tutes a composite body (protein frame + water of imbibition) and has the birefringence of rodlets, although experimentally not measurable. These considerations suggest that bone is really a complex system. Hence, it would be dangerous to apply to bone (as Dallemagne and Mélon do) a reasoning that is valid only for a two-component composite body.

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Brucella Ring Test Antigen Prepared by Reduction of a Tetrazolium Salt

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Brucella Ring Test is the name given the phenomenon which occurs when stained *Brucella* antigen is added to whole milk. If the milk contains *Brucella* agglutinins the stained antigen is agglutinated and rises with the cream to give an intensely colored cream layer, or ring. This test was first described by Fleischhauer (*2*) and is rapidly coming into general use as a quick and simple method of testing herds of cattle for evidence of brucellosis.

Present methods of staining antigen make use of hematoxylin, which frequently results in antigen unsatisfactory with respect to sensitivity, specificity, color intensity, and keeping quality. In addition the procedures are laborious and critical.

Tetrazolium salts have been used for staining plant and animal tissues. Several of these compounds were used to stain *Brucella* antigen, and the one found most suitable is 4,4′-bis(3,5-diphenyl-2-tetrazolinium)-biphenyl dichloride. This compound is reduced by living cells to an intensely colored violet-blue formazan. It appears that this reduction takes place inside the cell, and hence the antigenic specificity of the cell surface is not altered.

To prepare *Brucella* ring test antigen the organisms are grown using the method of Brown and Wood (*1*). To the heavy suspension of living cells in broth obtained by this method an aqueous solution of 4,4′-bis(3,5-diphenyl-2-tetrazolinium)-biphenyl dichloride is added to give a final concentration of one part in 16,000. This mixture is incubated at 37° C for 4 hr to permit the organisms to reduce the compound and thereby become stained. The organisms are then killed by heating to 60° C for 1 hr. The antigen is then concentrated by centrifugation and resuspended to the desired density in 0.85% NaCl solution containing 0.5% phenol as a preservative.

More than 100 different lots of antigen have been prepared over the last 2 years using this method, and all have been of uniform color intensity, specificity, and sensitivity. This antigen has remained stable over prolonged periods and under normal conditions of use and storage.

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Protection of Mice against X-Radiation by Thiourea

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It was reported previously (*4*) that thiourea protected desoxyribosenucleic acid (DNA) against x-ray depolymerization in aqueous solution and *in vivo*. Barron *et al.* (*1*) have reported that sulfhydryl-containing enzymes in aqueous solution were inactivated by x-radiation because of the oxidation of the sulfhydryl group and that the enzymes were reactivated by the addition of glutathione. Ephrati (*2*) found that the inactivation of tetanus toxin and of staphylococcus hemolysin by x-radiation in aqueous solution was inhibited by the presence of reducing agents such as ascorbic acid and glutathione, whereas oxidizing agents did not inhibit the action. Forssberg (*3*), however, found that reducing agents (cystein) enhanced the inactivation of catalase by x-radiation, whereas oxidizing agents (cystine) inhibited this action. Patt and his coworkers (*5*) have recently found that cysteine decreased the mortality of x-rayed rats, whereas cystine was ineffective. The protection by thiourea of a vital cellular constituent, such as DNA, therefore might be assumed to affect the mortality of x-rayed animals.

Male, white mice (Detwiler) weighing 18–22 g were allowed Fox Food Blox (Allied Mills) and water ad libitum. The mice were irradiated in groups of 20–25 with 650 r given at the rate of 100 r/min. The constants of the x-ray machine were 250 kv and 15 ma. A copper filter ½ mm thick was used, in addition to the inherent filtration of 3 mm of aluminum.

In a preliminary experiment an aqueous solution of thiourea (90 mg/ml) was injected intraperitoneally in a single dose (1,280 mg/kg) 5 min before irradiation. In a subsequent experiment thiourea was administered in the drinking water in a concentration of 1% for a period

of 6 days before irradiation. In a third experiment intraperitoneal injections (2,140 mg/kg) of an aqueous thiourea solution (90 mg/ml) were given 5 min before irradiation to one group and injections of the same dose 5 min before irradiation was most effective in increasing the number of survivals after irradiation ($p << 0.001$). Of the group injected with thiourea (2,140 mg/kg) before irradiation, 35.2% survived,

TABLE 1

EFFECT OF THIOUREA ON THE MORTALITY OF MICE X-RAYED WITH 650 R

Treatment	Time of treatment relative to irradiation	Dose mg/kg	No. of mice	Survived 1st week %	2nd week %	3rd week %	4th week %
Control			30	87	32	22	....
Thiourea—I.P.* injection	5 min before	1,280	30	60	37	37	....
Control			20	70	0	0	0
			"	100	10	10	10
			"	70	0	0	0
			"	80	0	0	0
			"	85	0	0	0
						mean	2
						σ†	4.472
						σ_M‡	1.999
Thiourea—I.P. injection	5 min before	2,140	20	80	70	70	60
" "	" " "	"	"	90	30	30	20
" "	" " "	"	"	90	40	15	10
" "	" " "	"	"	100	70	50	43
" "	" " "	"	"	95	80	60	43
						mean	35.2
						σ	20.000
						σ_M	8.944
Thiourea—I.P. injection	5 min after	2,140	20	75	15	10	10
" "	" " "	"	"	90	30	15	15
" "	" " "	"	"	70	5	0	0
" "	" " "	"	"	65	20	10	10
" "	" " "	"	"	75	10	5	0
						mean	7
						σ	6.708
						σ_M	3.000
Control			100	99	82	70	68
Thiourea—In drinking water	during 6-day period before	(1%)	100	98	95	88	86

* I.P.—intraperitoneal injection.

† Standard deviation, $\sigma = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N-1}}$

‡ Standard deviation of means, $\sigma_M = \frac{\sigma}{\sqrt{N}}$.

5 min after irradiation to another. Because thiourea had been found to be destroyed rapidly (*6*), the irradiation was carried out as soon as possible after injection.

The results of all the experiments are summarized in Table 1. When the thiourea dose was 1,280 mg/kg 37% of the mice injected before irradiation survived at the end of 3 weeks as compared to 22% of the irradiated controls. When 1% thiourea was added to the drinking water for 6 days before irradiation, 86% survived at the end of 4 weeks, but only 68% of the irradiated controls survived ($p << 0.01$). Although addition of thiourea to the drinking water caused a loss in body weight, the mice so treated gained weight after irradiation more rapidly than did the irradiated controls. The injection of 0.1% thiourea (2,140 mg/kg) 5 min after irradiation was only slightly effective, whereas injection of the same whereas only 7% of the group injected after irradiation and only 2% of the irradiated controls survived.

As a result of our experiments we believe that thiourea and possibly other reducing agents lower the mortality due to x-radiation because of the protection afforded to certain vital cellular constituents such as nucleic acid. It is possible that other easily oxidized constituents, such as sulfhydryl-containing enzymes, ascorbic acid, and glutathione, are protected in the same manner. Whether thiourea and related substances diminish the therapeutic effects of x-radiation besides lowering the mortality remains to be investigated.

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