19% survival, irradiated controls). We have obtained similar results in mice (*5*). On the other hand, the disulfide, cystine, did not influence survival. Cysteine-HCl administered at pH 1 seemed equally effective at each dose level. However, with the neutralized preparation, greatest protection was afforded when the highest dose (875 mg) was

used. This may possibly be explained by the fairly rapid oxidation of cysteine to cystine in a neutral medium before its administration. A similar reduction in mortality was observed when cysteine was given either 5 min or 1 hr before X irradiation. Significantly, injection of cysteine immediately after the exposure was ineffectual.

TABLE 2

INFLUENCE OF TIME OF INJECTION OF CYSTEINE ON SURVIVAL AFTER X IRRADIATION WITH 800 ROENTGENS*

Treatment group	Time of injection relative to X irradiation	Number of rats	% Survival after irradiation			
			1st week	2nd week	3rd week	4th week
Control	5 min before	15	73	20	13	13
Cysteine	5 min before	15	87	87	87	87
Control	1 hr before	15	80	20	20	20
Cysteine	1 hr before	15	100	87	80	80
Control	5 min after	16	88	19	13	6
Cysteine	5 min after	15	60	20	13	13

* Cysteine—875 mg/kg I.V., pH 7; controls received equivalent volume of 5% NaCl I.V.

These results are summarized in Tables 1 and 2. If all rats receiving cysteine at either pH 1 or 7 before their irradiation are considered as a single group, there are 92 survivors of 126 cysteine-treated animals (73%) as compared with 18 survivors of 134 irradiated controls (13%). These findings are highly significant statistically ($p \ll .001$).

Change in body weight was followed in a small group of rats pretreated with cysteine. Body weight is a fair prognostic sign of ultimate toxicity in the irradiated rat. Although the initial decrease was similar in the cysteine-pretreated and in the control animals, body weight recovered rapidly in the former.

We may conclude that cysteine, but not cystine, administered to rats prior to X irradiation in the nearly completely lethal range, greatly diminishes toxicity. This ameliorating influence may reside in the protection afforded certain critical cellular constituents against oxidation by the presence of cysteine or an intermediate of cysteine. A study of the protective influence of cysteine when administered at longer intervals before irradiation and by different routes as well as of other substances, such as glutathione, methionine, tryptophane, and ascorbic acid, is in progress. The metabolism of injected cysteine in the irradiated animal and its effect on the hematologic and histologic changes induced by radiation will be reported in detail in a subsequent communication.

References

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Humidifying Apparatus for Small Test Rooms

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The wood-element hygrostat illustrated in Fig. 1 was devised by Torgeson for use in an experimental lumber dry kiln at the U. S. Forest Products Laboratory, Madison, Wisconsin. Because of its simplicity and sturdiness it later was installed in each of two 600-cu-ft rooms used at the laboratory for accelerated testing of the resistance of wood and wood products to decay by pure cultures of wood-destroying fungi. As set, the hygrostat, in conjunction with the humidifier illustrated in Fig. 2, maintains a relative humidity of 70% with deviations of about ±2%. The temperature of the rooms is thermostatically kept at 80° F. Over several years, no adjustment in setting on account of changes in characteristics of the hygrostat has been needed. The reliable performance and simplicity of this humidification system should make it useful in many connections requiring humidity control within a moderately narrow range and at a level greater than that prevailing outside the space to be conditioned. It is already being used for biological work at a number of other laboratories.

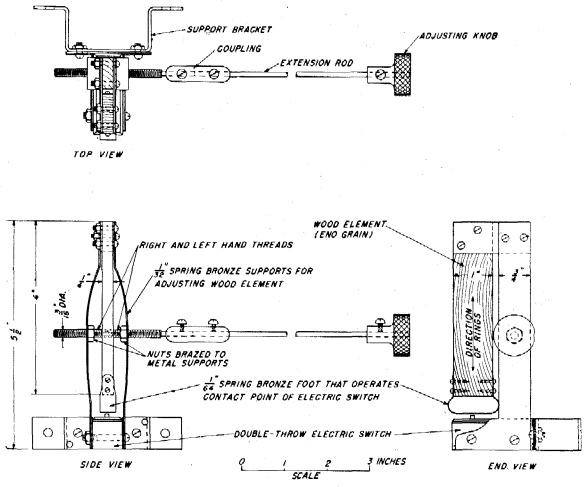

FIG. 1. Hygrostat.

The novel features of the hygrostat consist of the wood element and a microtype switch that is sensitive to a very slight movement of the element. The wood should be free of defects and have as flat a grain as possible, for the flatter the grain the greater will be the dimensional change lengthwise of the element with a given change in relative humidity, and thus a correspondingly greater operating sensitivity of the hygrostat will be provided. The sapwood of hardwood species, such as