COMPARATIVE KILLING EFFICIENCIES FOR DECAYS OF TRITIATED COMPOUNDS INCORPORATED INTO *E. COLI*

STANLEY PERSON

From the Graduate School Committee on Biophysics, Pennsylvania State University, University Park

ABSTRACT The killing efficiencies due to the decay of incorporated H^3-thymidine, H^3-uridine, and H^3-histidine in *E. coli* 15_{T-L-} have been determined. Decays from H^3-thymidine are 2.0 times as effective in producing lethality as those from H^3-uridine and 2.5 times as effective as those from H^3-histidine. Therefore, it seems that the greater part of damage from H^3-thymidine decays is due to chemical changes associated with nuclear transmutation.

INTRODUCTION

It has been shown that decays from incorporated H^3-thymidine in *E. coli* 15 auxotrophs cause death at a rate approaching that due to P^{32} decay (Person and Lewis, 1962). A similar result has been reported by Cairns (1961) for H^3-thymidine decays in bacteriophage, while Apelgot and Latarjet (1962) have reported a smaller rate of killing in bacteria. Although direct evidence is lacking, the relatively large killing efficiency for decays from H^3-thymidine suggests that lethality may be due to other factors in addition to radiation damage. If lethality is not caused exclusively by β-particle ionizations that accompany the nuclear transmutations it may be due to a chemical change in the thymidine molecule. The recoil He nucleus would not be suspect because it is biologically unimportant, chemically unreactive, and has an average energy of about 1 ev, which is probably too low to rupture covalent bonds.

It is the purpose of this communication to describe experiments which were designed to determine the extent of radiation damage due to decays from H^3-thymidine. Three H^3 compounds were used so the label would be incorporated into the bacterial DNA (H^3-thymidine), RNA (H^3-uridine) or protein (H^3-histidine). The highly radioactive bacteria were stored, and the efficiency of killing, the probability that a single radioactive decay will produce a lethal event, was determined for H^3 decay from each of these H^3 compounds.

If the major source of lethality is due to radiation damage we postulated that the killing efficiency, which is on a per decay basis, would be nearly the same for each of the three H^3 compounds. If factors such as chemical rearrangements are important we would expect a different killing efficiency for each H^3 compound.

We have found that decays from H^3-thymidine are 2.0 times as effective in producing lethality as those from H^3-uridine and 2.5 times as effective as those from H^3-histidine.

MATERIALS AND METHODS

A mutant of strain 15, 15_{T-L-}, which is thymidine- and leucine-deficient, was used in these experiments. Cultures of this mutant were obtained from Dr. F. Forro, Jr., Yale University. Stock cultures were grown on A-1 medium: 2 gm NH_4Cl, 6 gm anhydrous Na_2HPO_4, 5 gm NaCl, 0.115 gm Na_2SO_4, 0.34 gm $MgCl_2.6\,H_2O$, 4 gm glucose, 1 litter H_2O. Experimental cultures were supplemented with leucine at 40 μg/ml and with either thymidine or H^3-thymidine at 2 μg/ml.

The labeling procedure and the procedure for determining the number of H^3 atoms/bacterium have been described previously (Person and Lewis, 1962). Briefly, they are as follows: The desired quantity of a H^3 compound[1] was added to a log phase culture growing at 37°C with aeration. The culture was grown in the presence of the radioisotope for 4 to 5 cell divisions. At this time the remaining exogenous H^3 compound was separated from the cells and the cells stored at 4°C in A-1. Cell counts and H^3 counts were made directly on this highly radioactive culture from which N^*, the number of H^3 atoms/bacterium can be computed. Dilutions of stored cultures were made at various times in A-1 medium and platings for viability were on both A-1 supplemented and nutrient agar.

Cells labeled with H^3-thymidine or H^3-uridine were extracted with trichloroacetic acid (TCA) and NaOH to separate their nucleic acids. This procedure, due to Schmidt and Thannhauser (1945), involved 0.3 M TCA extraction at 4°C (acid-soluble fraction), overnight incubation at 37°C in 1 N NaOH (RNA degradation), heating in boiling water bath for 30 minutes in 0.3 M TCA (DNA degradation), and the uptake of the remaining residue in 1 N NaOH. Enzymatic digestion with 100 μg/ml of RNAase and/or 50 μg/ml of DNAase at 37°C for 30 minutes also used in conjunction with cold TCA extraction.

RESULTS

Survival curves for H^3 decay in bacteria are "single hit" and satisfy the relation $S/S_0 = \exp(-\alpha_{H^3}\,\lambda N^* t)$ or $\ln S/S_0 = -\alpha_{H^3}\lambda N^* t$ where S is the number of cells viable at time t and S_0 is the number originally present at $t = 0$. α_{H^3} is the probability of a lethal event being produced by a single H^3 decay. Since α_{H^3} reflects the

---

[1] H^3-thymidine, 3.0 c/mmole; H^3-uridine, 0.9 c/mmole; and H^3-histidine, 1.7 c/mmole were all obtained from Schwarz Bioresearch, Orangeburg, New York. H^3-thymidine and H^3-histidine were used at the above specific activities, but H^3-uridine was diluted to a specific activity of 0.4 c/mmole. Final concentrations were H^3-thymidine, 2 μg/ml; H^3-urdine, 28 μg/ml; H^3-histidine, 4.5 μg/ml.

lethal yield per decay it has come to be known as the killing efficiency. λ is the decay constant for H^3, and N^* is the number of H^3 atoms/bacterium at time t. However, N^* is constant with time in these experiments because $\lambda = 1.52 \times 10^{-4}$/day and the experiments last only about 8 days. The value of N^* is determined by measuring the cells/ml and the H^3/ml for an aliquot of stored radioactive cells.

When λ is expressed as the fraction of radioactive atoms that decay per hour, λN^* is the number of decays/hour/bacterium; so that $\lambda N^* t$ is the number of decays/bacterium at any time t in hours. Therefore, if we plot the fraction of cells surviving on a logarithmic scale (S/S_0) as a function of decays/cell ($\lambda N^* t$), a straight line results whose slope is α_{H^3}. α_{H^3} may be calculated directly since the assay for viability measures S/S_0 as a function of time; λ is a constant, and N^* is a constant for any one experiment. When the data are plotted in this way differences in the slopes of survival curves reflect different killing efficiencies. This procedure was used to determine α_{H^3} for the three H^3-labeled compounds.

Fig. 1 is a semilogarithmic plot of the surviving fraction of $15_{\text{T-L-}}(S/S_0)$, labeled with H^3-thymidine, H^3-uridine or H^3-histidine, as a function of the number of decays/bacterium ($\lambda N^* t$). After growth in medium containing one of these compounds the bacteria are stored in A-1 at 4°C without supplements. Data showing the viability of an unlabeled culture over the same time period are also shown in Fig. 1. The average killing efficiencies as well as typical values for H^3 atoms/bacterium and decays/hour/bacterium are listed below.

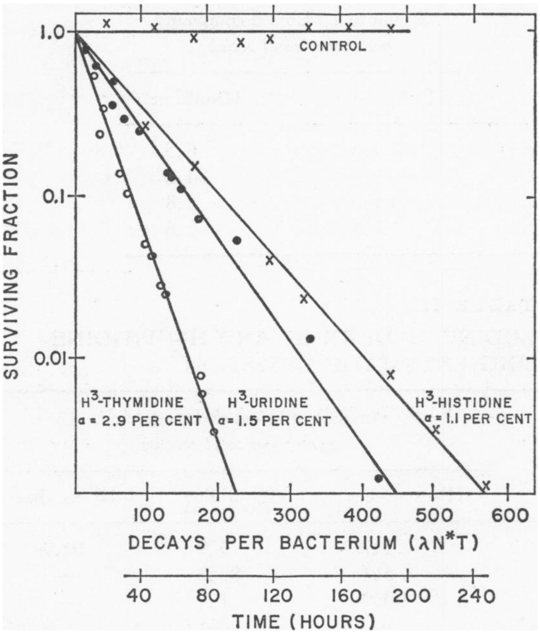

FIGURE 1 The survival of $15_{\text{T-L-}}$ as a function of exposure to decays from H^3-thymidine, H^3-uridine, or H^3-histidine. After growth in medium containing one of these H^3 compounds the bacteria are stored at 4°C in A-1. Since the exposure to decay is plotted as decays/bacterium ($\lambda N^* t$) the differences in slopes of the survival curves reflect different killing efficiencies. It is clear that the killing efficiency for H^3-thymidine decays is markedly greater than for that from either H^3-uridine or H^3-histidine.

Quantity	H^3-thymidine	H^3-uridine	H^3-histidine
N^*(H^3 atoms/bacterium)	3.3×10^5	2.4×10^5	1.05×10^6
λN^*(H^3 decays/hr./bacterium)	2.1	1.5	6.7
α_{H^3}(*per cent*)	2.9	1.5	1.1

The probability of producing lethality per decay, under these conditions, is 2.9 per cent for decays from H^3-thymidine, 1.5 per cent for decays from H^3-uridine, and 1.1 per cent for decays from H^3-histidine.

The results of cell fractionation studies are shown in Tables I and II. Radioactivity determinations were made in a liquid scintillation counter.

TABLE I

THE DISTRIBUTION OF H^3-THYMIDINE AND H^3-URIDINE IN *E. COLI* 15_{T-L-} USING THE SCHMIDT-THANNHAUSER PROCEDURE

	Amount of H^3-labeled compound, *per cent of total activity*	
Treatment and fraction	H^3-thymidine	H^3-uridine
Soluble in cold TCA (acid-soluble)	5.6	6.5
Soluble in warm NaOH (RNA)	1.7	84.2
Soluble in hot TCA (DNA)	85.0	8.8
Residual fraction	7.8	0.6

TABLE II

THE DISTRIBUTION OF H^3-THYMIDINE, H^3-URIDINE, AND H^3-HISTIDINE IN *E. COLI* 15_{T-L-} USING ENZYMATIC DIGESTION

	Amount of H^3-labeled compound, *per cent of total activity*		
Treatment and fraction	H^3-thymidine	H^3-uridine	H^3-histidine
Soluble in cold TCA (acid-soluble)	1.7	9.9	10.9
Soluble in RNAase (RNA)	5.4	88.3	—
Soluble in DNAase (DNA)	90.9	1.7	—
Soluble in RNAase and DNAase (DNA + RNA)	—	—	1.9
Residual fraction (protein)	1.9	0.1	87.8

DISCUSSION

The data presented above show that decays from H^3-thymidine are more effective in producing lethality than those from either H^3-uridine or H^3-histidine. Since the killing efficiencies for the different H^3 compounds are not the same we feel that radiation damage is not the sole cause of killing for the H^3-thymidine decays. If we assume that the smallest α_{H^3}, for H^3-histidine decays, is entirely due to radiation damage, then we can calculate the fraction of killing for H^3-thymidine and H^3-uridine decays that is not due to radiation damage. Since α_{H^3} (thymidine) = 2.9 per cent, α_{H^3} (uridine) = 1.5 per cent, and α_{H^3} (histidine) = 1.1 per cent; 1.8/2.9 of the H^3-thymidine–induced damage and 0.4/1.5 for H^3-uridine–induced damage would have a cause other than radiation. On the other hand, radiation damage would account for 1.1/2.9 for H^3-thymidine– and 1.1/1.5 for H^3-uridine–induced lethality. It would seem that about ⅔ of the damage from H^3-thymidine decays is not radiation-induced, but is more likely due to chemical changes in the thymidine molecules. H^3-thymidine decays are probably more efficient than those from H^3-uridine or H^3-histidine because of their strategic location in the "genetic part" of the cell's DNA. While in all cases approximately 85 per cent of the H^3-labeled compounds used are incorporated into the expected macromolecular fractions, it is possible that a small fraction of damage from H^3-uridine decays is due to a chemical alteration of the uridine molecules following H^3 decay. If true, this could be due to the conversion of a small amount of H^3-uridine to H^3-deoxycytidine with its subsequent incorporation into DNA. Caro and Forro (1961), using $15_{\text{T-U-}}$, reported that 8 per cent of H^3-uridine was incorporated into DNA, and all of this was in the cytosine fraction. Our data are in agreement with this figure.

It is a pleasure to acknowledge discussion of the above data with Professor Ernest Pollard, Mrs. Hazel Lewis, and Dr. Gordon F. Whitmore. I also acknowledge the technical assistance of Mrs. Esther Porter.

This work was aided by a grant from the United States Public Health Service and the United States Atomic Energy Commission.

Received for publication, August 18, 1962.

REFERENCES

Apelgot, A., and Latarjet, R., *Biochim, et Biophysica Acta,* 1962, **55,** 40.

Cairns, J., *J. Mol. Biol.,* 1961, **3,** 756.

Caro, L. G., and Forro, F., Jr., *J. Biophysic. and Biochem. Cytol.,* 1961, **9,** 555.

Person, S., and Lewis, H. L., *Biophysic. J.,* 1962, **2,** 451.

Schmidt, G., and Thannhauser, S. J., *J. Biol. Chem.,* 1945, **161,** 83.