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ULTRAVIOLET AND CHEMICAL INDUCED DNA REPAIR IN HUMAN CELLS ASSAYED BY BROMODEOXYURIDINE PHOTOLYSIS OR CYTOSINE ARABINOSIDE ARREST

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ABSTRACT

The bromodeoxyuridine photolysis assay of DNA damage in human cells permits an estimate of both the number of repaired regions in the DNA and the size of the average repaired region - the "patch" size. The antineoplastic agent arabinofuranosyl cytosine (ara-C) can also be employed to assay the magnitude of repair since this agent appears to block rejoining of single-strand incisions made in the DNA during the initial step of repair. Thus, the number of incisions can be accumulated. The ara-C effect is dependent on the presence of hydroxyurea. Both assays can be employed for the study of physical or chemical DNA damages. Results comparing these assays are presented.

INTRODUCTION

The bromodeoxyuridine (dBrU) photolysis assay is a useful technique for determining the magnitude of excision repair in cells and culture (1). We developed the technique sometime ago and have used it mainly to study human cells (2). It is an assay which is relatively rapid and gives information both on the number of repaired regions in the DNA and an estimate of the average size of the repaired regions. The technique can be used for studies of repair after ultraviolet (UV) or ionizing radiation and we have used it extensively to study repair induced by chemical carcinogens and mutagens after treatment of human cells with these agents (3).

We have recently developed another technique which employs the chemotherapeutic agent cytosine arabinoside (ara-C) to estimate the magnitude of repair in human cells (4). This assay is advantageous because it does not require a powerful source of monochromatic 313 nm light as is required for the dBrU photolysis assay. We have used the ara-C technique to study the magnitude of repair among the various complementation groups of xeroderma pigmentosum (XP) after treatment with UV radiation. The ara-C method is useful for studying the amount of excision repair occurring in human cells after treatment with chemical carcinogens, e.g., benzo(a)pyrene derivatives. In this communication we present data comparing results of dBrU photolysis and of ara-C experiments after ultraviolet and chemical damage to human cell DNA.

METHODS AND MATERIALS

The details of the dBrU photolysis assay have been presented in several publications (1-3). Fig. 1 shows rationale for the technique. The principle is simply to permit the cells to repair damaged DNA in medium containing dBrU, which is incorporated into the repaired regions. These regions can then be

photolyzed with monochromatic 313 nm light, becoming alkaline labile. The cells are lysed on, and the DNA sedimented through alkaline sucrose gradients. Thus, the number and size of repaired regions can be estimated.

The ara-C technique has also been described in detail in a recent publication (4). This technique relys on the cells repairing their damaged regions in cytosine arabinoside. Apparently, due to configurational distortions that occur when an amount of cytosine arabinoside is incorporated into the DNA, the repair-induced strand breaks remain open and thus one can get a direct estimate of the number of repaired regions in the DNA. The ara-C technique is dependent on hydroxyurea being in the medium. Apparently, with the inhibition of ribonucleotide reductase by hydroxyurea, the pool of deoxyribonucleotides available for repair is so small that sufficient ara-C is incorporated to keep the repair breaks open, permitting an estimate of the number of repaired regions.

RESULTS

Figure 2 shows the results of a typical dBrU photolysis assay with normal human fibroblasts (5). The insult to the DNA in this case is 200 ergs/mm^2 254 nm UV. In the left panel, No 313 nm was given and the two DNAs sedimented together.

In the right panel photolyzing dose of 313 nm was given. The dBrU-containing DNA shifted to the right. The magnitude of this shift provides an estimate of the number of repaired regions in the DNA. If one gives increasing exposures of 313 nm light to cells that have been damaged and subsequently incubated in dBrU, one can estimate the number and the size of the repaired regions. In Fig. 3, we compare human repair capacity with that of hamster, mouse and XP cells (5). The figure shows that human cell DNA under these conditions is very sensitive to the 313 nm light. Calculations from the kinetics of curves like these indicate that the patch size is about 80 nucleotides per repaired region. The hamster

has approximately the same number of nucleotides in the average repaired region but the numbers of these regions are much smaller. Mouse has an even smaller number of repaired regions.

Table 1 shows data from ara-C experiments with normal and XP cells following UV radiation. This technique permits an estimate of the number of DNA single-strand breaks per 10^8 daltons. Alkaline sucrose gradients profiles demonstrated weight-average molecular weights smaller (24.33×10^6 daltons) than those of control cells (240.37×10^6 daltons). The number of incised regions equals the number of single-strand breaks (7.39) and correlates well with our results from dBrU photolysis experiments. The ara-C-induced repair inhibition is hydroxyurea dependent. Experiments performed without hydroxyurea yielded only (0.30 breaks/10^8 daltons or 4.1% of the inhibition observed with hydroxyurea. In cells from excision-defective XP complementation groups, decreases in the number of strand-breaks were observed (0.22, 1.09, 0.55, 0.58 and 4.57 respectively). In SGL, an XP cell line as yet uncharacterized as to complementation group, the number of strand-breaks (0.40) indicated highly defective excision of dimers similar to results with Group A XP cells.

One extraordinary finding of these experiments was that the XP variant (6) (the form of xeroderma pigmentosum having a normal excision repair but defective postreplication repair) showed a unusual number of single-strand breaks compared to normal cells in this assay. For presently unknown reasons, it appears that the XP variant makes considerably more repair incisions in the DNA than normal cells.

We have also performed cesium chloride density sedimentation analysis of the inhibitory effect of ara-C on repair replication in normal skin fibroblasts. Results of these experiments are shown in Fig. 4. The details of the experiment are presented in the figure legend. Ara-C (10 μM) caused a significant reduction in repair synthesis in cells exposed to 20 J/m^2 of UV

radiation and allowed to repair for 18-20 hr (Fig. 4A). Incorporation of ^{3}H-ara-C was demonstrated (Fig. 1B fractions 13-16) during the repair period.

Figure 5 shows the effect of deoxycytidine (dCyd) on the repair inhibition caused by ara-C. While repair inhibition at lower doses of UV ($\sim$5 J/m^2) could readily be reversed by simply removing the ara-C from the medium, at higher UV doses (10-20 J/m^2) reversal was incomplete, e.g., only 42% after 15 hr following a UV dose of 20 J/m^2. If ara-C was removed after two hours and cells were incubated in medium containing 100 μM dCyd, the inhibition was reversed at all UV doses by 85% in three hours and >95% in 15 hr.

Figure 6 shows a direct comparison of the dBrU photolysis assay and the ara-C arrest method when the insult to the human cellular DNA is a chemical carcinogen which induces "long-patch" or UV-like repair - benzo(a)pyrene biol-expoxide. Findings with benzo(a)pyrene and it's metabolites with regard to DNA repair have been published in detail elsewhere (7, 8). In Fig. 6, the number of single-strand breaks detected by each assay can be determined and expressed as the reciprocal of the weight-average molecular weight ($1/M_w$). The magnitude of repair can then be expressed by $\Delta\ 1/M_w = (1/M_w)_{dBrU} - (1/M_w)_{dt}$ in the case of dBrU photolysis or $\Delta\ 1/M_w = (1/M_w)_{ara\text{-}C} - (1/M_w)_{control}$ in the case of ara-C arrest. For data in Fig. 6 indicate good agreement in the results from these two types of assays.

In summary, we have presented results of DNA repair assays in human cells using either ara-C arrest or dBrU to estimate the extent of repair. The ara-C arrest is applicable not only to studies of radiation-induced damage to DNA but also to DNA damage-induced by a variety of chemical mutagens and carcinogens. Quantitatively similar results are obtained with either assay. We believe the ara-C arrest technique could be a rapid, inexpensive screening test for DNA-damaging, environmental carcinogens.

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FIGURE LEGENDS

Fig. 1. Rationale of the dBrU photolysis assay (5).

Fig. 2. dBrU photolysis assay of excision repair in normal human fibroblasts after ultraviolet irradiation (5).

Fig. 3. Sensitivity to 313 nm photolysis of various mammalian cell DNAs after UV irradiation and incubation in dBrU (5).

Fig. 4. Cesium chloride density sedimentation analysis of the effect of ara-C on repair replication in normal human skin fibroblasts (4).

Cells were incubated for two hours in the presence of 10 μM dBrU and 1 μM FdUrd, then given a UV dose of 20 J/m^2. Repair was allowed to take place for 19-20 hr in the presence of 2 mM hydroxyurea, 1 μM FdUrd, and either (A) 12.5 μCi/ml [^{3}H]dBrU (20 Ci/mmole) with or without ara-C or (B) 10 μM dBrU and [^{3}H]ara-C. The DNA was extracted and analyzed on CsCl gradients. (A) ▲, no UV; △, UV without ara-C; O, UV 1 μM ara-C; ●, UV 10 μM ara-C, (B) O, UV 1 μM [^{3}H]ara-C (15 Ci/mmole); ●, UV 10 μM [^{3}H]ara-C (3 Ci/mmole).

Fig. 5. Effect of dCyd on DNA repair inhibition by ara-C in UV-irradiated human skin fibroblasts (4).

Cells labeled with [^{3}H]dThd were exposed to different doses of 254 nm radiation followed by a two-hour repair period in the presence of 2 mM hydroxyurea and 10 μM ara-C. The ara-C was removed, and repair was allowed to continue for either three or 15 hr growth medium with hydroxyurea with or without 100 μM dCyd. M_w's of DNA were analysed on alkaline sucrose gradients. Experimental molecular weight values were compared with those of unirradiated control cells labeled with [^{14}C]dThd. □, ara-C for two hours; ▲, ara-C + three hours in E-90/cs; △, ara-C + 15 hr in E-90/cs; ●, ara-C + three hours in E-90/cs with dCyd; O, ara-C + 15 hr in E-90/cs with dCyd.

FIGURE LEGENDS (cont'd)

Fig. 6. Repair of DNA damaged by 7β, 8α-dihydroxy-9α, 10α-epoxybenzo(a)pyrene (BP-diol-epoxide).

The DNA of human skin fibroblasts was labeled overnight with either [^{3}H]-thymidine (O) or [^{14}C]-thymidine (●). Following labeling, all cultures were treated with 1 μM BP-diol-epoxide for 60 min and allowed to undergo repair for 20 hr. The following compounds were also present during exposure and repair, depending upon the assay employed: ara-C inhibition assay - cells labeled with ^{3}H were exposed to 10 μM ara-C and 2 mM hydroxyurea. Cells labeled with ^{14}C served as controls without ara-C and hydroxyurea. Bromodeoxyuridine (dBrU) photolysis assay - cells labeled with ^{3}H received 100 μM dBrU, whereas ^{14}C labeled cells received 100 μM thymidine. Following repair, cells were harvested and exposed to 10^{5} J/m^{2} of 313 nm radiation (dBrU assay only). DNA molecular weight analysis was performed using alkaline sucrose sedimentation.

TABLE 1

Measurement of DNA repair by means of inhibition by ara-C in normal and XP cells following UV irradiation

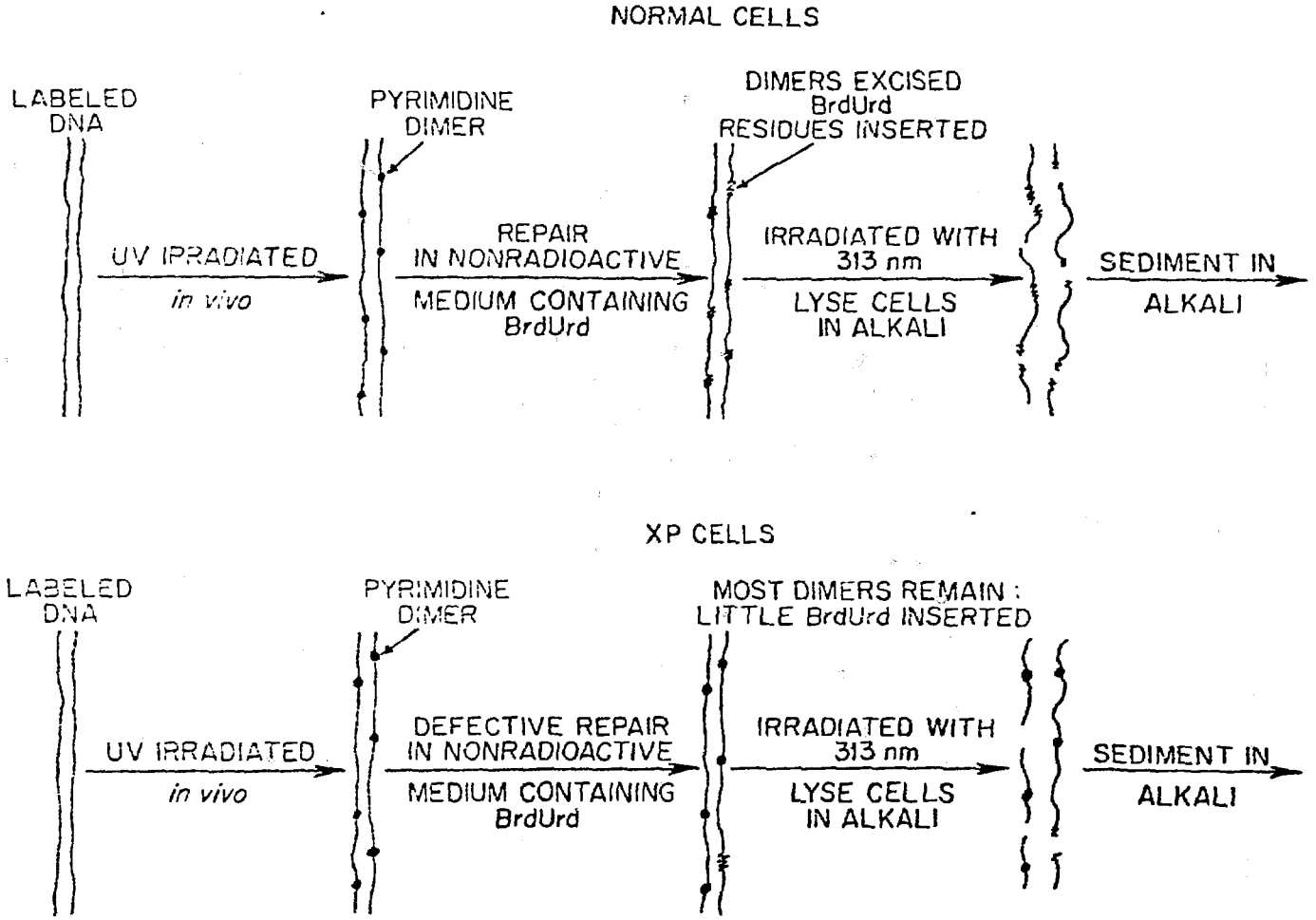
Cell cultures grown for 48 hr were then labeled for 24 hr in E-90/cs containing [^{3}H]dThd or [^{14}C]dThd. The label was removed, and cultures were incubated in unlabeled medium for 2 hr. Cells were exposed to 20 J/m^2 of 254-nm radiation and allowed to undergo repair for 18–20 hr in medium containing 2 mM hydroxyurea and 10 μM ara-C. Cultures labeled with [^{14}C]dThd served as unirradiated controls. Control and experimental cultures were suspended together in saline – 0.12% ethylenediaminetetraacetic acid, and the cellular DNA was analyzed on alkaline sucrose gradients (see METHODS).

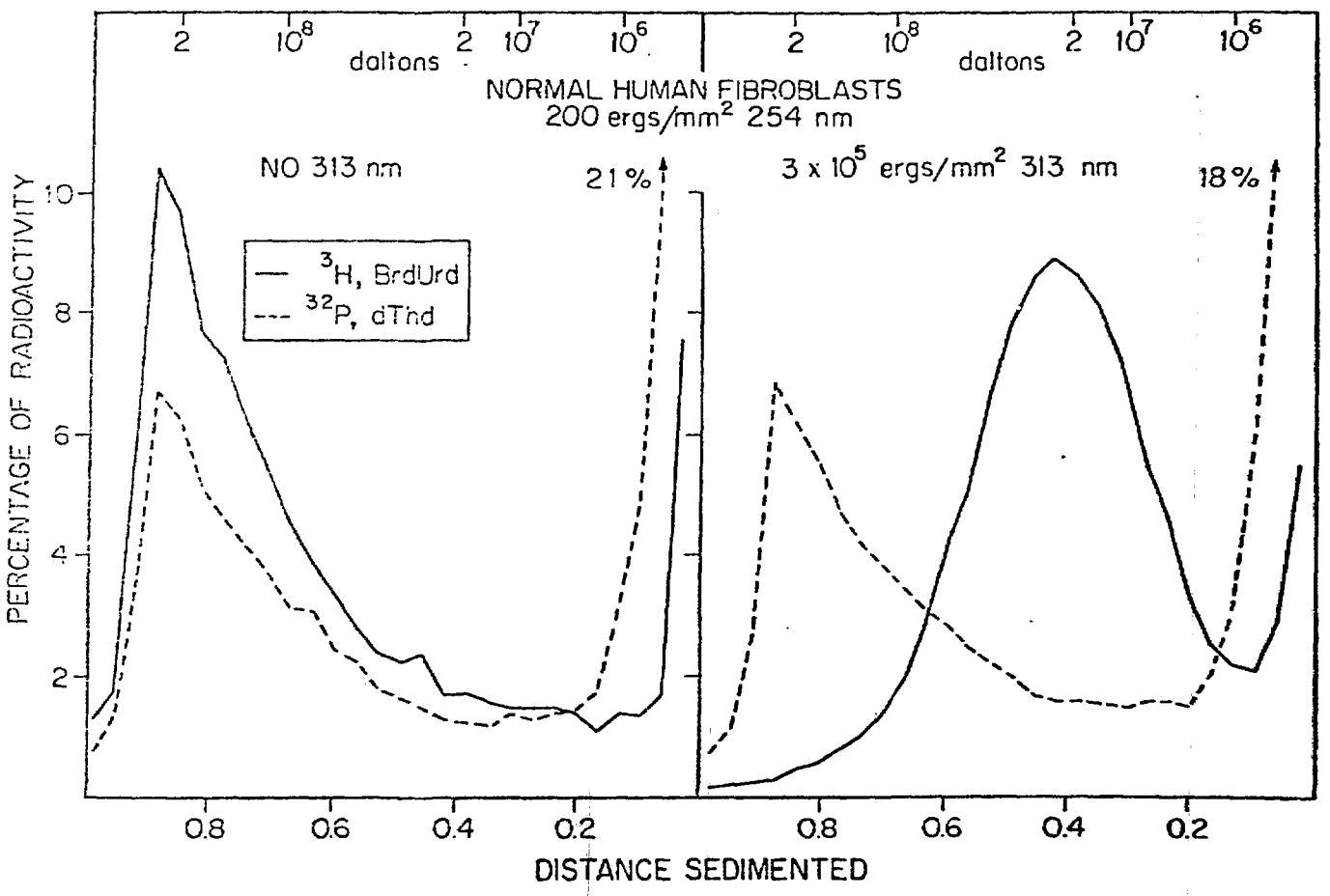
Cell line	Description	XP complementation group	DNA weight average molecular weight (M_w) × 10^6 daltons		DNA single-strand breaks/10^8 daltons[a]	Percent of normal incision
			without UV	with UV		
HSBP	Normal	—	240.37	24.33	7.39	100.00
	human skin	—	259.81[b]	187.75[b]	0.30[b]	—
	fibroblasts	—	272.48[c]	254.56[c]	0.05[c]	—
SGL	XP	?	252.65	167.91	0.40	5.41
CRL 1223 (Jay Tim)	XP	A	293.77	221.33	0.22	2.98
CRL 1199 (PoCo)	XP	B	281.27	110.93	1.09	14.75
CRL 1158 (PeAr)	XP	C	310.16	166.87	0.55	7.44
CRL 1157 (CayWen)	XP	D	303.59	161.63	0.58	7.85
CRL 1159 (XP-2)	XP	E	264.86	37.54	4.57	61.84
CRL 1258 (PeHay)	XP	XP-variant	221.87	15.60	11.92	161.30

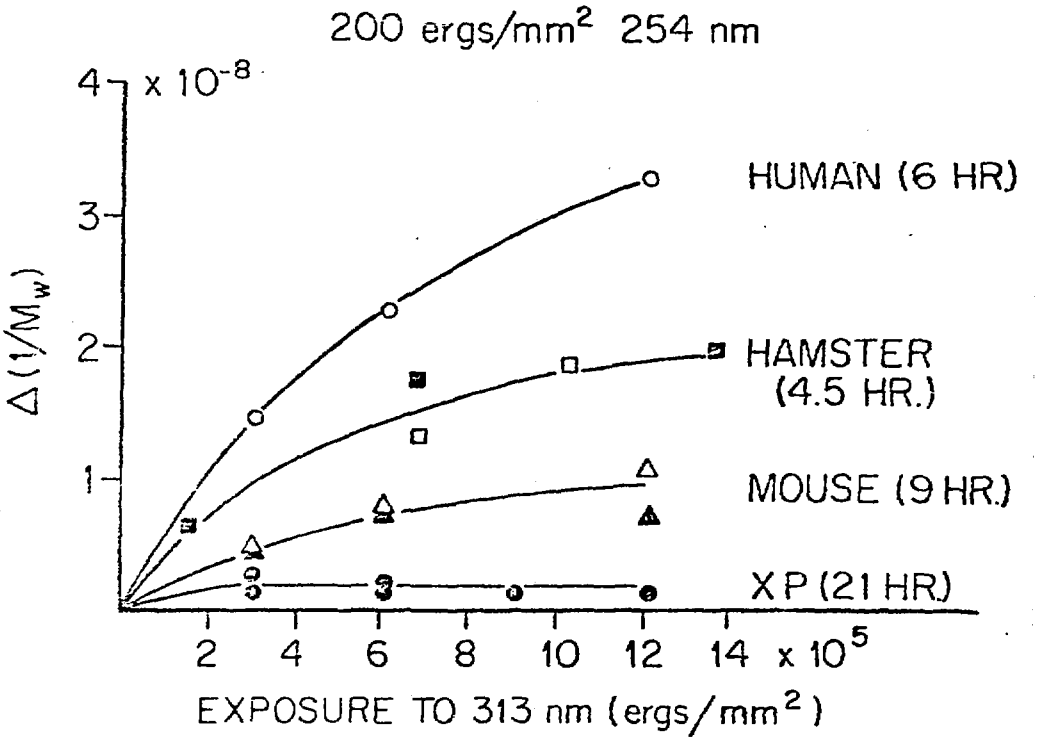
[a] Number of DNA strand breaks = $[2(1/M_{w\text{with UV}} - 1/M_{w\text{without UV}})]$.

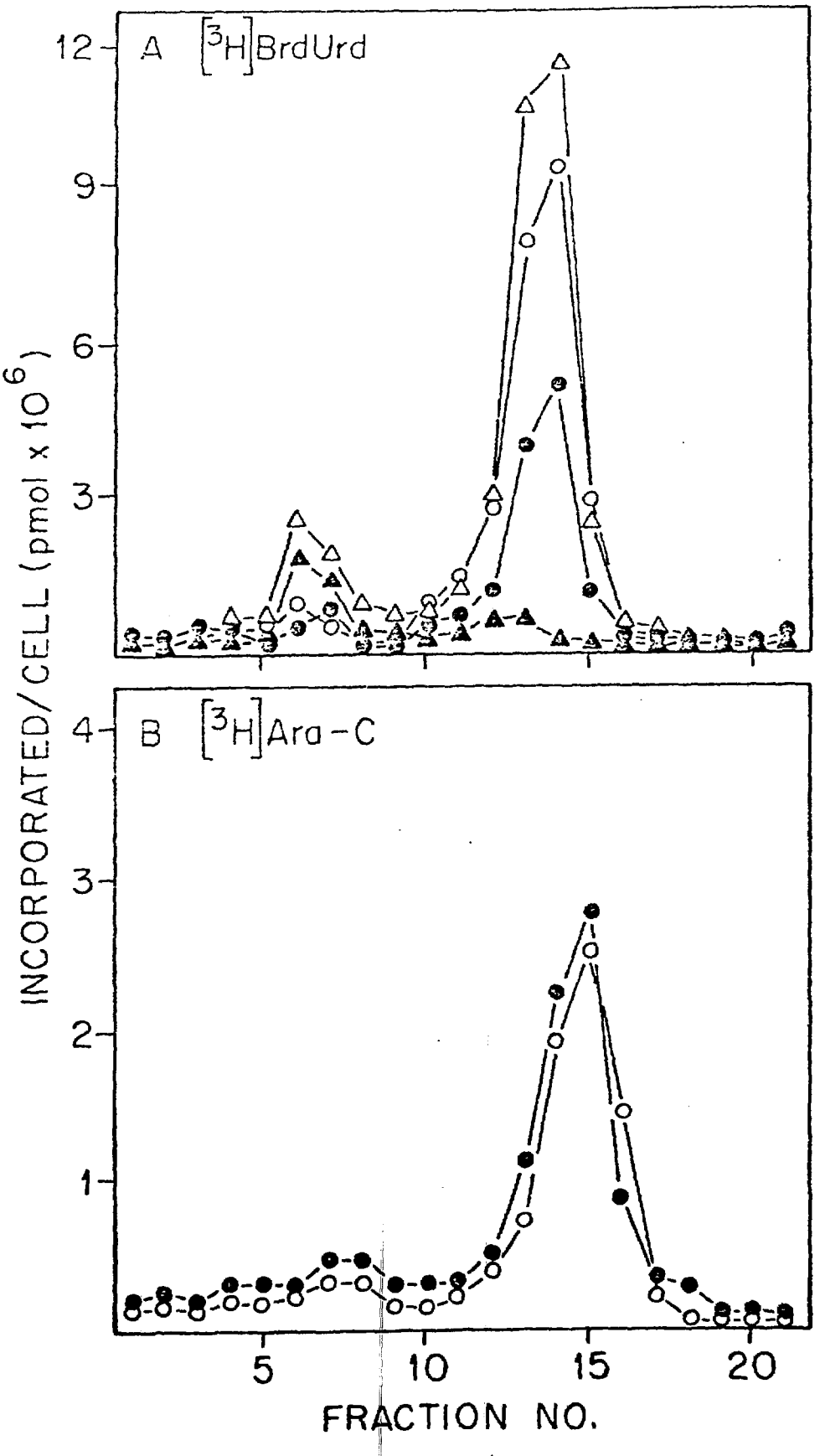
[b] Cells received no hydroxyurea during repair.

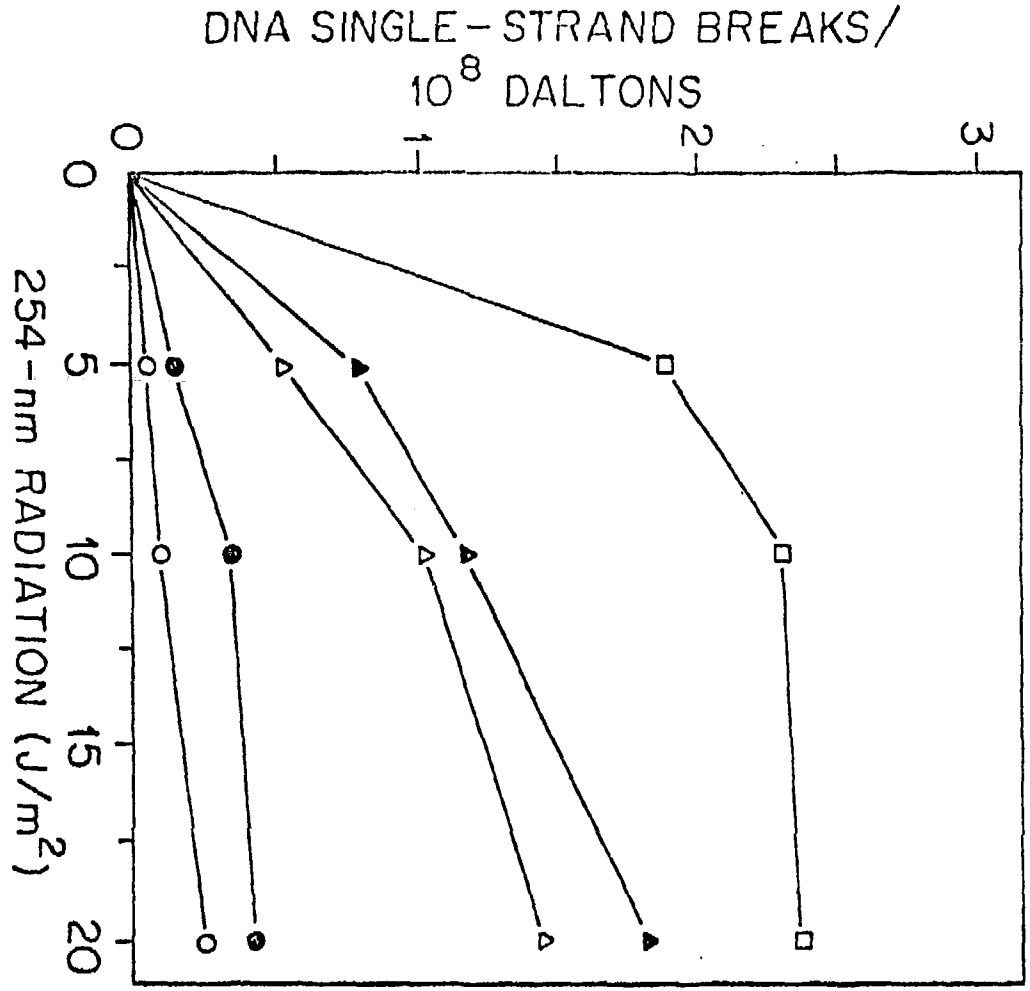
[c] Cells received no ara-C during repair.


NORMAL CELLS
LABELED DNA
UV IRRADIATED
in vivo
PYRIMIDINE DIMER
REPAIR IN NONRADIOACTIVE MEDIUM CONTAINING BrdUrd
DIMERS EXCISED BrdUrd RESIDUES INSERTED
IRRADIATED WITH 313 nm
LYSE CELLS IN ALKALI
SEDIMENT IN ALKALI
XP CELLS
LABELED DNA
UV IRRADIATED
in vivo
PYRIMIDINE DIMER
DEFECTIVE REPAIR IN NONRADIOACTIVE MEDIUM CONTAINING BrdUrd
MOST DIMERS REMAIN : LITTLE BrdUrd INSERTED
IRRADIATED WITH 313 nm
LYSE CELLS IN ALKALI
SEDIMENT IN ALKALI


2 10^8 daltons 2 10^7 10^6
2 10^8 daltons 2 10^7 10^6
NORMAL HUMAN FIBROBLASTS
200 ergs/mm² 254 nm
NO 313 nm
21%
3 x 10^5 ergs/mm² 313 nm
18%
³H, BrdUrd
³²P, dThd
PERCENTAGE OF RADIOACTIVITY
10
8
6
4
2
0.8 0.6 0.4 0.2
0.8 0.6 0.4 0.2
DISTANCE SEDIMENTED


200 ergs/mm² 254 nm
4
x 10⁻⁸
3
2
1
Δ(1/Mw)
HUMAN (6 HR)
HAMSTER
(4.5 HR.)
MOUSE (9 HR.)
XP (21 HR)
2
4
6
8
10
12
14
x 10⁵
EXPOSURE TO 313 nm (ergs/mm²)


A [3H]BrdUrd
B [3H]Ara-C
INCORPORATED/CELL (pmol x 10^6)
FRACTION NO.
12
9
6
3
4
3
2
1
5
10
15
20


DNA SINGLE-STRAND BREAKS/
10^8 DALTONS
0
1
2
3
0
5
10
15
20
254-nm RADIATION (J/m^2)

Repair of DNA Damaged by

7β,8α-dihydroxy-9α,10α-epoxybenzo(a)pyrene