

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-di-

phenyl-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 deoxyribosenucleic 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 (cystine) enhanced the inactivation of catalase by x-radiation, whereas oxidizing agents (cystine) inhibited this action. Patt and his co-workers (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 $\frac{1}{4}$ 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