FRAGMENTATION OF CHROMATIN WITH ^{125}I RADIOACTIVE DISINTEGRATIONS

GAIL N. TURNER, P. NOBIS, *and* W. C. DEWEY

From the Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, Colorado 80523

ABSTRACT The DNA in Chinese hamster cells was labeled first for 3 h with [^{3}H]TdR and then for 3 h with [^{125}I]UdR. Chromatin was extracted, frozen, and stored at −30°C until 1.0×10^{17} and 1.25×10^{17} disintegrations/g of labeled DNA occurred for ^{125}I and ^{3}H, respectively. Velocity sedimentation of chromatin (DNA with associated chromosomal proteins) in neutral sucrose gradients indicated that the localized energy from the ^{125}I disintegrations, which gave about 1 double-strand break/disintegration plus an additional 1.3 single strand breaks, selectively fragmented the [^{125}I] chromatin into pieces smaller than the [^{3}H] chromatin. In other words, ^{125}I disintegrations caused much more localized damage in the chromatin labeled with ^{125}I than in the chromatin labeled with ^{3}H, and fragments induced in DNA by ^{125}I disintegrations were not held together by the associated chromosomal proteins. Use of this ^{125}I technique for studying chromosomal proteins associated with different regions in the cellular DNA is discussed. For these studies, the number of disintegrations required for fragmenting DNA molecules of different sizes is illustrated.

INTRODUCTION

Studies (Burki et al. 1973; Feinendegen, 1975) have shown that when ^{125}I-labeled deoxyuridine or ^{3}H-labeled thymidine are incorporated into DNA, the ^{125}I disintegrations (18.9 keV) are much more effective than ^{3}H disintegrations (5.7 keV) in causing biological damage. Under these conditions, some 7 to 25 ^{3}H disintegrations are required to produce the same reduction in cell survival as one disintegration of ^{125}I (a rad dose 2.4 to 10 times greater for ^{3}H than ^{125}I). This factor has been reported to be between 22 and 44 for killing of *E. coli* and about 2 for the number of single strand breaks per disintegration in the DNA of mammalian cells (4-5/2.3 from Burki et al., 1975; Cleaver et al., 1972; Painter et al., 1974). The most interesting observation, undoubtedly related to the ^{125}I toxicity, is that ^{125}I contained in either the DNA of frozen phage or in frozen DNA extracted from phage caused, respectively, 1.05 or 1.0 double-strand breaks for each ^{125}I disintegration (Krisch and Ley, 1974; Krisch and Sauri, 1975) and an additional 1.0–1.6 or 0.2 single-strand breaks (Schmidt and Hotz, 1973; Krisch and Sauri, 1975) for a total of 3.1–3.7 or 2.2 strand breaks per disintegration. This breakage frequency for ^{125}I is much greater than reported for ^{3}H disintegrations occurring in frozen DNA, i.e., 0.2–0.3 single-strand breaks per ^{3}H disintegration (Burki et al., 1975; Cleaver et al., 1972; Rosenthal and Fox, 1970) and less than 0.01 double-strand

breaks per ^{3}H disintegration (Rosenthal and Fox, 1970). This relatively large amount of damage from the ^{125}I disintegration apparently results from the Auger effect associated with electron capture which leads to the simultaneous emission of 6 electrons (0.5–34.6 keV) which can deposit as much as 1000 eV within a range of 250 Å (Burki et al., 1973; Feinendegen, 1975). Also, the +6 charge on the daughter ^{125}Te might lead to destruction of the DNA molecule in the region where the disintegration occurs (Burki et al., 1973; Feinendegen, 1975).

The question posed in the present study is whether or not the relatively large amount of localized energy deposited from the ^{125}I disintegration, which in turn results in one double-strand break per disintegration, will cause breaks in chromatin, i.e., DNA with its associated histones and nonhistones. Thus, Chinese hamster cells were labeled with [^{3}H]TdR and/or [^{125}I]UdR, and both the chromatin from cells containing ^{3}H and ^{125}I and the chromatin from cells containing ^{3}H pooled with cells containing ^{125}I were frozen and stored at −30°C. After there was an approximately equal number of ^{125}I and ^{3}H disintegrations, the chromatin was thawed and placed on a sucrose gradient at neutral pH to determine whether [^{125}I] chromatin existed in smaller pieces than [^{3}H] chromatin. This comparison revealed that the localized energy from the ^{125}I disintegration indeed selectively fragmented the chromatin containing ^{125}I.

MATERIALS AND METHODS

Culturing and Labeling Conditions

Asynchronous cultures of Chinese hamster ovary (CHO) cells (Dewey and Miller, 1969) were cultured in Falcon 75 cm^2 plastic flasks (Falcon Plastics, Oxnard, Calif.) in McCoy's modified 5a medium without bactopeptone, but supplemented with dialyzed 10% calf serum and 5% fetal calf serum. After a 24-h incubation period of exponential growth at 37°C with a generation time of about 12 h, the medium was aspirated from three flasks and replaced either with 3 ml of fresh medium (culture A-2) or medium containing 1 μCi/ml [^{3}H]TdR (specific activity of 20 Ci/mmol, New England Nuclear, Boston, Ma.) (cultures A-1 and B). After 3.0 h of incubation, the medium was aspirated from the flasks, the cells were washed with 10 ml of medium, and 15 ml of fresh medium was added for a 1-h incubation period. Then, the medium was aspirated from the three flasks and replaced with either 3 ml of fresh medium (culture A-1) or 3 ml of medium containing 6.3 μCi/ml [^{125}I]UdR (specific activity of 7.6 Ci/mmol, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N.Y.) (cultures A-2 and B). After 3.0 h of incubation, the medium was aspirated, and 2 ml of 0.25% trypsin in McCoy's 5a medium (with 10% calf serum only) was added and immediately aspirated. Another 2 ml of the trypsin solution was added for 15 s and aspirated. After 3 min, the cells had detached, and 1 ml of egg white ovalbumin trypsin inhibitor (10 mg) was added, followed by 9 ml of medium. Single cell suspensions were produced by utilizing small bore pipettes, and 0.1 ml aliquots were taken for determinations of radioactivity (sampled in triplicate) and for determinations of cell number (8.5×10^{6} cells per flask) by particle counting (Coulter Electronics Inc., Hialeah, Fla.). Culture A-1 (labeled with ^{3}H) and culture A-2 (labeled with ^{125}I) were pooled to give a single culture A. Note that culture B contained ^{3}H and ^{125}I in the same cells.

Preparation of Chromatin and Exposure to ^{3}H and ^{125}I Disintegrations

The low ionic strength detergent method of Hancock (1974) was used, with all procedures being conducted at 4°C. Briefly, the cells were washed by centrifugation through a hypotonic solu-

tion (0.1 M sucrose, 0.7 mM NaH_2PO_4 at pH 7.5), and then resuspended in 1 ml of the above hypotonic solution at pH 8.5. To lyse the cells, 1 ml of 0.5% Non-idet P40 (a detergent supplied by Particle Data Labs, Elmhurst, Ill.) in 0.2 mM EDTA at pH 7.5 was added slowly to the cell suspension. The chromatin in the resulting cell lysate was washed 4 times by sedimentation through the hypotonic sucrose solution at pH 8.5. Finally, the resulting chromatin gel was resuspended in 1.0 ml of 0.2 mM EDTA (pH 7.0) and sheared with 50 strokes in a Dounce homogenizer (7 ml, type B with a tight piston, supplied by Kontes Glass Co., Vineland, N.J.). The sheared solubilized chromatin was transferred to a Beckman Spinco polyallomer tube (2" × ½"), the volume was increased to 2.0 ml with 0.2 mM EDTA, and the chromatin was centrifuged in a SW 50.1 rotor (4°C) at 13,000 rpm for 3 min. The resulting supernatant (containing chromatin at a concentration of 50–100 μg/ml, with about 50% of the ^{125}I and ^{3}H cellular radioactivity) was sampled (25 μl) for radioactivity, and the remainder was frozen and stored at −30°C.

After ^{125}I and ^{3}H disintegrations were allowed to occur in the frozen chromatin over a 395-day period, the chromatin was thawed and again sheared with 50 strokes in the Dounce homogenizer. This second homogenization was necessary to prevent aggregation. Aliquots (containing 3 to 8 μg of chromatin) were taken for sucrose gradient analyses, and the remainder was frozen again for subsequent analyses. For subsequent analyses (second thaw), the Dounce homogenization procedure was always repeated, again to prevent aggregation.

In one case, an aliquot of chromatin A (second thaw) was further sheared in a French press (Amicon Corp., Lexington, Ma.) at 14,000 psi. One-half of the aliquot was then fixed with formaldehyde (6% in 5 mM NaH_2PO_4 at pH 7.0) (Hancock, 1970) in order to compare the velocity sedimentation of unfixed chromatin with the velocity sedimentation of chromatin in which the chromosomal proteins were fixed to the DNA.

Sedimentation of Chromatin and DNA

For velocity sedimentation of chromatin under conditions which do not remove the protein from the DNA, the chromatin (2–4.5 μg in 0.5–0.7 ml) contained in 0.2 mM EDTA in 0.1 mM Tris-HCl at pH 7.0 was layered on top of 36 ml of a 5–20% linear sucrose gradient preformed at 4°C in polyallomer centrifuge tubes containing 0.2 mM EDTA in 0.1 mM Tris-HCl at pH 7.4). (Crystalline sucrose which was ribonuclease free was obtained from Nutritional Biochemical Co., Cleveland, Ohio) Immediately, the tubes were centrifuged (4°C) in the SW 27 rotor at 27,000 rpm for variable time periods. After centrifugation, the bottoms of the tubes were punctured with a needle, and 20–23 equal volume fractions (~1.6 ml) were collected.

For velocity sedimentation of double-stranded DNA under conditions which removed the protein from the DNA, the neutral gradient system of Cole et al. (1974) was used, and the number average molecular weight was calculated as described (Cole et al., 1974). Briefly a 2 ml lysing solution consisting of 1% sodium dodecylsarcinosate, 0.1 M Na_2SO_4, 0.05 M EDTA at pH 9.6 was layered over a 37.0 ml linear 5–20% sucrose gradient in polyallomer tubes containing 5 M NaCl, 0.1 M Na_2SO_4, and 0.025 M EDTA (24°C) at pH 9.6. Chromatin containing approximately 2 μg of DNA (0.2 ml of chromatin) was added to the lysing solution for 2 h, and then centrifuged at 27,000 rpm for 7.5 h at 20°C in a Beckman SW 27 rotor. After centrifugation, 33 equal volume (~1.2 ml) fractions were collected by piercing the bottom of the tube.

For velocity sedimentation of single-stranded DNA under conditions which removed the protein from the DNA, the alkaline-sucrose system of Lett et al. (1970) was used, and the number average molecular weight was calculated as described (Lett and Sun, 1970). Briefly, a 0.5 ml lysing solution of 0.5 N NaOH, 0.02 M EDTA was gently layered over a linear 37 ml, 5–20% sucrose gradient in polyallomer tubes containing 0.1 N NaOH, 0.9 M NaCl and 0.01 M EDTA at pH 13. Chromatin containing approximately 2 μg of DNA (0.5 ml) was added to the lysing solution for 10 min and then centrifuged at 27,000 rpm for 12 h at 20°C in a Beckman SW 27

rotor. After centrifugation, 23 equal volume fractions (~1.6 ml) were collected as described above.

Radioactive Assay

Cell, chromatin, and gradient samples, collected as described above, were added to 10 ml of 10% trichloroacetic acid (TCA), containing 100 μg of calf thymus DNA added for carrier. After at least 2 h were allowed for precipitation, the samples were filtered onto GF/B filters, washed with 20 ml of cold 5% TCA followed by 3 ml of 95% cold ethanol, and dried. The ^{125}I was counted by placing plastic tubes containing the filters into the well of a NaI crystal gamma counter (efficiency of 54%). The filters were then placed in Kimble glass scintillation vials, 1 ml of hydroxide of Hyamine was added, and the tightly capped vials were incubated overnight at 55°C. The vials were then cooled, 10 ml of toluene-based scintillation fluid was added, and after the samples were agitated, they were counted in a liquid scintillation counter (^{125}I efficiency in the ^{3}H channel/^{125}I efficiency in the gamma counter was 0.45, and the efficiency for counting ^{3}H was 17%). Although the efficiency (24.3%) for counting ^{125}I in the ^{3}H channel was higher for ^{125}I than for ^{3}H, only about 10% of the counting rate in the ^{3}H channel was due to ^{125}I because ^{125}I had decayed considerably by the time it was counted (see legends of Figs. 1 and 2). The recovery of radioactivity from the gradients varied between 50 and 90%.

RESULTS

Preliminary studies indicated that chromatin prepared by the low ionic strength detergent method could be centrifuged through a neutral sucrose gradient in order to determine relative sizes of chromatin pieces. First, the chromatin had a density of 1.385, as determined by CsCl equilibrium centrifugation of the chromatin fixed with formaldehyde, and thus should have relative masses of DNA:histone:nonhistone of about 1.0, 1.0, and 0.5, respectively (Hancock, 1974; Garrard et al., 1974; Chalkley and Jensen, 1968). Second, the identical sedimentation patterns of unfixed chromatin and of formaldehyde-fixed chromatin (data not shown) revealed that the unfixed chromatin could be sedimented satisfactorily through a neutral 5–20% sucrose gradient without losing its chromosomal proteins.

Next, sedimentation studies of chromatin through a neutral sucrose gradient demonstrated that chromatin was fragmented by ^{125}I disintegrations. Specifically, chromatin containing ^{125}I in its DNA sedimented less rapidly than chromatin containing ^{3}H in its DNA. This is illustrated in Fig. 1 for chromatin obtained from a pooled population of cells consisting of cells labeled with ^{3}H and cells labeled with ^{125}I, and again in Fig. 2 for chromatin obtained from cells labeled first with ^{3}H and then with ^{125}I. Immediately after the 3-h labeling period for each isotope, the cells contained 1.86 disintegrations/min/cell (dpm/cell) for ^{125}I and 0.50 dpm/cell for ^{3}H which gave 2.28×10^{5} disintegrations/cell for ^{125}I and 2.83×10^{5} disintegrations/cell for ^{3}H during the 395-day storage period at −30°C. It should be emphasized that although the chromatin contained both ^{125}I and ^{3}H, the two isotopes were contained in different regions of the DNA. Therefore, the separation of [^{125}I]chromatin from [^{3}H]chromatin in the sucrose gradient indicates that at least some of the ^{125}I disintegrations were causing fragmentation of the chromatin in regions close to the sites of the disintegrations.

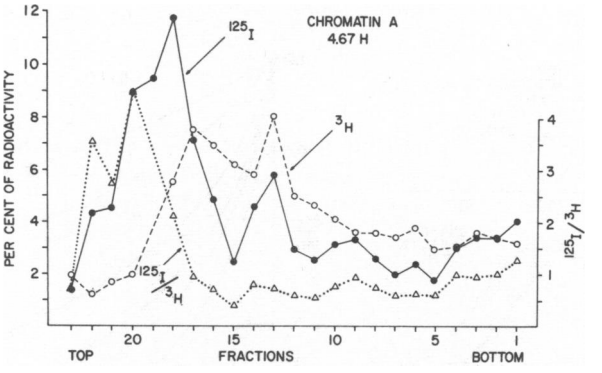

FIGURE 1 A neutral 5 to 20% sucrose gradient of chromatin (second thaw) obtained from cells labeled for 3 h with [^{3}H]TdR and then pooled with cells labeled for 3 h with [^{125}I]UdR. About 1.2 μg of DNA with its associated chromosomal protein was applied to the gradient, without any lysing solution, and the rotor was spun at 27,000 rpm (4°C) for 4.67 h. Total counts/min (cpm) for ^{125}I were 1458 and for ^{3}H were 6210.

As would be expected, DNA containing ^{125}I also sedimented less rapidly than DNA containing ^{3}H. When the chromatin used in Fig. 1 was lysed on top of an alkaline sucrose gradient, the number average molecular weights of single-stranded DNA were 1.4×10^6 and 3.2×10^6 for ^{125}I and ^{3}H, respectively, for a ratio of 0.44 for ^{125}I relative to ^{3}H (Fig. 3—middle panel). A similar analysis on a neutral gradient of chromatin used in Fig. 2 gave molecular weights of 4×10^6 and 8×10^6 for ^{125}I and ^{3}H, respectively, for a ratio of 0.50 for ^{125}I relative to ^{3}H (Fig. 3—upper panel). Although the molecular weights were not determined precisely under well-calibrated conditions, the ratios should be quite accurate.

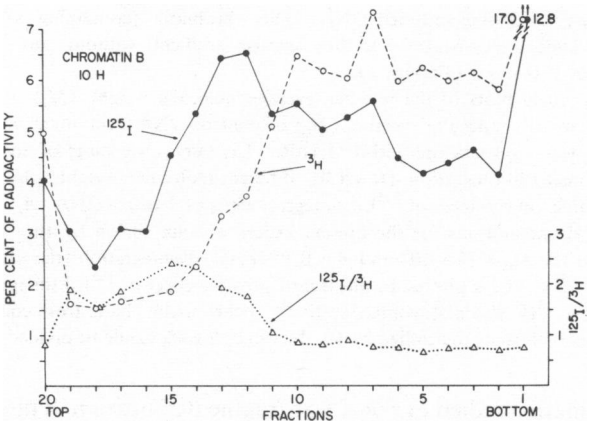

FIGURE 2 A neutral 5 to 20% sucrose gradient of chromatin (first thaw) obtained from cells labeled first for 3 hr with [^{3}H]TdR and then for 3 h with [^{125}I]UdR. Other details were identical to those listed in the legend for Fig. 1, except that about 3.1 μg of DNA with its associated chromosomal protein was applied, and the rotor was spun for 10.0 h. Total cpm for ^{125}I were 3606 and for ^{3}H were 15,522.

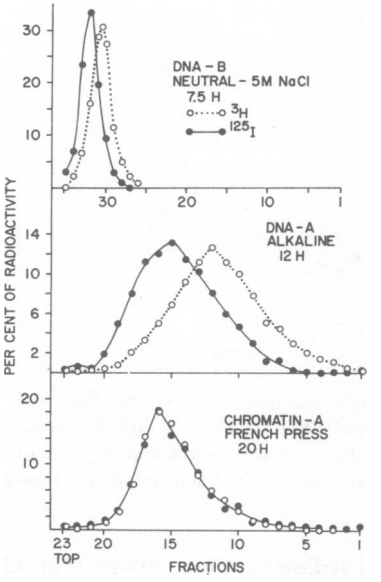

FIGURE 3

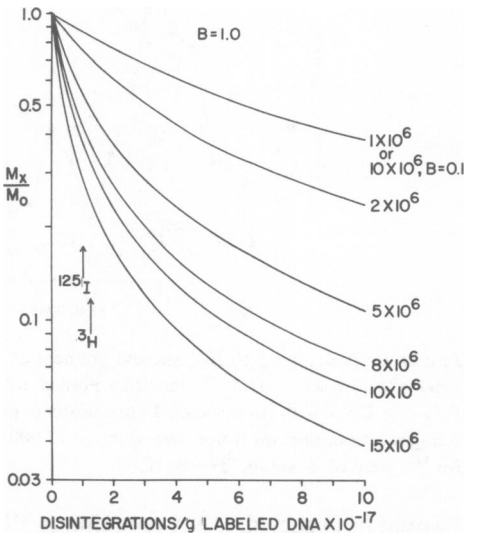

FIGURE 4

FIGURE 3 *Upper panel*: Chromatin B (second thaw of that studied in Fig. 2) was layered on top of a neutral, high salt, 5 to 20% sucrose gradient, and the rotor was spun at 27,000 rpm (20°C) for 7.5 h. The number average molecular weights of the double-stranded DNA were calculated to be 4×10^6 and 8×10^6 daltons for ^{125}I and ^{3}H, respectively. *Middle panel*: Chromatin A (studied in Fig. 1) was layered on top of an alkaline 5 to 20% sucrose gradient, and the rotor was spun at 27,000 rpm (20°C) for 12 h. The number average molecular weights of the single-stranded DNA were calculated to be 1.4×10^6 and 3.2×10^6 daltons for ^{125}I and ^{3}H, respectively. *Lower panel*: Chromatin A, the same as that studied in the middle panel and in Fig. 1, was sheared through a French Press to an estimated molecular weight of about $1\text{–}2 \times 10^6$ daltons for double-stranded DNA. This chromatin (containing about 1.2 μg of DNA) was then applied to a neutral 5 to 20% sucrose gradient without any lysing solution and centrifuged at 27,000 rpm (4°C) for 20 h.

FIGURE 4 Theoretical plots of the number average molecular weights (M_x) of DNA containing ^{125}I relative to the molecular weights (M_0) of control DNA containing no isotope (could be identified by low level labeling with [^{3}H]TdR. The curves are plots of equation 4 for 1.0 double-strand break/disintegration ($B = 1.0$), different molecular weights (M_0) of the control DNA, and for different numbers of ^{125}I disintegrations/g of labeled DNA (A_L in equation 4). The number of disintegrations for the present experiment are shown by the ^{125}I and ^{3}H arrows. The curve for $M_0 = 10 \times 10^6$ and $B = 0.10$ breaks/disintegration (the same curve as for $M_0 = 1 \times 10^6$ and $B = 1.0$) is plotted to illustrate a possible effect of ^{125}I disintegrations on [^{3}H]-DNA contained in [^{3}H] chromatin mixed with [^{125}I] chromatin. Note from equation 4, that if the values on the abscissa are multiplied by 10, the values for M_0 would be divided by 10.

When the chromatin studied in Fig. 1 was fragmented into small pieces prior to sedimentation (molecular weight of double-stranded DNA of $1\text{–}2 \times 10^6$, as estimated from our gradients and the shearing studies of Chalkley and Jensen (1968)), no separation between ^{125}I and ^{3} was observed (Fig. 3—lower panel). Therefore, the fragmentation of chromatin by the ^{125}I disintegrations can be detected only when the chromatin exists in pieces of sufficient size.

DISCUSSION

Although this study was not intended to quantify precisely the relationship between number of ^{125}I disintegrations and number of breaks in DNA and chromatin, some semi-quantitative information is available. This can be obtained from estimates of molecular weights of DNA contained in the chromatin samples lysed on top of alkaline or neutral sucrose gradients in order to remove chromosomal proteins prior to sedimentation. The relationship between the number average molecular weights (M_x for ^{125}I or ^{3}H, and M_0 for control DNA containing no isotope-induced breaks), the number of disintegrations per gram of labeled DNA (A_L), and the number of strand breaks per labeled DNA molecule per disintegration (B) can be shown to be:

$$B = 6 \times 10^{23}(1 - M_x/M_0)/M_xA_L, \quad (1)$$

which is based on the commonly used equation (Painter et al., 1974)

$$\text{Breaks/molecule} = M_0(1/M_x - 1/M_0). \quad (2)$$

The value of A_L is obtained from the number of disintegrations/cell occurring in the chromatin during the storage period divided by: total grams of DNA/cell multiplied by the duration of labeling/generation time.

For example, for our CHO cells, $A_L = 2.28 \times 10^5/(9.0 \times 10^{-12}$ g/cell $\times$ 3 h/12 h) = 1.01×10^{17} disintegrations of ^{125}I/g of labeled double-stranded DNA. This same calculation for ^{3}H gives 1.25×10^{17} disintegrations of ^{3}H/g of labeled double-stranded DNA. For labeled single-stranded DNA studies (alkaline gradients), these same values were used by assuming that there were as many undetected breaks in the unlabeled strand as detected breaks in the labeled strand, shown to be true for ^{3}H (Burki et al., 1975); if all the breaks occurred only in the labeled strand, the values for A_L should be multiplied by 2. Also, the number of disintegrations/cell for ^{125}I (half-life of 60 days) equals:

$$\text{dpm/cell}/8.02 \times 10^{-6} \times (1 - e^{-8.02 \times 10^{-6}t}), \quad (3)$$

where t is the storage time in minutes after the initial dpm/cell was determined.

Thus, the number average molecular weights for single-stranded DNA (M_S), estimated to be 1.4×10^6 and 3.2×10^6 for ^{125}I and ^{3}H, respectively, when substituted for M_x in equation 1 give 3.04 single-strand breaks/disintegration (2.76–3.44) for ^{125}I and 0.54 single strand breaks/disintegration (0.30–0.86) for ^{3}H. (The mean value assumes a M_0 of 5×10^6 daltons, and the range is for M_0 of $4 \times 10^6 - 7.5 \times 10^6$.) The number average molecular weights for double-stranded DNA (M_D), estimated to be 4×10^6 and 8×10^6 for ^{125}I and ^{3}H, respectively, when substituted for M_x in equation 1 give 0.89 double-strand breaks/disintegration (0.74–1.09) for ^{125}I and 0.12 (0–0.28) double-strand breaks/disintegration for ^{3}H. (The mean value assumes a M_0 of 10×10^6 daltons, and the range is for M_0 of $8 \times 10^6 - 15 \times 10^6$.) Note, that the values of about 1 double-strand break/disintegration of ^{125}I and 1.27 and 0.30 single-strand breaks/disintegration of ^{125}I and ^{3}H, respectively, after subtracting two times the appropriate number of double-strand breaks (i.e. $B_S = 6 \times$

$10^{23}(M_D - 2M_S)/M_S M_D A_L$), agree well with reported values (see Introduction). This, our results are consistent with the concept of each ^{125}I disintegration causing one double-strand break very near the site of the disintegration, and since sedimentation of ^{3}H chromatin relative to ^{125}I chromatin was similar to that observed for single-stranded and double-stranded DNA (compare Figs. 1, 2, and 3), a large fraction of the double-strand breaks in the DNA and possibly some of the single-strand breaks must also lead to breaks in the chromatin. Of course, these breaks in chromatin may not actually exist until they are produced physically as the chromatin is subjected to shear in the Dounce homogenizer.

Future work should be oriented in two general directions, one to further understand the radiobiology of the ^{125}I effect on chromatin, and the other to utilize the ^{125}I labeling technique for separating chromatin containing ^{125}I from chromatin containing ^{3}H, for example, the separation of chromatin containing DNA synthesized in early S phase from chromatin containing DNA synthesized in late S phase.

In the first case, careful studies comparing ^{125}I effects with ^{3}H effects on chromatin in DNA irradiated either in the frozen cell prior to extraction of chromatin or in solution after extraction should be carried out to determine whether double-strand breaks from ^{125}I are more effective than double-strand breaks from ^{3}H, and whether or not single-strand breaks also lead to fragmentation of chromatin. Since the ratio of single-strand breaks:double-strand breaks will be much higher for ^{3}H than ^{125}I (Feinendegen, 1975; Schmidt and Hotz, 1973), this question can be answered by exposing chromatin to different numbers of ^{3}H or ^{125}I disintegrations. We wish to emphasize that in the present study we have shown only that the ^{125}I disintegrations in the DNA of isolated chromatin lead to fragmentation of DNA and chromatin in or near the regions where the disintegrations occur, i.e., not in adjacent ^{3}H-labeled regions. The possibility that ^{3}H, ^{14}C, or ^{32}P disintegrations in the DNA also induce some fragmentation of chromatin remains to be investigated.

In the second case, the sucrose gradient technique needs to be refined, primarily by developing reproducible methods for shearing chromatin to different sizes. These studies also should provide information on whether or not breaks in chromatin exist prior to physical shearing. As illustrated in Figs. 3 and 4, the relative size of the pieces of [^{125}I]DNA relative to [^{3}H]DNA will depend greatly on the degree of shearing and number of ^{125}I disintegrations occurring in the labeled DNA. The curves in Fig. 4 are plots of the equation:

$$M_x/M_0 = 6 \times 10^{23}/(6 \times 10^{23} + BM_0A_L) \quad (4)$$

which is a rearrangement of equation 1. Since chromatin is most likely fragmented at the loci where double-strand breaks have occurred, curves in Fig. 4 were calculated for $B = 1.0$ double-strand break/^{125}I disintegration. Possibly, the ionization from the ^{125}I disintegrations also will cause a few double-strand breaks in the [^{3}H]DNA, and this effect needs to be quantified; however, this effect should not be great because: (1) in our experiment, the molecular weight of ^{125}I-labeled double-stranded DNA relative to

^{3}H-labeled double-stranded DNA was 0.5 and similar to the calculated values plotted in Fig. 4 for the control molecular weights of 8–10 × 10^6 daltons, and (2) the number of double-strand breaks in [^{3}H]DNA per ^{125}I disintegration should not exceed 0.07 as calculated by Krisch and Sauri (1975) or 0.1 as calculated for a maximum of 0.01 double-strand breaks/^{3}H disintegration (Rosenthal and Fox, 1970) × 3 for the ^{125}I energy relative to ^{3}H energy (Feinendegen, 1975) × a maximum of 3 for a possible RBE effect (Burki et al., 1973; Feinendegen, 1975). This relatively small effect that a value of 0.1 would have on separating ^{3}H and ^{125}I chromatin is illustrated by comparing, for example, the curve for 10 × 10^6, B = 1.0, with the curve for 10 × 10^6, B = 0.1. Thus, if the number of ^{125}I disintegrations in the labeled DNA were increased 10- to 350-fold above the level used in the present experiment by increasing the concentration and specific activity of [^{125}I]UdR (available at greater than 200 Ci/mmole), and possible by using FUdR to block endogenous synthesis, it might be possible to separate ^{125}I chromatin from ^{3}H chromatin containing pieces of DNA as small as 1 × 10^6 daltons (if A_L = 350 × 10^{17}, M_x/M_0 = 0.017). Furthermore, the separation should be better than that obtained by separating chromatin containing BUdR or IUdR from normal chromatin by density gradient centrifugation (Hancock, 1974; Jackson and Chalkley, 1974).

In these studies separating small molecules of about 0.5–1.0 μm in length, the DNA could be labeled with [^{125}I]UdR during a 30-s pulse, which should give labeled regions of about 10^6 daltons. Then, the chromatin could be isolated, frozen, and stored for about 60 days. After the chromatin is thawed, sheared to about 10^6–10^7 daltons, and fractionated, the chromosomal proteins associated with this replicating DNA could be isolated and studied.

Appreciation is expressed to Dr. Peter Corry and Mr. Edward P. Clark for assistance in the analyses of neutral and alkaline sucrose gradients, respectively, and to Dr. Ronald Hancock for suggesting the present experiment.

This research was supported in part by U.S. Public Health Service National Institutes of Health Grant No. CA 08618.

Received for publication 18 March 1976 and in revised form 10 May 1976.

REFERENCES

BURKI, H. J., S. BUNKER, M. RITTER, and J. E. CLEAVER. 1975. DNA damage from incorporated radioisotopes: Influence of the ^{3}H location in the cell. *Radiat. Res.* **62**:299–312.

BURKI, H. J., R. ROOTS, L. E. FEINENDEGEN, and V. P. BOND. 1973. Inactivation of mammalian cells after disintegrations of ^{3}H or ^{125}I in cell DNA at −196°C. *Int. J. Radiat. Biol.* **24**:363–375.

CHALKLEY, R., and R. H. JENSEN. 1968. A study of the structure of isolated chromatin. *Biochemistry.* **7**: 4380–4387.

CLEAVER, J. E., G. H. THOMAS, and H. J. BURKI. 1972. Biological damage from intranuclear tritium: DNA strand breaks and their repair. *Science (Wash., D.C.).* **177**:996–998.

COLE, A., W. G. COOPER, F. SHONKA, P. M. CORRY, R. M. HUMPHREY, and A. T. ANSEVIN. 1974. DNA scission in hamster cells and isolated nuclei studied by low-voltage electron beam irradiation. *Radiat. Res.* **66**:1–33.

DEWEY, W. C., and H. H. MILLER. 1969. X-ray induction of chromatid exchanges in mitotic and G1 Chinese hamster cells pretreated with colcemid. *Exp. Cell. Res.* **57**:63–70.

FEINENDEGEN, L. E. 1975. Biological damage from the Auger effect, possible benefits. *Radiat. Environ. Biophys.* **12**:85–99.

GARRARD, W. T., W. R. PEARSON, S. K. WAKE, and J. BONNER. 1974. Stoichiometry of chromatin proteins. *Biochem. Biophys. Res. Commun.* **58**:50–57.

HANCOCK, R. 1970. Separation by equilibrium centriguation in CsCl gradients of density-labelled and normal deoxyribonucleoprotein from chromatin. *J. Mol. Biol.* **48**:357–360.

HANCOCK, R. 1974. Interphase chromosomal deoxyribonucleoprotein isolated as a discrete structure from cultured cells. *J. Mol. Biol.* **86**:649–663.

JACKSON, V., and R. CHALKLEY. 1974. Separation of newly synthesized nucleohistone by equilibrium centrifugation in cesium chloride. *Biochemistry.* **13**:3952–3956.

KRISCH, R. E., and R. D. LEY. 1974. Induction of lethality and DNA breakage by the decay of iodine-125 in bacteriophage T4. *Int. J. Radiat. Biol.* **25**:21–30.

KRISCH, R. E., and C. J. SAURI. 1975. Further studies of DNA damage and lethality from the decay of iodine-125 in bacteriophages. *Int. J. Radiat. Biol.* **27**:553–560.

LETT, J. T., E. S. KLUCIS, and C. SUN. 1970. On the size of DNA in the mammalian chromosome. Structural subunits. *Biophys. J.* **10**:277–292.

LETT, J. T., and C. SUN. 1970. The production of strand breaks in mammalian DNA by x-rays: At different stages in the cell cycle. *Radiat. Res.* **44**:771–787.

PAINTER, R. B., B. R. YOUNG, and H. J. BURKI. 1974. Non-repairable strand breaks induced by ^{125}I incorporated into mammalian DNA. *Proc. Natl. Acad. Sci. U.S.A.* **71**:4836–4838.

ROSENTHAL, P. N., and M. S. FOX. 1970. Effects of disintegration of incorporated ^{3}H and ^{32}P on the physical and biological properties of DNA. *J. Mol. Biol.* **54**:441–463.

SCHMIDT, A., and G. HOTZ. 1973. The occurrence of double-strand breaks in coliphage T1-DNA by iodine-125 decay. *Int. J. Radiat. Biol.* **24**:307–313.