# SPURIOUS PHOTOLABILITY OF DNA LABELED WITH [$^{14}$C]-THYMIDINE

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SUMMARY: Thymidine labeled in the methyl position with $^{14}$C (Lot No. 824-137, Compound NEC-568, New England Nuclear Corporation) was used to label the DNA in V79 Chinese hamster cells and bacteriophage T4. In both cases, abnormally high ultraviolet and near ultraviolet light photolability resulted. A 1-2% contamination with 5-bromodeoxyuridine of the total thymidine in a [$^{3}$H]-thymidine stock produced an equivalent photolability. Neutron activation analysis showed that Lot No. 824-137 contained 1-2% Br relative to thymidine.

## INTRODUCTION

In the course of a study of the changes produced in the DNA from V79 Chinese hamster cells exposed to ultraviolet (UV, 254 nm) or near ultraviolet (NUV, $\sim$ 315 nm) light, a particular lot of [$^{14}$C]-thymidine was employed (Compound NEC-568, New England Nuclear Corporation) which yielded quite different results from those obtained with other batches of [$^{14}$C]- or [$^{3}$H]-thymidine. A number of possible causes for this observation were checked (i.e., changes in the serum, antibiotics, and/or cells) to no avail. Since the photolability of DNA that results when V79 cells are grown in the presence of 5-bromodeoxyuridine (BUdR) had been studied (1), the possibility was considered that Lot No. 824-137 might be contaminated with this thymidine analog. This possibility was supported by: 1) the fact that V79 cells incubated after UV or NUV exposure repaired DNA damage rapidly (within $\sim$ 60 min) in similarity to earlier work (1); and 2) the information on the data sheet for Compound NEC-568 that it is made enzymatically by the incubation of [$^{14}$C]-thymine with a "pyrimidine deoxyriboside." We present here evidence supporting the probability that the [$^{14}$C]-thymidine batch in question was contaminated with BUdR; other labeled thymidines made in the same way by New England Nuclear Corporation or other manufacturers also may be so contaminated.

## MATERIALS AND METHODS

For the observations with cultured Chinese hamster cells, sublines of V79 cells were used. The details of the growth, labeling, and sedimentation of the DNA from V79 cells and the bacteriophage T4 have been published (2-5); for present purposes, essential further details are given in the figure legends.

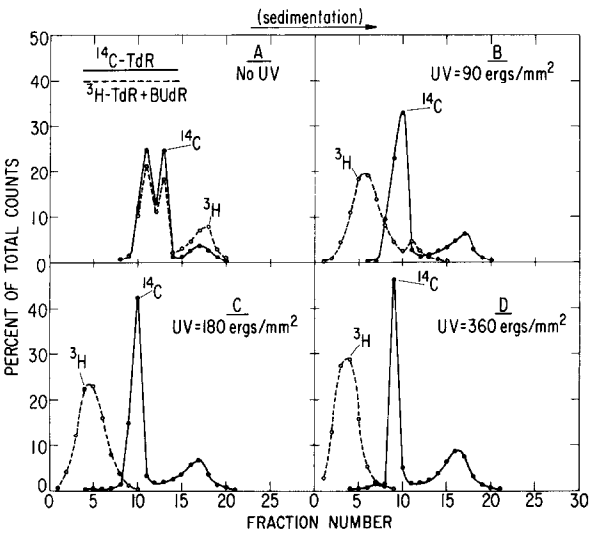


Fig. 1. Chinese hamster cells, subline V79-753B, were grown overnight (about 2 doublings) in the presence of 0.3 μCi/ml [$^{14}$C]-thymidine (not Lot No. 824-137). Following a 30 min chase, the cells were incubated for 3 hrs in a medium containing 2.0 μCi/ml [$^{3}$H]-thymidine (20.5 Ci/mM), 5 x $10^{-7}$ M thymidine, and 1 x $10^{-6}$ M BUdR. A second chase in medium containing 1 x $10^{-6}$ M thymidine was followed by exposure to UV (254 nm) (3 ergs mm$^{-2}$ sec$^{-1}$) and then by the suspension of cells by trypsin treatment at ∿ 0°C followed by pipetting. About 1 x $10^{4}$ cells were lysed for 30 min at ∿ 25°C on top of 5-20% alkaline sucrose gradients before their contents were sedimented in a Beckman Sw-50.1 rotor at 36,000 rpm for 90 min at ∿ 0°C (2,3). Sedimentation from left to right.

## RESULTS

Figure 1 was obtained with DNA labels which, by themselves, did not confer any appreciable photolability and illustrates two points: 1) the usual relative insensitivity of the DNA in V79 cells to the production of lesions by UV which appear as single-strand breaks under alkaline conditions (pH ≃ 13); and 2) the enhanced UV sensitivity conferred by BUdR incorporation. Cells were

grown overnight in the presence of [$^{14}$C]-thymidine (not Lot No. 824-137). Because the doubling time of these cells for the conditions used was 9-10 hrs (DNA synthesis, 5-6 hrs) and because of the experimental conditions (see legend, Fig. 1), most of the DNA became labeled in one DNA strand by $^{14}$C and for long stretches of the complementary DNA strand by $^{3}$H and Br. Thus, the $^{3}$H label served to indicate DNA photolability due to the presence of bromouracil (1). The DNA from unirradiated V79 cells typically appears as a bimodal pattern* after sedimentation in an alkaline sucrose gradient (2-4). [Occasionally, the peak that has been called the "complex" (1) appears itself to be bimodal as in panel A, left two peaks.] The relative positions and, to a lesser extent, the shapes of the DNA peaks are strongly dependent on speed of rotation (3,6). This is similar to the speed and molecular weight dependencies proposed by Zimm for duplex DNA sedimenting in neutral gradients (7). Under alkaline conditions, however, the dependence of sedimentation rate on rotational speed is presumably made more pronounced by the larger number of free-draining ends that results from the incomplete denaturation of ends of single-strands emanating from non-complementary breaks in duplex DNA. Except for the split in the complex, panel A shows a typical distribution of DNA label after high speed sedimentation (36,000 rpm). Since graded small doses of a bond-breaking treatment like X-irradiation shifts label progressively from the "complex" to the "main peak" (right peak in A, fractions 17 and 18) (2,3), and since essentially all the DNA placed on a gradient is accounted for, the complex must be larger in gross molecular weight than that of the main peak, $\sim 2 \times 10^{8}$ daltons (3,6). The conclusion that the complex consists of high molecular weight, partially denatured duplex DNA comes from the observation that for a given backbone-breaking X-ray dose, interstrand cross-links retard the resolution of the complex into the main peak (8,9), and hasten the reformation of the complex

*Similar patterns are observed with mouse lymphoma cells, HeLa cells, and fibroblasts obtained from normal humans as well as from individuals suffering from xeroderma pigmentosum (data not presented).

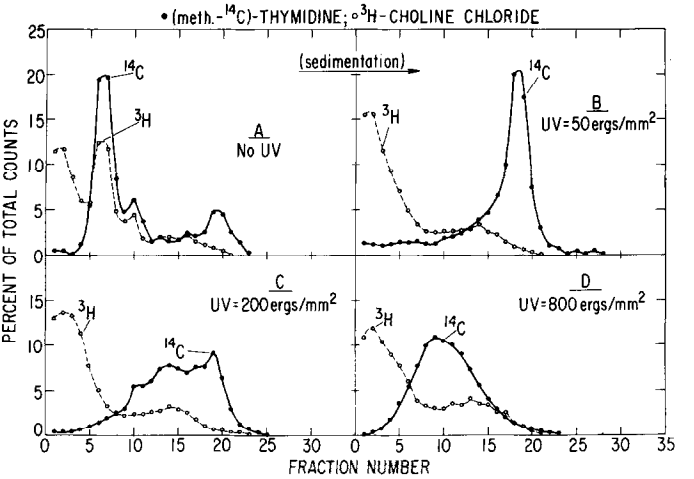


Fig. 2. Chinese hamster cells, subline V79-S465A, grown overnight in the presence of 0.44 μCi/ml of [methyl-$^{14}$C]-thymidine (NEC-568, New England Nuclear Corporation, Lot No. 824-137) plus 0.88 μCi/ml [$^{3}$H]-choline chloride (16.5 Ci/mM, Amersham/Searle). Following a 30 min chase period, cells were UV irradiated (4.4 ergs/mm$^2$•sec), suspended, and sedimented as for Fig. 1. Sedimentation from left to right.

from the main peak during cell incubation for repair (10). [Similar conclusions about the duplex nature of the complex have been reached by others (11,12).]

Figure 1 shows that with increasing doses of UV, the BUdR strand is selectively degraded. Other data indicate that doses even smaller than 90 ergs/mm$^2$ first shift the BUdR-DNA from the complex to the main peak before the main peak DNA is degraded. Thus, the results in Figure 1 show both the photolability conferred by BUdR as well as the relative insensitivity of normal DNA to UV induced alkali-labile lesions. They also indicate that a low frequency of single-strand breaks are induced in the $^{14}$C-containing strand due to the UV photolysis of its complement.

Figure 2 shows the result of labeling cells with [$^{14}$C]-thymidine, Lot No. 824-137. In this case [$^{3}$H]-choline chloride was used as a label for membranous structures. Qualitatively, panel A is consistent with earlier results (4) that showed that the DNA complex cosediments with, presumably, the lipid

in the nuclear envelope with little if any lipid cosedimenting with main peak DNA (fractions 19-20). However, it is evident that after 50 ergs/mm$^2$ the complex is completely converted into the main peak with a loss of cosedimentation of the two labels [reminiscent of cells exposed to X-rays (4)], and that larger doses result in a progressive degradation of main peak DNA. Similar results were obtained with NUV (exposure to FS20 Westinghouse Sun Lamps, $\sim$ 315 nm). When V79 cells labeled with Lot No. 824-137, were incubated after UV or NUV light, they reversed the degradative effect of the exposure on main peak DNA and with further incubation returned the DNA from the main peak to the complex.

On the assumption that the photolability in Figure 2 resulted from a BUdR contaminant in Lot No. 824-137, simulation experiments were done with V79 cells and bacteriophage T4. Compounds labeled with $^{14}C$ generally have low specific activity because of the long half-life of this isotope. In these experiments, as is frequently the case, the [$^{14}C$]-thymidine was about 50 mCi/mM. To examine the effect of a possible BUdR contamination using V79 cells, we initially simulated the thymidine concentration in the medium by adding unlabeled thymidine to high specific activity [$^{3}H$]-thymidine. The concentration of thymidine chosen was that equal to what we estimated it to be in Lot No. 824-137 from the absorption at 265 nm. Thus, the BUdR concentrations in Figure 3 are in addition to 6.6 x $10^{-6}$ M thymidine in the medium of V79 cells grown for periods equal to those used to label these cells with the radioactivity in Lot No. 824-137. The data show that for 1 x $10^{-7}$ M BUdR, the reciprocal of the number average molecular weight ($1/M_n$) increased linearly with a slope equal to that observed with cells labeled with the $^{14}C$ from Lot No. 824-137. These results are consistent with a 1.5% contamination of the thymidine in Lot No. 824-137 with BUdR. A similar conclusion was reached from data similar to those in Figure 3 but involving V79 cells exposed to NUV. In other experiments with V79 cells, we showed that the effect of 1 x $10^{-9}$ M BUdR could be readily detected after a UV dose of only 2400 ergs/mm$^2$; i.e., a 0.015% contamination of thymidine with BUdR.

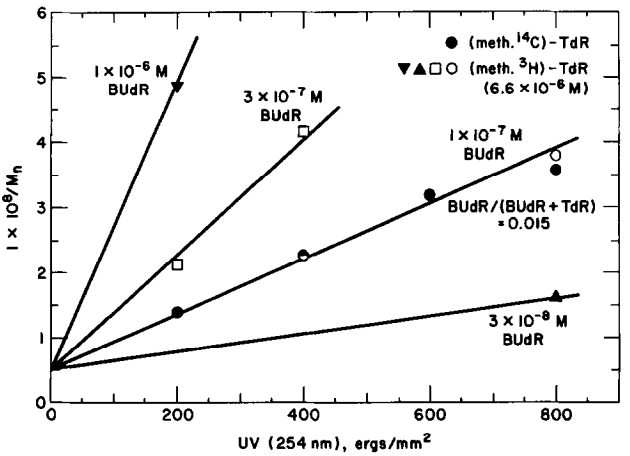


Fig. 3. The dependence of the reciprocal of the number average molecular weight ($M_n$) as a function of UV dose for cells labeled with [$^{14}$C]-thymidine (NEC-568 as in Fig. 2), or with 0.67 μCi/ml [$^{3}$H]-thymidine plus unlabeled thymidine to make 6.6 x 10$^{-6}$ M and to which BUdR was added as shown. $1/M_n$ is proportional to the number of breaks (1-3).

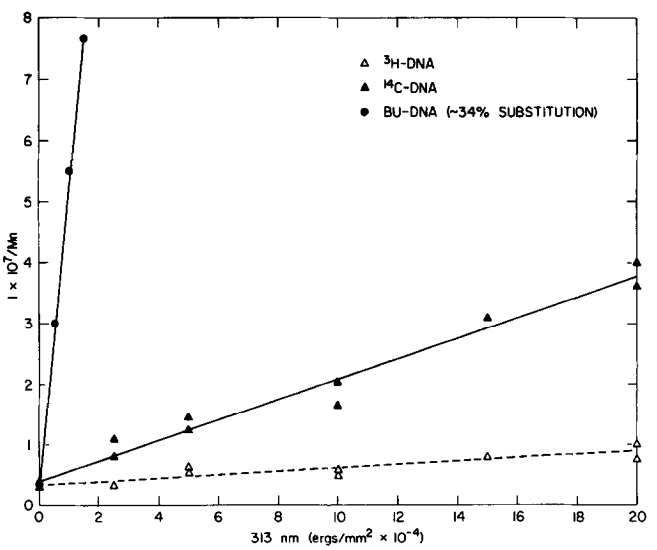


Fig. 4. Dependence of the reciprocal of the number average molecular weight ($M_n$) as a function of exposure of bacteriophage T4 DNA to 313 nm radiation. Labeled coliphage T4 was produced in E. coli B/r, trp thy grown in the presence of 2 x 10$^{-5}$ M [methyl-$^{14}$C]-thymidine (NEC-568, Lot No. 824-137), 4.1 x 10$^{-5}$ M $^{3}$H-thymine (0.5 Ci/mM), or a mixture of $^{3}$H-thymine (4.1 x 10$^{-5}$ M) and unlabeled bromouracil (2.6 x 10$^{-5}$ M). The 313 nm radiation, obtained from a monochrometer (5), was passed through a thin Mylar film. DNA solutions were exposed at room temperature; incident fluence rate 600 ergs/mm$^2$•sec. $M_n$'s were determined from velocity sedimentation: Beckman SW-S6 rotor; 40,000 rpm; and 100 min at 20°C (5).

Figure 4 shows the relative photolability at 313 nm of bacteriophage T4 DNA labeled with [$^{3}$H]-thymine, with [$^{14}$C]-thymidine (Lot No. 824-137), or with a mixture of [$^{3}$H]-thymine and bromouracil (BU). T4 DNA in the last instance was estimated, from isopycnic sedimentation in CsCl density gradients, to have 34% of its thymine replaced by BU. From the molecular weight reductions observed after velocity sedimentation in alkaline sucrose gradients, we calculated breakage efficiencies of $0.28 \times 10^{-12}$, $1.65 \times 10^{-12}$, and $48.8 \times 10^{-12}$ per dalton per erg/mm$^{-2}$ for the respective labelings described above. If the enhanced photolability resulting from labeling with Lot No. 824-137 reflects BUdR incorporation, from the slopes of the lines in Figure 4 we estimate the BU replacement of thymine to be $\sim$ 1%. This suggests that Lot No. 824-137 contained at least 1% BUdR.

A solution of Compound NEC-568 is nominally an aqueous solution of thymidine. A sample of Lot No. 824-137 was sealed in a quartz vial and placed in the CP-5 Research Reactor at the Argonne National Laboratory for neutron activation. From an energy analysis of the induced $\gamma$-ray activity as a function of time relative to that from samples containing known amounts of Br activated at the same time as the unknown, we concluded that Lot No. 824-137 contained between 1.8-3.8 $\mu$g/ml Br. If all the Br was in BUdR, the BUdR contamination level would be 1-2%.

## CONCLUSIONS AND RECOMMENDATION

Our evidence supports the likelihood that: 1) the pyrimidine deoxyriboside used by the New England Nuclear Corporation for the synthesis of [methyl-$^{14}$C]-thymidine from [methyl-$^{14}$C]-thymine was 5-bromodeoxyuridine; and 2) the manufacturing process can result in as much as a 1-2% contamination with BUdR. Because the spectral red shift produced by the substitution of Br for $CH_3$ at the 5 position of thymidine is not large enough to detect this level of contamination spectroscopically, the absorbance ratios furnished on the data sheet that accompanies a given lot of NEC-568 are inadequate to assure chemical purity. Indeed, for photobiological and photochemical work with DNA, a

detection system sensitive to less than 0.015% BUdR in thymidine is needed.

In sedimentation studies of DNA, [$^{14}$C]-thymidine is considered by some authors preferable to [$^{3}$H]-thymidine because, for the same degree of detectability, $^{14}$C decay is thought to produce less internal radiation damage. However, it is also known that the growth of cells in the presence of BUdR can produce unexpected results such as the release of a virus in addition to enhanced radiation sensitivity. When a thymidine DNA label is required, and when specific tests for photolability similar to those described here are not feasible or practical, we recommend that unlabeled thymidine be added to a source of high specific activity [$^{3}$H]-thymidine to reduce the specific activity to the range of values encountered with [$^{14}$C]-thymidine. Typically, this would dilute any BUdR relative to thymidine by 100- to 1000-fold.

## ACKNOWLEDGMENTS

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