supplemented with only vit. B_{12} and folacin. However, liver fat of the SD strain rats was 33% (dry basis) as compared to 62% for the AES strain rats. (3) The addition of vit. B_{12} and folacin to the basal diet supplemented with suboptimal choline chloride markedly suppressed the accumulation of fat in the liver, reduced the incidence of renal damage and increased the choline content of the livers of the SD strain of rats. Liver fat values averaging 12.8% (dry basis) were obtained with a 0.20% choline chloride supplement in the presence of vit. B_{12} and folacin as compared to a requirement of 0.40% choline chloride in the absence of vit. B_{12} and folacin.

Received June 25, 1951. P.S.E.B.M., 1951, v77.

Reduction of X-ray Sensitivity of *Escherichia coli* B/r by Sulfhydryl Compounds, Alcohols, Glycols, and Sodium Hydrosulfite.* (18874)

W. T. BURNETT, JR., G. E. STAPLETON, M. L. MORSE, AND ALEXANDER HOLLAENDER.

From the Biology Division, Oak Ridge National Laboratory, Oak Ridge.

It has been shown(1) that oxygen has a marked effect on the survival of X-irradiated *Escherichia coli* B/r. A striking increase in survival was attained by saturating the bacterial suspensions with nitrogen before exposure to X-radiation. In this study, the X-ray sensitivity of *E. coli* B/r in the presence of 2,3 dimercaptopropanol (BAL), sodium hydrosulfite, and ethanol has been examined.

Suspensions of *E. coli* B/r cultured aerobically 20-24 hours in nutrient broth (Difco) were washed and resuspended in 1/15 M phosphate buffer. A 0.1 ml portion containing about 10^{10} cells was introduced into each of several 2 ml test tubes together with 0.9 ml of the test compound in phosphate buffer. Buffer solutions 0.04 M in sodium hydrosulfite and solutions 3.5 M in ethanol were used in examining the protective ability of these compounds. Dispersions equivalent to 0.04 M BAL were prepared by shaking in buffer a short time before addition to the suspensions. These were shown in other experiments(2) to be the maximally effective concentrations of these agents.

The diluted suspensions were cooled to 2°C and maintained at this temperature during the exposure to X-radiation. The source of X-rays was a Maxitron 250 unit operated at 250 kvp at 30 ma (3 mm added aluminum filter). The test tubes were held in a slanting position, at a 35° angle from the horizontal, under the X-ray tube by a circular lucite holder containing ice and ice water. The irradiated suspensions were diluted with nutrient broth and aliquots transferred to several broth agar plates, the average number of colonies developing in 24 hours at 37°C being used to calculate the number of surviving cells.

The log of the survival of *E. coli* B/r grown under aerobic conditions is a linear function of the X-ray dose, at least over the range 15,000 to 90,000 r. This relationship is illustrated in Fig. 1 for untreated suspensions and for suspensions which have been bubbled for 15 minutes with nitrogen immediately before the exposure period. As shown in Fig. 1-2, a similar relationship also obtains in the presence of BAL, sodium hydrosulfite, and ethanol.

The protective action of a chemical agent can be expressed simply as the ratio of the X-ray dose required to kill a given number of cells in the presence of the compound to that required in the absence of the compound. This method of presenting data is essentially that used by Kelner(3). "Isoefficiency ra-

* Work performed under Contract No. W-7405-eng-26 for the Atomic Energy Commission.

1. Hollaender, A., Stapleton, G. E., and Martin, F. L., *Nature*, 1951, v167, 103.

2. Burnett, W. T., Jr., Morse, M. L., Burke, A. W., and Hollaender, A.; Unpublished data.

3. Kelner, A., *J. Bact.*, 1949, v58, 511.

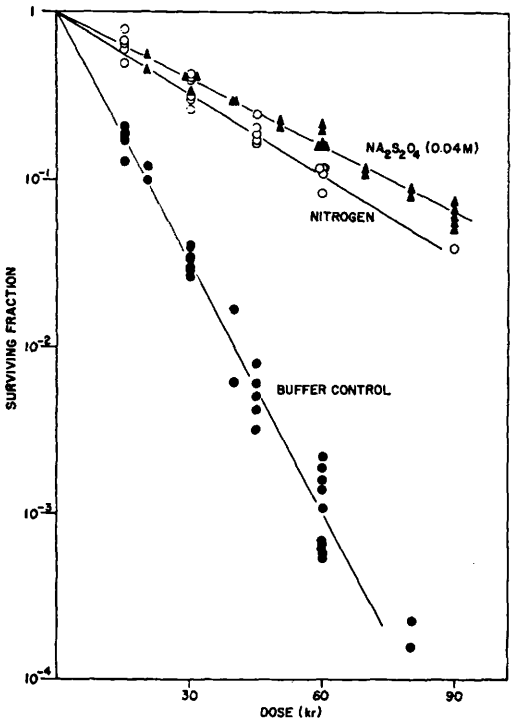

FIG. 1. Effect of nitrogen saturation and sodium hydrosulfite on survival of *E. coli* B/r suspensions X-irradiated at 2°C.

tios" calculated from data presented graphically in Fig. 1-2, are given in Table I.

The "isoefficiency ratio" for BAL, which has 2 sulfhydryl groups, is about twice that found for other compounds which have only one sulfhydryl group, *e.g.*, cysteine, mercaptosuccinic acid, mercaptopyruvic acid, and 2(2-mercaptoethoxy) ethanol using equivalent molarities. It might be considered that the sulfhydryl compounds have the ability to protect the enzyme systems in bacterial cells, since Barron(4) has shown that cysteine and glutathione protect X-irradiated enzyme substrates. However, the entrance of these compounds into the bacterial cell has not been shown. The protective action of cysteine in mice under certain conditions has been demonstrated by Patt(5).

Because sodium hydrosulfite is a strong reducing agent and combines rapidly with molecular oxygen in aqueous solution, its mode of action may be simply a removal of oxygen from the bacterial suspensions. Reference has already been made to the finding in this laboratory that a reduction in the oxygen tension of *E. coli* B/r suspensions before X-irradiation increases the survival. It may be speculated that the removal of molecular oxygen, either by a chemical agent or by bubbling an inert gas through the suspension, interferes with the production of highly reactive radiodecomposition products(6-13).

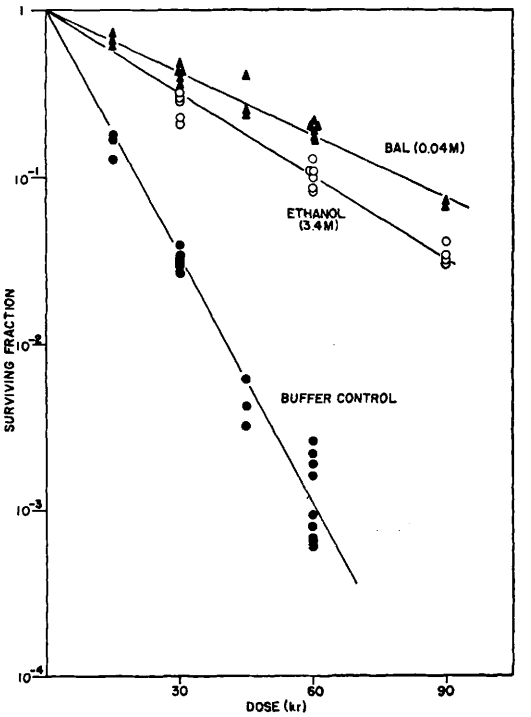

FIG. 2. Effect of ethanol and BAL on survival of *E. coli* B/r suspensions X-irradiated at 2°C.

4. Barron, E. S. G., Dickman, S., Muntz, J. A., and Singer, T. P., *J. Gen. Physiol.*, 1947, v32, 537.

5. Patt, H. M., Tyree, E. B., Straube, R. L., and Smith, D. E., *Science*, 1949, v110, 213.

6. Allen, A. O., *J. Phys. Colloid Chem.*, 1948, v52, 479.

7. Burton, M., *J. Phys. Colloid Chem.*, 1947, v51, 611.

8. Stein, G., and Weiss, J., *Nature*, 1948, v161, 650.

9. Bonet-Maury, P., and Lefort, M., *Nature*, 1948, v162, 381.

10. Weiss, J., *Nature*, 1944, v153, 748; 1946, v157, 184.

11. Fricke, H., *J. Chem. Phys.*, 1935, v3, 364.

12. Dainton, F. S., *J. Phys. Colloid Chem.*, 1948, v52, 490.

13. Lea, D. E., Action of Radiations on Living Cells, 1947, The Macmillan Co., New York.

TABLE I. Isoefficiency Ratios* for Nitrogen, BAL, Ethanol, and Sodium Hydrosulfite Calculated from Data Presented in Fig. 1 and 2.

	Nitrogen	BAL (0.04 M)	Ethanol (3.5 M)	Sodium hydrosulfite (0.04 M)
Dose ratio ($\rho = 10^{-1}$ survival)	3.2	4.0	3.1	3.7

* Isoefficiency ratio $= \frac{\text{kr required to give } \rho \text{ survival in presence of agent}}{\text{kr required to give } \rho \text{ survival without agent}}$.

However, since the "isoefficiency ratio" for sodium hydrosulfite is higher than that found by saturating the suspension with nitrogen, it seems worth while to further investigate the protective action of sodium hydrosulfite.

Methanol; 2-propanol; 1-butanol; 2-methyl-1-propanol; 2-methyl-2-propanol; 1,2,3-propanetriol; 1,2-propanediol; and 1,2-ethylenedioxydiol are about as effective as ethanol on a molar basis.

The concentration of ethanol necessary to effect the large increase in cell survival of *E. coli* B/r (Fig. 2) is much higher than the concentrations reported to interfere with the X-ray inactivation of trypsin(14). However, ethanol in lower concentrations (0.02-0.1 M) has been found to increase cell survival if the suspensions are incubated in its presence at higher temperatures (25-37°C) prior to irradiation.

Summary The X-ray sensitivity of *E. coli* is reduced if the cells are irradiated in the presence of certain chemical agents. BAL, ethanol, and sodium hydrosulfite increase the dose required to kill a given number of cells in suspensions at 2°C by a factor of 3-4. Other sulfhydryl compounds, glycols, and low-molecular-weight alcohols also increase the survival of *E. coli* suspensions.

The authors are indebted to Dr. C. T. Bahner, Carson-Newman College; Mr. L. E. Klein, Monsanto Chemical Company; and Drs. D. G. Doherty and A. H. Doermann, Biology Division, Oak Ridge National Laboratory for valuable suggestions. The technical assistance of Mr. A. W. Burke is acknowledged.

Received June 25, 1951. P.S.E.B.M., 1951, v77.

14. Kaufmann, B. P., McDonald, M. R., Gay, H., Belser, N. O., Pennoyer, J. M., Lennihan, M. R., and Judge, J. T., Carnegie Institution of Washington Year Book, 1950, No. 49, 175.

Maintenance of Normal Thyroid Activity after Transplantation of Thyroid Gland into Spleen or Kidney. (18875)

PHILIP K. BONDY.

From the Department of Medicine, Emory University School of Medicine, Atlanta, Ga.

Shortly after the intravenous injection of thyroxin labelled with radioactive iodine, much of the radioactivity can be demonstrated in the liver, gastrointestinal contents and feces(1,2). Most of the gastrointestinal and fecal iodine is no longer in the form of thyroxin(1). Since thyroid hormone appears to circulate in the form of thyroxin(3) it might be concluded that the liver normally plays an important part in the isolation, destruction and excretion of circulating thyroid hormone. However, the sudden introduction of a test material into the bloodstream may cause a transient elevation in the concentration of the

1. Gross, J., and LeBlond, C. P., *J. Biol. Chem.*, 1947, v171, 309.

2. Gross, J., and LeBlond, C. P., *J. Biol. Chem.*, 1950, v184, 489.

3. Taurog, A., and Chaikoff, I. L., *J. Biol. Chem.*, 1948, v176, 639.