Quantitative Aspects of the Protective Action of Cysteine Against X-Radiation. (17624)

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We have previously reported(1,2) that cysteine administration either orally or intravenously prior to the exposure of rats to lethal dosages of X-radiation greatly increases their chances for survival. As part of a series of studies pointed toward the characterization of this action of cysteine, experiments were designed for the purpose of revealing whatever quantitative relationships exist between cysteine dosage, X-ray dosage, and the level of the protective action of cysteine. The results of such experiments are presented in this paper.

Methods. In general the methods were similar to those reported previously(1). Control and experimental animals were irradiated simultaneously, caged together and otherwise similarly handled. Male Sprague-Dawley rats weighing 150 to 250 g were employed. Cysteine was administered as a 10% solution of the hydrochloride neutralized to pH 7-8 with 2 N sodium hydroxide. The material was injected into a tail vein within 5 minutes prior to irradiation. Animals were irradiated in pairs, one having been injected with cysteine and the other with an equivalent volume of 5 per cent sodium chloride. The irradiation consisted of a single total-body exposure to X-rays (250 kv, 15 ma, and 0.5-mm Cu and 3.0 mm Bakelite filters, 26.5 cm target distance, 1.5 mm Cu half-value layer and 210 to 220 r per minute dose rate). Survival at 30 days post irradiation was the chief criterion employed to evaluate cysteine protection. In addition, however, time to 50% mortality and the average survival time of animals dying within the 30-day post irradiation period were noted. Three types of experiments were performed: 1) the relative protective action of various dosages of cysteine (50 mg per kg to 950 mg per kg) was determined for given X-ray dosages (800 r and 1000 r); 2) the protective action of a given dosage of cysteine (950 mg per kg) was evaluated at various X-ray dosages (600 r to 1800 r); 3) since 950 mg per kg was found to be the maximum dose of cysteine tolerated by rats, the protection afforded by a dual injection whereby the total dosage of cysteine was increased, was evaluated for an X-ray dose of 1200 r. The dual injection consisted of a dose of 475 mg per kg followed in 45 to 60 minutes by a second dose of 950 mg per kg. The rats were irradiated immediately after the second injection (15 controls, 18 cysteine treated).

Results. The degree of protection afforded by cysteine against a particular dosage of X-radiation is roughly proportional to the cysteine dose (Fig. 1)

The degree of protection afforded by a given dosage of cysteine decreases as the X-ray dosage is increased (Fig. 2). A plot of the

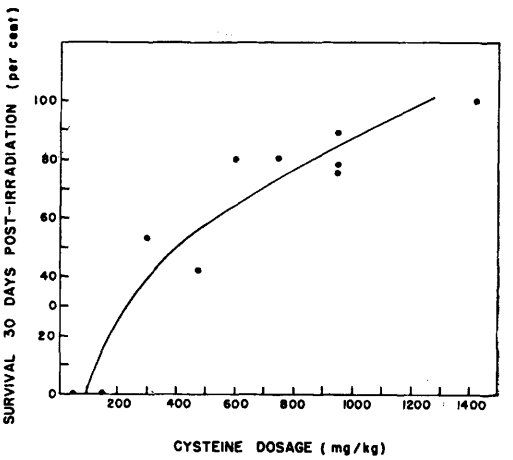

FIG. 1.

The influence of cysteine dosage upon the survival of rats exposed to total-body X irradiation (1000 r). Each point represents 9 to 18 rats. The 1425 mg/kg of cysteine point represents the dual injection experiment.

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

2. Patt, H. M., Smith, D. E., Tyree, E. B., and Straube, R. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 18.

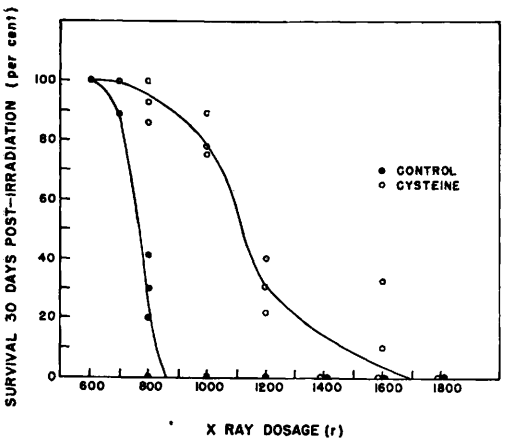

FIG. 2. The influence of X-ray dosage upon the survival of rats pretreated with cysteine. Each point represents 6 to 18 rats.

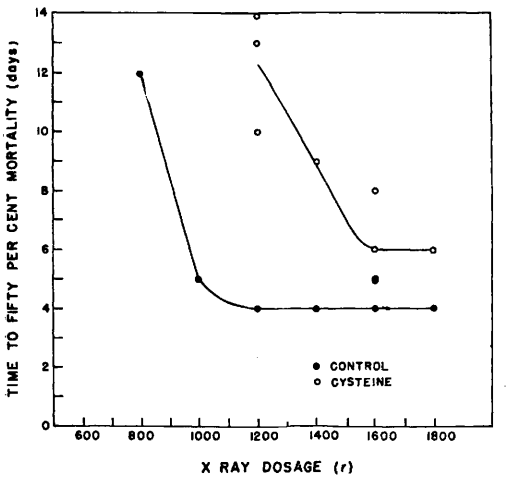

FIG. 3. The influence of X-ray dosage on time to 50% mortality of rats pretreated with cysteine. Each point represents 6 to 17 rats.

data upon logarithmic-probit graph paper yields straight lines.

A cysteine dosage of 950 mg per kg raises the LD_{50} from about 765 r to about 1120 r and almost doubles the LD_{100} (Fig. 2).

Cysteine increases the time to 50% mortality (Fig. 3). The average survival time of animals dying within 30 days post irradiation is also significantly increased.

Increasing the dose of cysteine 50% above the maximum tolerable single dose by means of dual injections 45 to 60 minutes apart protects 100% of animals exposed to 1200 r.

Discussion. The results of our experiments employing various cysteine doses at given levels of irradiation demonstrate the difficulties attending the evaluation of the effectiveness of substances which might alter radiosensitivity. Although exposure to 800 r in this laboratory usually kills about 80% of rats within 30 days, the range of mortality is from 60 to 100%. Only a rough indication of the relationship between cysteine dosage and protective action is obtained when the 30-day % survival is taken as an expression of cysteine protection at 800 r. The difficulties presented by the variation in control mortality after 800 r are somewhat lessened, however, and a better idea of the cysteine dose-cysteine protection relation is gained, if one considers the difference in per cent survival between control and treated animals which were irradiated simultaneously. Since an X-ray dose of 1000 r kills all control animals, it would seem from the above considerations and from the work of Clark and Uncapher(3) that testing at this level would allow one a better chance to assess the ability of materials to alter sensitivity to irradiation. The results of our experiments bear out this premise.

A more concise picture of the ability of cysteine to alter sensitivity to irradiation was gained from the series of experiments where the protection afforded by a given dosage of cysteine was evaluated for various X-ray dosages. Fig. 2 clearly demonstrates that the protective power of a given cysteine dose decreases as the amount of radiation is increased. When in addition to the data discussed above one considers the results of the dual injection experiment (100% survival at 30 days post 1200 r) it is clear that the level of protection afforded by cysteine against X-radiation is dependent upon cysteine dosage and radiation dosage.

The practicality of employing time to 50% mortality and average survival time as criteria for assessing the effectiveness of procedures which might alter radiosensitivity is well demonstrated in the present experiments. It is clear that cysteine can prolong life in instances where it does not save the animal from death.

3. Clark, W. G., and Uncapher, R. P., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 214.

Summary. Experiments in which cysteine and X-ray dosage were varied over a wide range revealed that the protective action afforded by cysteine is proportional to the dosages of cysteine and radiation employed and that cysteine increases the time to 50% mortality and the average survival time of animals dying within 30 days post irradiation.

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Bacterial Inhibition by a Penicillin Moiety.* (17625)

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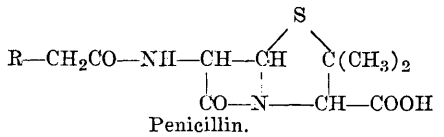
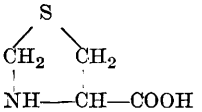
Little is known concerning the exact mode of action of most natural antibiotics, other than that they produce several general changes in bacterial morphology and respiration. Since biological activity can often be referred to specific groups in the molecule, metabolic studies involving smaller moieties of antibiotic molecules might possibly provide an approach to this problem. A preliminary attempt along these lines involving the nucleus of the penicillin molecule is described in this paper.

Experimental. 4-Thiazolidinecarboxylic acid, thioproline, was synthesized by the method of Armstrong and du Vigneaud(1). The organ-



Penicillin.



Thioproline.

ism, medium, and technics employed were those previously described and extensively used in analogue-metabolite inhibition studies(2). The results are summarized in Table I. Thioproline effectively inhibits the growth of *Escherichia coli* in mineral salts-glucose medium, and the inhibition is reversed to a limited extent by proline, despite the fact that the inhibitor is not isosteric with proline. To a somewhat lesser extent, a large number of other amino acids can also reverse the inhibition. Vitamins, purines, pyrimidines, and increased levels of glucose, however, are inactive in this regard. Combinations of amino acids manifest an even greater reversing ability than any single substance.

Discussion. A consideration of the data suggests that thioproline may inhibit bacterial growth by preventing the conversion of a large number of amino acids to some product or products essential for growth. It is apparently most effective in preventing proline utilization, perhaps because of its structural similarity to this amino acid. The known facts regarding penicillin action are not out of line with the possibility that penicillin prevents bacterial protein synthesis.

* Part of this work was conducted under Dr. William Shive at the University of Texas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

1. Armstrong, M. D., and du Vigneaud, V., *J. Biol. Chem.*, 1947, v168, 377.

2. Beerstecher, E., Jr., and Shive, W., *J. Biol. Chem.*, 1946, v164, 53.

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