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Rachmeler, Martin Ph.D.
Northwestern University Medical School

THE EFFECTS OF TRITIUM DECAY ON DNA

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MASTER

PROGRESS REPORT

This report covers the period from 1 Jan. 1967 through 31 December 1967.

INTRODUCTION

Our ultimate goals in this project are (1) to determine the nature of the effective agent(s) that cause the biological properties observed after tritium decays in a cell, (2) to determine the nature of the defects and (3) to determine the extent of damage necessary before phenotypic and genotypic effects can be observed.

The format in our experiments is twofold: (1) to incorporate tritium into the various macromolecules of cells and to study the effects of their decays on viability, enzyme forming ability, and mutation type and frequency; In addition the physical integrity of the DNA of the labeled cells would be examined, and (2) to label DNA with H^3 in vivo, isolate and examine it after varying periods of storage to determine heterogeneity as caused by double and single stranded breaks.

MATERIALS AND METHODS

These are essentially the same as in the previous Progress Report and thus only differences or modifications will be detailed here.

Organisms

In addition to 15T⁻ and 15THU we are using E. coli K12 SH-28 a thymidine phosphorylase negative mutant (Fangman and Novick, 1966) and E. coli AB2487. The latter is a recombination deficient (rec⁻), thymine requiring mutant (Howard-Flanders and Theriot 1966) that is sensitive to UV irradiation.

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Enzyme studies

These were carried out according to previously published techniques: tryptophanase (Pardee and Prestidge, 1961); serine deaminase (Pardee and Prestidge 1955) and B-galactosidase (Riley et al 1960). These techniques all involve assays of whole cells broken by toluene and sodium lauryl sulfate treatment.

Preparation of DNA and Density Gradient Centrifugations

The procedure of Thomas (1966) was used for DNA isolation. In the preparation of DNA for in vitro storage, in order to achieve a fairly homogeneous preparation for study, it was necessary to centrifuge the final product in a 5-20% sucrose gradient for 16 hr at 25,000 rpm in an SW 25.2 rotor. The DNA which moved in a peak near to the bottom was pooled, precipitated in alcohol, redissolved in SSC and stored.

CsCl gradients were also used in some later work. A 55% solution of CsCl was prepared (density = 1.6814), sample added and centrifuged at 45,000 rpm (165,000 xg) for 16 hr in an SW 50L rotor. Fractions were collected by puncturing the bottom of the tube and collecting drops; OD's were read at 260 and 280 mμ and aliquots counted in the Packard Tri-Carb liquid scintillation counter.

In some instances aliquots were tested for hyperchromicity by heating at 100°C for 10' and rereading OD₂₆₀.

Viability studies

RESULTS

The viability of two different strains of *E. coli* was compared, *E. coli* 15 and *E. coli* K12 and it was found (Table 1) that there was a strain difference regarding sensitivity to tritium decays. In both, decays in DNA were more efficient in leading to loss in viability than decays in RNA or protein but the arguments concerning the geography of the decays in the cell could possibly account for this difference. Of further interest were the results obtained with

E. coli K12 AB2487 rec⁻. It was found that this strain was especially susceptible to tritium decays (Table 1). This is undoubtedly related to deficiency in repair mechanism (Howard-Flanders and Theriot 1966) as a study of in vitro DNA from the strain (see Fig. 2) reveals no special sensitivity to decays as compared with other strains.

Density gradient studies on DNA

In vivo:

DNA, prepared from E. coli K12SH-28 and AB2487 stored in the cold, differed in degree of degradation from approximately equal numbers of decays. Where the SH DNA remained stable after ~ 140 decays/cell, the AB DNA apparently developed many breaks after a similar number of decays. This undoubtedly reflects the impaired DNA repair mechanism in the AB2487 strain.

In vitro:

DNA was prepared from K12SH-28 and AB2487 and stored in small aliquots at -65°C in glycerol storage media (Riley et al., 1960). Aliquots were thawed after varying periods of time in storage and put on 5-20% sucrose gradients. The SH-28 DNA had 37×10^6 dpm/OD₂₆₀ while the AB strain had 20.4 dpm/OD₂₆₀. Initially, the DNA from the AB strain appeared closer to the bottom than the SH DNA gradients (Fig. 2). With time both moved further toward the bottom, at first maintaining some homogeneity, then becoming heterogeneous. With continued storage, the DNA's now appeared to develop lighter heterogeneous segments; this probably reflects breakage into short single stranded fragments.

Mutation and Enzyme studies

In a series of extensive experiments in which enzyme forming ability was studied with time, only a slight decrease in enzyme forming ability could be detected, using E. coli K12 SH-28. The efficiency of loss of enzyme activity was $> 1/1000$. These findings are not in agreement with some earlier studies

using E. coli 15T⁻ (Rachmeler and Pardee, 1963). Although one could invoke strain differences as a basis for this difference, studies must be conducted to settle this matter.

An interesting finding regarding mutational activity was observed. Even at the point where the viability had decreased more than two log units, we were unable to find substantial numbers of mutations. This appears more similar to work with x-rays rather than UV; in the latter a great proportion of survivors after extensive UV irradiation are mutants, whereas, with x-rays, altho mutation rates are increased more chromosomal aberrations occur leading to lethal mutations. We have, however, succeeded in isolating 10 auxotrophic mutants which will be examined in reversion studies by mutagenic agents. We plan to continue our mutant hunts.

Thymine Loss from DNA

In addition to the studies reported, attempts were made to determine if thymine is released from DNA after extensive degradation. The DNA was precipitated by TCA and the supernatant concentrated and the TCA removed by lyophilization. The dried material was dissolved in a small amount of water and aliquots chromatographed in various solvent systems with and without the addition of labeled TDR and thymine. In no instance could we detect significant amounts of thymine or TDR. It would appear that our premise that the deoxyribose-thymine bond is labilized as a result of decays does not play a substantial role, if any, in the strand breakages.

DISCUSSION

It appears that tritium decays in a cell lead to single and double strand breaks in the DNA. This, undoubtedly, causes an interruption in normal replication and thus leads to loss in viability. The fact that at least some of these effects can be slowed by cysteine suggests that radiation plays an important

causative role. The significance of transmutation has not been determined as yet; a quantitative evaluation of the effects of radioprotective agents is necessary. Such studies on both in vivo and in vitro DNA are now underway.

The observed higher sensitivity of the E. coli AB2487 to tritium decays, both as regards viability and DNA degradation, supports the thinking that the damage in the cell is due to breaks in the DNA, since this strain is defective in its DNA repair mechanism. There is an implication inherent in this finding that at least some damage to a normal cell by tritium decay is repairable. This may mean that each decay is sufficiently active to cause a single break but that the repair mechanism of the cell is able to cope with many of these. It is our intention to determine the efficiency of decays in vitro where this repair mechanism can play no role. Our current studies have provided us with the techniques necessary to quantitate single and double strand breaks in the DNA. Using AB2487 cells and assuming its increased UV sensitivity is related quantitatively to its faulty DNA repair mechanism, perhaps we can establish the "true" efficiency of tritium decays in regard to alterations of the physical integrity of DNA.

The data gathered regarding enzyme forming ability of cells was fairly clear cut over the term of the experiments - there was only slight loss of activity, however, in contrast to earlier experiments there was great variability observed in the assays for a given day. These experiments must clearly be repeated.

Our inability to find thymine in the medium after extensive DNA degradation indicates that an earlier proposed mechanism for DNA degradation, e.g. labilization of the C-N bond between thymine and deoxyribose due to tritium decay plays only a minor role, if any, in leading to breaks in the DNA structure. However, the finding by Person and Bockrath (1965) that UR-5 was much more effective as a mutagenic agent than UR-6 does suggest that transmutation plays a role, at least in mutagenesis. This facet will be explored by using thymine labeled in

the ring vs label in the methyl group.

It is our present feeling that the objectives of our original proposal regarding the effects of H^3 - decay on DNA and the biological properties of cells can for the most part be reached within the coming year.

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Table 1

Efficiency of tritium decays in causing loss of
viability in various strains of E. coli

Site of H ³	<u>E. coli</u> strains		
	15THU	K12SH28	K12 AB2487 rec ⁻
DNA	1/120	1/735	1/25
RNA	1/400	1/850	1/170
Protein	1/880	-----	1/108

Efficiency calculated as reciprocal of decays required to bring
about a 37% loss in viability.

Fig. 1. Cells were labeled with H^3 -TDR and stored in the refrigerator. At various times DNA was prepared from aliquots and centrifuged for 3 hr. at 49,000 rpm on a 5-20% sucrose density gradient in a SW50L rotor.

Fig. 2. DNA was prepared from E. coli strains and stored at -65°C . Aliquots were thawed at various times and centrifuged for 3 hrs at 49,000 rpm in a 5-20% sucrose density gradient in a Spinco SW 50L rotor. The tubes were punctured at the bottom and fractions collected.