(1978) *Biochim. Biophys. Acta 506*, 281-287.
Orr, G. A., Brewer, C. F., & Heney, J. (1982) *Biochemistry 21*, 3202-3206.
Papahadjopoulos, D., Poste, G., Schaeffer, B. E., & Vail, W. J. (1974) *Biochim. Biophys. Acta 352*, 10.
Portis, A. R., Newton, C., Pangborn, W., & Papahadjopoulos, D. (1979) *Biochemistry 18*, 780-790.
Stewart, T. P., Hui, S. W., Porties, A. R., & Papahadjopoulos, D. (1979) *Biochim. Biophys. Acta 556*, 1-16.
Van Dijck, P. W. M., de Kruijff, B., Verkleij, A. J., van Deenen, L. L. M., & de Gier, J. (1978) *Biochim. Biophys. Acta 512*, 84-96.
Vasilenko, I. A., de Kruijff, B., & Verkleij, A. J. (1982) *Biochim. Biophys. Acta 685*, 144-152.
Wilschut, J., Duzgunes, N., Fraley, R., & Papahadjopoulos, D. (1980) *Biochemistry 19*, 6011-6021.

Rearrangements of Chromatin Structure in Newly Repaired Regions of Deoxyribonucleic Acid in Human Cells Treated with Sodium Butyrate or Hydroxyurea†

Michael J. Smerdon

ABSTRACT: The rate and extent of redistribution of repair-incorporated nucleotides within chromatin during very early times (10–45 min) after ultraviolet irradiation were examined in normal human fibroblasts treated with 20 mM sodium butyrate, or 2–10 mM hydroxyurea, and compared to results for untreated cells. Under these conditions, DNA replicative synthesis is reduced to very low levels in each case. However, DNA repair synthesis is stimulated by sodium butyrate and partially inhibited by hydroxyurea. Furthermore, in the sodium butyrate treated cells, the core histones are maximally hyperacetylated. Using methods previously described by us, it was found that treatment with sodium butyrate had little or no effect on either the rate or the extent of redistribution of repair-incorporated nucleotides during this early time interval. On the other hand, there was a 1.7–2.5-fold decrease in the rate of redistribution of these nucleotides in cells treated with hydroxyurea; the extent of redistribution was unchanged in these cells. Since hydroxyurea has been shown to decrease the rate of completion of "repair patches" in mammalian cells, these results indicate that nucleosome rearrangement in newly repaired regions of DNA does not occur until after the final stages of the excision repair process are completed. Furthermore, hyperacetylation of the core histones in a large fraction of the total chromatin prior to DNA damage and repair synthesis does not appear to alter the rate or extent of nucleosome core formation in newly repaired regions of DNA.

It is now clear that rearrangements of chromatin structure take place following DNA repair synthesis in human cells damaged by ultraviolet (UV)[1] radiation (Smerdon & Lieberman, 1978, 1980; Smerdon et al., 1979, 1982a; Williams & Friedberg, 1979; Bodell & Cleaver, 1981; Cleaver, 1982) or UV-mimetic chemicals (Tlsty & Lieberman, 1978; Oleson et al., 1979; Zolan et al., 1982). Immediately after repair synthesis, the newly repaired DNA is rapidly digested to acid-soluble form by both staphylococcal nuclease and DNase I. This DNA is markedly reduced in nucleosome core particles and does not yield the familiar ~10-base repeat pattern on gels following digestion with DNase I. Subsequently, the newly repaired DNA becomes increasingly nuclease resistant, is associated with nucleosome cores, as well as histone H1, and yields an ~10-base repeat pattern following DNase I digestion (Smerdon & Lieberman, 1980; Smerdon et al., 1982a). These results led us to propose that this redistribution process, which we have termed "nucleosome rearrangement", may involve the refolding of newly repaired regions of DNA into their original (or near-original) nucleosome structures following a perturbation of these structures by the excision repair process (Lieberman et al., 1979). An alternative explanation for these results is that core histones are continuously changing their positions along the DNA ("sliding") and that damaged DNA bases are not "fixed" to their original positions within a nucleosome unit. The repair enzymes could then perform the excision of these lesions, as well as resynthesis, only when the lesions were "presented" in the linker region. The rearrangement that we observe would then be due to the continuous sliding of core histones in these newly repaired regions of DNA which would serve to randomize the distribution of this DNA between linker and core regions. We have classified this form of nucleosome rearrangement as "constitutive rearrangement", and it represents a marked contrast to the first proposal given above, which we have called "induced rearrangement" (Smerdon & Lieberman, 1978).

Data on the initial chromatin distribution and subsequent removal of labeled chemical adducts in intact cells support the induced rearrangement model (Oleson et al., 1979; Kaneko & Cerutti, 1980; Jack & Brookes, 1982). For example, the nonrandom distribution between linker and core DNA of the adducts formed by *N*-acetoxy-2-(acetylamino)fluorene (Kaneko & Cerutti, 1980) and *r*-7,*t*-8-dihydroxy-*t*-9,10-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (Jack & Brookes, 1982) remains unchanged for periods of 24–48 h in repair-deficient

† From the Biochemistry/Biophysics Program, Washington State University, Pullman, Washington 99164-4630. *Received January 5, 1983.* This study was supported by National Institutes of Health Grant ES 02614. M.J.S. is the recipient of a National Institutes of Health Research Career Development Award.

[1] Abbreviations: UV, ultraviolet; dThd, thymidine; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; BrdUrd, 5-bromo-2′-deoxyuridine; bp, base pair(s).

0006-2960/83/0422-3516$01.50/0 © 1983 American Chemical Society

xeroderma pigmentosum human fibroblasts. However, to date there has been no direct evidence that the rearrangements of chromatin structure in newly repaired regions of DNA are, in fact, "coupled" to the excision repair process. One approach to addressing this question is to examine these rearrangements of chromatin structure in cells in which the completion of "repair patches" is inhibited. There is now convincing evidence that such an inhibition occurs during very early times after UV irradiation in mammalian cells treated with millimolar concentrations of hydroxyurea (Ben-Hur & Ben-Ishai, 1971; Collins et al., 1977, 1982; Collins, 1977; Erixon & Ahnstrom, 1979; Collins & Johnson, 1981).

In a previous study, we reported that treatment of human fibroblasts with 10 mM hydroxyurea did not markedly affect the rate and extent of redistribution of nucleotides incorporated during a 30-min pulse-labeling period following UV irradiation (Smerdon et al., 1979). Although small differences were observed between hydroxyurea-treated cells and untreated cells in the distribution of these nucleotides immediately after the pulse period [see Table I and Figure 2 of Smerdon et al. (1979)], there was little or no difference in the distributions measured during the subsequent 1–25.5-h chase period. Therefore, in those studies, it was difficult to assess the significance of the differences observed immediately after the pulse-labeling period. In light of the more recent evidence demonstrating a partial inhibition of the final step of excision repair by hydroxyurea, I have examined the effects of this drug on the rate and extent of redistribution of repair-incorporated nucleotides occurring during the very early time interval of 10–45 min after irradiation and report these results here.

I have also examined the effects of sodium butyrate on the rate and extent of nucleosome rearrangements in newly repaired regions of DNA in human cells. Treatment of these cells with this short-chained fatty acid, under the conditions used here, results in a massive hyperacetylation of the core histones and a marked decrease in the levels of DNA replicative synthesis (Smerdon et al., 1982b). Furthermore, there is a 1.5–2-fold increase in repair synthesis during early times (i.e., 0–2 h) after UV irradiation (Smerdon et al., 1982b). Thus, it is of interest to examine the chromatin redistribution characteristics of nucleotides incorporated during this enhanced level of repair synthesis in cells containing hyperacetylated core histones.

Materials and Methods

Cell Culture. Human diploid fibroblasts (strain AG 1518) were grown in culture as previously described (Smerdon et al., 1982b). All cells were between passage 11 and passage 17. For nuclease digestion experiments, cells were split 1:3 and prelabeled for 1 week with 20–50 nCi/mL [^{14}C]dThd (50 mCi/mmol; New England Nuclear) or 10 nCi/mL [^{3}H]dThd (50–80 Ci/mmol; New England Nuclear), depending on the experiment. The medium was then changed with fresh medium (not containing labeled nucleotides), and the cells were incubated for 1 more week before they were irradiated and labeled during repair synthesis. For experiments measuring the suppression of DNA replicative synthesis by UV radiation, cells were split 1:4 and prelabeled for 3 days with 1 nCi/mL [^{14}C]dThd. The medium was changed, and the cells were irradiated 1 week after passage.

Irradiation and Labeling during Repair Synthesis. Prior to irradiation, some cells were treated with 20 mM sodium butyrate for 48 h (Smerdon et al., 1982b) or 2–10 mM hydroxyurea for 45 min. Cells were irradiated with 12 J/m^2 UV light (predominantly 254 nm; 2 W/m^2) in an enclosed room heated to 37 °C as previously described (Smerdon et al., 1978). Immediately after the irradiation step, [^{3}H]dThd (2–10 μCi/mL final concentration) was then added to the medium, and the cells were placed in incubators for 10–45 min (see text).

Nuclei Preparation and Nuclease Digestions. Nuclei were prepared as described in Smerdon et al. (1979). In experiments involving both ^{3}H- and ^{14}C-prelabeled cells, all of the cells were combined during the first step of the procedure, and the nuclei were coisolated. Suspended nuclei [(1.6–4.1) × 10^6/mL] were incubated at 37 °C with either staphylococcal nuclease (0.3–1.6 units/10^6 nuclei; Worthington) or DNase I (1.1 μg/10^6 nuclei; Sigma) in 10 mM Tris, pH 7.8, 0.1 mM $CaCl_2$, and 0.25 M sucrose as described previously (Smerdon et al., 1978, 1982a). For the generation of difference curves (see text) with a sufficient number of data points to accurately describe the initial and final slopes, staphylococcal nuclease was added to the digestion solution at two different times as described in Smerdon et al. (1982a). Measurement of acid-soluble radioactivity, data analysis, and agarose gel electrophoresis have been described in detail elsewhere [e.g., see Smerdon & Lieberman (1980)]. The initial and final slopes of the difference curves were determined by a linear regression analysis of the data points corresponding to values of $f(^{14}C) < 0.10$ and >0.50, respectively. Values for ξ (see text) were obtained from the difference curves by using the equation $\xi = (b_f - b_i)/(M_i - M_f)$, where b_i and b_f are the ordinate intercepts for the lines defined by the initial slope (M_i) and the final slope (M_f), respectively.

Measurement of DNA Replicative Synthesis. Cells, prelabeled with [^{14}C]dThd, were treated with 50 μM BrdUrd 90 min prior to irradiation. The cells were irradiated and labeled with 10 μCi/mL [^{3}H]dThd (in the presence of BrdUrd) as described above. The procedures for DNA preparation, isopycnic centrifugation on alkaline CsCl gradients, and determination of the radioactivity profiles have been described elsewhere (Smerdon et al., 1979, 1982b).

Results

UV Suppression of DNA Replicative Synthesis. For examination of the rate and extent of redistribution of DNA repair-incorporated nucleotides at very early times after UV irradiation, the contribution of DNA replicative synthesis to the total incorporation of labeled nucleotides must be determined. Therefore, I examined the levels of replicative synthesis in both irradiated and unirradiated (control) cells by using the BrdUrd density-shift method. Human fibroblasts were prelabeled with [^{14}C]dThd, grown to "near confluence", and labeled with [^{3}H]dThd (in the presence of BrdUrd) during repair synthesis. Figure 1 shows typical profiles for the alkaline CsCl gradients obtained from irradiated and control cells labeled for 10 min (Figure 1A,B) or 45 min (Figure 1C,D) with [^{3}H]dThd, starting immediately after irradiation in each case. As can be seen, the amount of "heavy" (newly replicated) DNA decreases following irradiation for both labeling times. From the ratio of total ^{3}H dpm in the heavy peak to the total ^{14}C dpm, the time course of DNA replicative synthesis was determined for both irradiated and control cells (Figure 2A). The data in each case give a good approximation of a straight line over this relatively short time interval. The ratio of the slopes of these lines is 0.39. Thus, over the time interval of 10–45 min after irradiation, the level of replicative synthesis is reduced to ~39% of the level found in unirradiated cells. This value for the UV suppression of replicative synthesis was used in all chromatin distribution analyses (see below).

The time course for repair synthesis can also be determined from the alkaline CsCl gradient profiles (e.g., Figure 1). The

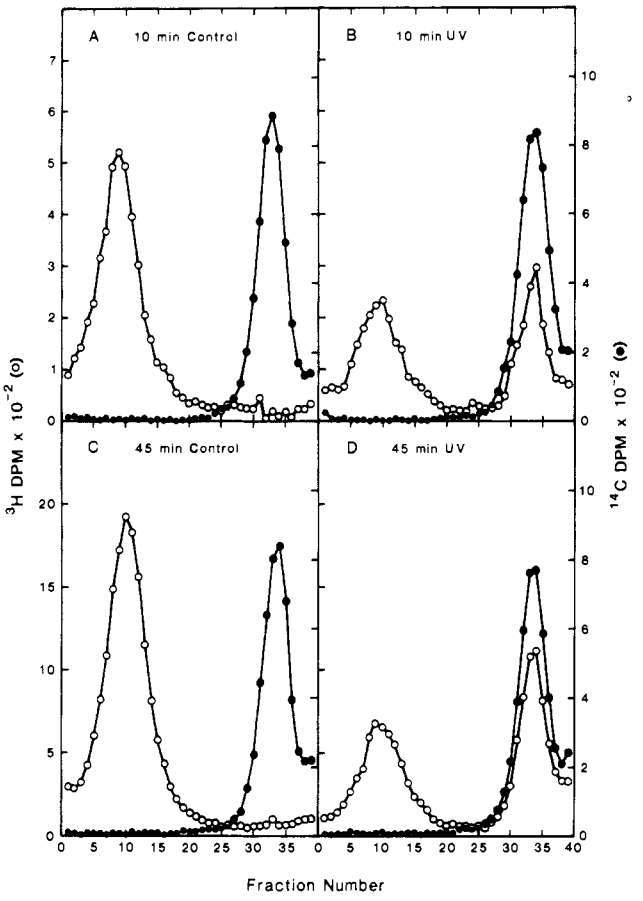

FIGURE 1: Alkaline CsCl gradient profiles of DNA from UV-irradiated and unirradiated (control) human fibroblasts. Near-confluent cells, prelabeled with [^{14}C]dThd, were irradiated (panels B and D) or mock irradiated (panels A and C) with 12 J/m^2 UV light and labeled with [^{3}H]dThd and BrdUrd for 10 (panels A and B) or 45 (panels C and D) min immediately after irradiation. The DNA was isolated and banded in alkaline CsCl gradients (Materials and Methods). The density decreases from left to right in each panel.

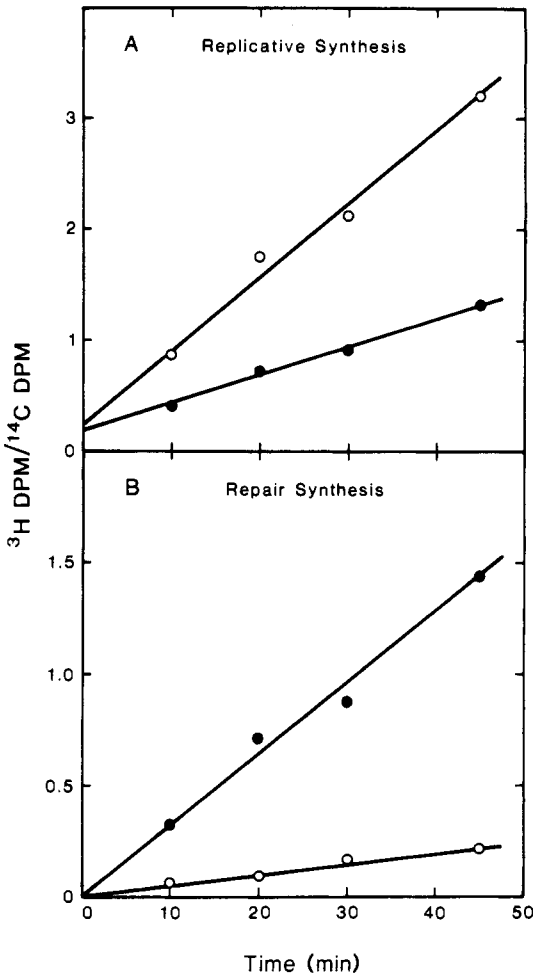

FIGURE 2: Time course of DNA replicative synthesis (A) and DNA repair synthesis (B) in UV-irradiated (●) and unirradiated (○) human fibroblasts. Cells were treated as described in the legend to Figure 1 and labeled with [^{3}H]dThd and BrdUrd for the times shown. Alkaline CsCl gradient profiles (e.g., Figure 1) were obtained for each labeling time. The levels of replicative synthesis, or repair synthesis, were determined from the ratios of the sum of the ^{3}H dpm in the heavy peak (replicative synthesis), or light peak (repair synthesis), to the sum of the ^{14}C dpm. The ^{3}H dpm/^{14}C dpm values in panel B for unirradiated cells (○) represent the background levels of ^{3}H dpm in the light peak.

ratio of the total ^{3}H dpm in the "light" peak (repaired DNA) to the total ^{14}C dpm is shown in Figure 2B. Once again, the data are linear with time, indicating that this time interval is completely within the initial rapid phase of repair synthesis [e.g., see Smerdon et al. (1978)].

Analysis of Nuclease-Sensitive DNA. The analysis of the relative nuclease sensitivity of repair-incorporated nucleotides in cells treated with sodium butyrate and hydroxyurea is dependent on the rate of digestion of the bulk DNA in nuclei from these cells. Therefore, to compare the rate of redistribution of repair-incorporated nucleotides in sodium butyrate treated cells to that of hydroxyurea-treated cells and untreated cells, it was necessary to determine the effect of sodium butyrate treatment on the kinetics of nuclease digestion of bulk DNA. Indeed, several investigators have shown that the rate of digestion of DNA to acid-soluble form by DNase I is greater in hyperacetylated chromatin (i.e., from sodium butyrate treated cells) than in chromatin from untreated cells (Nelson et al., 1978; Simpson, 1978; Vidali et al., 1978; Perry & Chalkley, 1981). A similar observation was reported for very low extents of digestion by staphylococcal nuclease (Kitzis et al., 1980). Therefore, the time course of digestion for both DNase I and staphylococcal nuclease was determined for a mixture of nuclei from sodium butyrate treated cells (prelabeled with [^{3}H]dThd) and nuclei from untreated cells (prelabeled with [^{14}C]dThd). As shown in Figure 3, neither enzyme preferentially digests the bulk DNA in nuclei from sodium butyrate treated cells to acid-soluble fragments under the conditions used in these experiments. These results are in agreement with those of Perry & Chalkley (1981), who showed that the enhanced DNase I sensitivity of bulk DNA in hyperacetylated chromatin disappeared when the salt concentration of the digestion buffer was as low as that used in the present experiments. It was previously shown that treatment with hydroxyurea (at least under the conditions used here) also does not alter the kinetics of digestion of bulk DNA in nuclei from human fibroblasts (Smerdon et al., 1978, 1979).

The relative staphylococcal nuclease sensitivity of newly repaired regions of DNA was analyzed by a method previously described by us (Smerdon et al., 1978, 1979; Smerdon & Lieberman, 1980). In using this method, one first obtains nuclease digestion data analogous to that shown in Figure 3 for nuclei from cells prelabeled with [^{14}C]dThd. Figure 4 shows examples of such data for the staphylococcal nuclease digestion of nuclei from both control (Figure 4A) and sodium butyrate treated (Figure 4B) cells labeled for 30 min with [^{3}H]dThd immediately after irradiation. It is important to note that the digestion conditions should be chosen such that many data points can be obtained for both very low (i.e., <10%

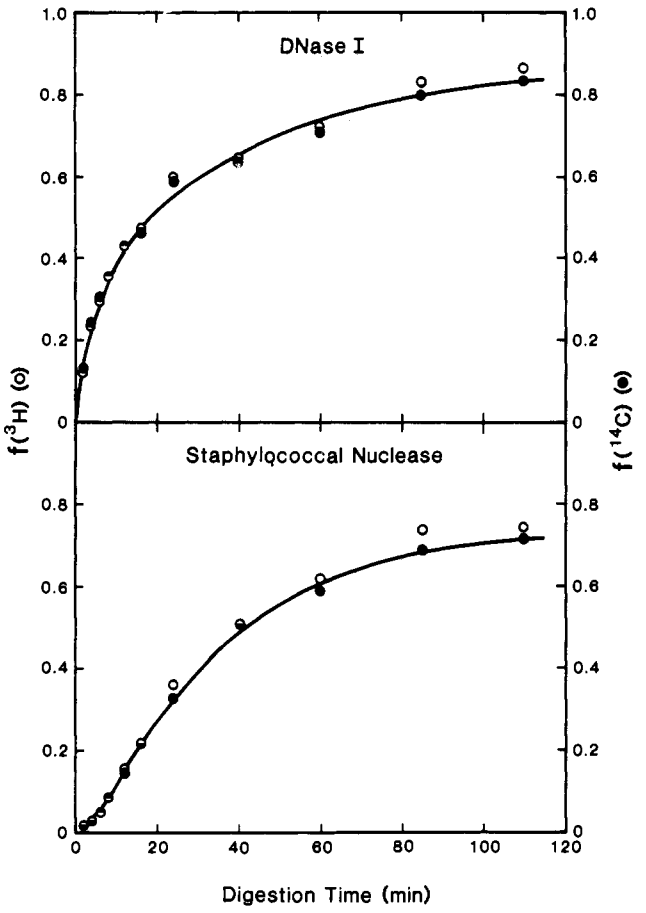

FIGURE 3: Fraction of bulk DNA in nuclei, from sodium butyrate treated or untreated cells, rendered acid soluble as a function of time of digestion with DNase I (upper panel) or staphylococcal nuclease (lower panel). Cells were either prelabeled with [^{3}H]dThd and exposed to 20 mM sodium butyrate for 48 h prior to harvest (O) or prelabeled with [^{14}C]dThd and not exposed to sodium butyrate (●). Nuclease digestions were carried out on a mixture of nuclei from each set of cells (Materials and Methods). The 100% values for ^{3}H and ^{14}C were 5386 and 5783 dpm, respectively. The fits shown are to the data for nuclei from untreated cells.

of the bulk DNA rendered acid soluble) and very high (i.e., >50% of the bulk DNA rendered acid soluble) extents of digestion. This will facilitate the accurate determination of the initial and final slopes of the difference curves described below.

The next step in this method is to plot the difference between the fractions of ^{3}H dpm and ^{14}C dpm rendered acid soluble [$f(^3H) - f(^{14}C)$] vs. the fraction of ^{14}C dpm rendered acid soluble [$f(^{14}C)$]. From these difference curves, one can obtain values for the fraction of repair-incorporated nucleotides that is nuclease sensitive (f_s) and the fraction of bulk DNA that is nuclease sensitive (ξ). The ratio f_s/ξ is determined from the initial slope (M_i) of these curves by using the following equation (Smerdon et al., 1978, 1979):

$$f_s/\xi = \frac{(M_i + 1)\sigma - 1}{\sigma - 1}$$

where the factor σ represents a correation for the amount of [^{3}H]dThd incorporated by DNA replicative synthesis and is determined from

$$\sigma = \frac{H_{100\%}/C_{100\%} \text{ for nuclei from irradiated cells}}{H_{100\%}/C_{100\%} \text{ for nuclei from control cells}}$$

where $H_{100\%}$ and $C_{100\%}$ are the total ^{3}H dpm and ^{14}C dpm in

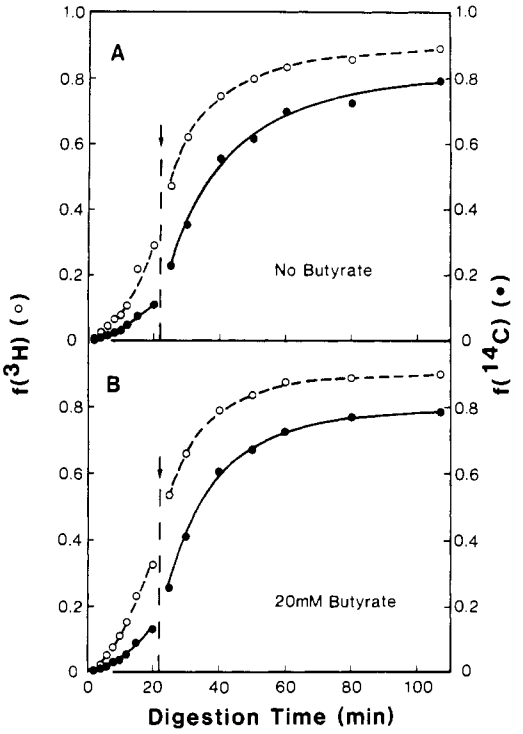

FIGURE 4: Fraction of newly repaired DNA (O) and bulk DNA (●) in nuclei, from sodium butyrate treated and untreated cells, rendered acid soluble as a function of time of digestion with staphylococcal nuclease. Confluent cells, prelabeled with [^{14}C]dThd, were treated with (B) or without (A) 20 mM sodium butyrate, irradiated with 12 J/m^2 UV light and labeled for 30 min with [^{3}H]dThd immediately after irradiation. Nuclei were suspended in digestion buffer and incubated with staphylococcal nuclease, which was added both at time zero and at t = 22 min (arrow; see Materials and Methods). The 100% values for ^{3}H were 6506 (A) and 9304 dpm (B). The 100% values for ^{14}C were 4080 (A) and 3635 dpm (B).

the nuclei in each case (Smerdon et al., 1978). As discussed in Smerdon et al. (1979), this value does not account for any suppression of DNA replicative synthesis by the DNA damaging agent. This can have a measurable effect on the value one obtains for f_s/ξ when σ is relatively small (i.e., <5). I have routinely obtained values of σ <5 for confluent cells not treated with sodium butyrate or hydroxyurea. Therefore, the σ values obtained for each set of cells in these experiments were divided by 0.39 (i.e., the UV suppression factor obtained from the BrdUrd density-shift method; see above). Finally, the value of ξ is determined by measuring the value of $f(^{14}C)$ coinciding with the intercept of the lines defined by the initial and final slopes of the difference curves (see Materials and Methods).

The difference curves obtained for the data in Figure 4 are shown in Figure 5. As can be seen, there is little difference between the curves obtained in each case. However, when cells were treated with 2–10 mM hydroxyurea in the presence or absence of sodium butyrate, a small, yet reproducible, difference in these curves was observed. Examples of the difference curves obtained for cells treated with 2 mM hydroxyurea are shown in Figure 6. These data are for cells labeled for 20 min after irradiation and are compared to the results obtained for both untreated cells (Figure 6A) and cells treated with sodium butyrate alone (Figure 6B). The difference between the curves in Figure 6A could be explained by the fact that the untreated cells have higher levels of DNA replicative synthesis than the hydroxyurea-treated cells. [The nucleotides incorporated by DNA replicative synthesis are uniformly distributed in chromatin and yield a value of zero for all values of $f(^{14}C)$ on these curves (Smerdon et al., 1978).] This con-

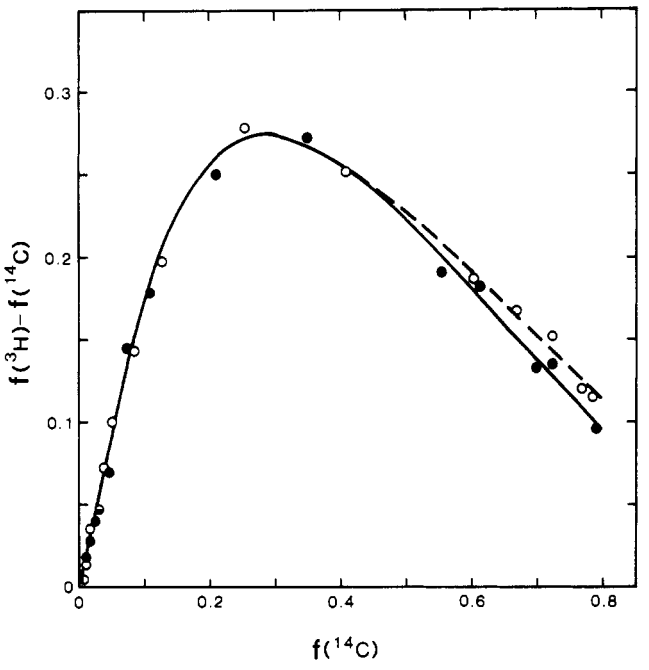

FIGURE 5: Difference curves for human fibroblasts treated with (○) or without (●) sodium butyrate. The data represent the difference between the fraction of ^3H dpm [$f(^3H)$; repaired DNA] and ^{14}C dpm [$f(^{14}C)$; bulk DNA] rendered acid soluble by staphylococcal nuclease as a function of the fraction of ^{14}C dpm rendered acid soluble for the data in Figure 4.

tribution will vary from experiment to experiment and can have little or no effect on the results (i.e., when σ is relatively large), as in Figure 5, or it can significantly reduce the differences between $f(^3H)$ and $f(^{14}C)$ (i.e., when σ is relative small). However, in sodium butyrate treated cells, the amount of DNA replicative synthesis is reduced to very low levels under these conditions (Smerdon et al., 1982b), and the data in Figure 6B can be directly compared. Thus, these data suggest that treatment of cells with 2 mM hydroxyurea in the presence or absence of 20 mM sodium butyrate results in an increase in the relative staphylococcal nuclease sensitivity of nucleotides incorporated during a 20-min repair period.

For examination of the rate of redistribution of repair-incorporated nucleotides, the ideal method is to "pulse" the cells with [^3H]dThd for a short period of time and then "chase" the cells with unlabeled dThd. However, since the repair times examined in these studies are very short, and since there is a rapid rate of repair synthesis during these times, a significant amount of [^3H]dThd is incorporated during the chase period following a 10-min pulse. Therefore, I have measured values of f_s/ξ for cells labeled *continuously* with [^3H]dThd for varying times during the 10–45-min postirradiation interval. Thus, the rate of redistribution of repair-incorporated nucleotides that is measured in these studies is *less* than the actual rate of redistribution in the intact cell, although previous studies indicate that the difference between these rates is small (Tlsty & Lieberman, 1978).

Difference curves were regenerated for each labeling time for untreated cells and cells treated with sodium butyrate or 2 mM hydroxyurea. Typical results are shown in Figure 7 for hydroxyurea-treated cells (upper panel) and sodium butyrate treated cells (lower panel) which were labeled for 10, 15, 20, 30, or 45 min after irradiation. These data represent just the initial portion of the difference curves in each case and indicate the differences in initial slopes that were observed for the two sets of cells. From data such as these, the values of f_s/ξ were determined for each labeling time following the

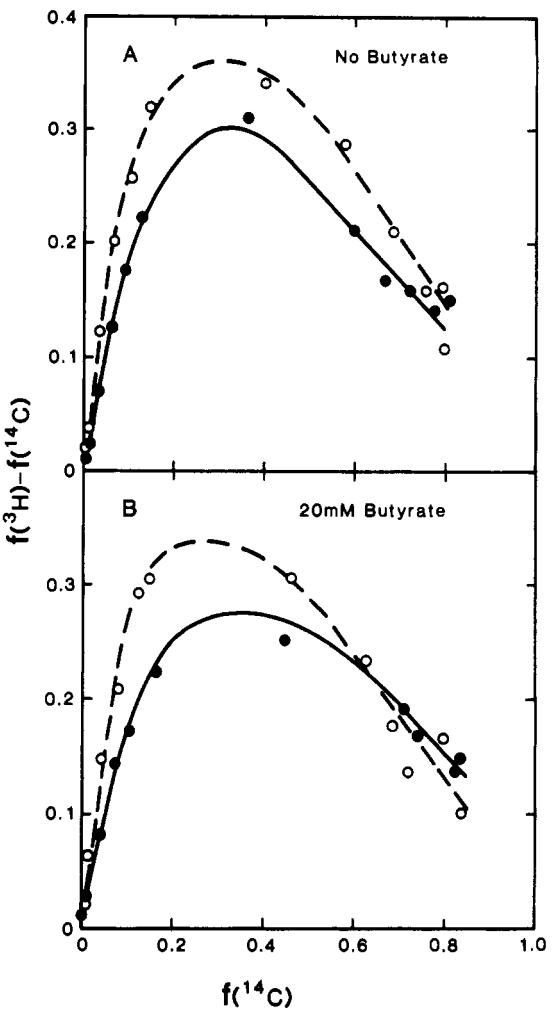

FIGURE 6: Difference curves for human fibroblasts treated with (○) or without (●) hydroxyurea. Prior to exposure to hydroxyurea, cells were treated with (B) or without (A) 20 mM sodium butyrate. Half of the untreated (no butyrate) and sodium butyrate treated cells were exposed to 2 mM hydroxyurea for 45 min prior to irradiation and during the 20-min [^3H]dThd labeling period. Staphylococcal nuclease digestions were performed as described under Materials and Methods (also see Figure 4). The 100% values for ^3H varied from 6523 dpm (for cells treated with both sodium butyrate and hydroxyurea) to 11 317 dpm (for cells treated with sodium butyrate alone). The 100% values for ^{14}C varied from 1973 to 3172 dpm.

different treatments. As shown in Figure 8A the values of f_s/ξ for the sodium butyrate treated cells are similar to those for untreated cells at each time point, whereas these values are significantly higher for the hydroxyurea-treated cells. One explanation of these results is that prior to nucleosome rearrangement the repair-incorporated nucleotides in nuclei from hydroxyurea-treated cells are digested more rapidly by staphylococcal nuclease than those in nuclei from untreated (or sodium butyrate treated) cells. This would result in a decreased value of ξ for hydroxyurea-treated cells and, hence, an increased value of f_s/ξ for these cells. Thus, a determination of ξ is required for each set of cells before the differences in Figure 8A can be assessed. The average values of ξ that were obtained from the difference curves were 0.17, 0.20, and 0.19 for hydroxyurea-treated cells, sodium butyrate treated cells, and untreated cells, respectively, with a range of ~0.01 in each case. The dashed lines in Figure 8A show extrapolations of the data to the zero time values predicted by these values of ξ, assuming that newly repaired DNA is initially *totally* nuclease sensitive (i.e., $f_s = 1$ at zero time). As can be seen from these extrapolated values, the ratio f_s/ξ is quite sensitive to

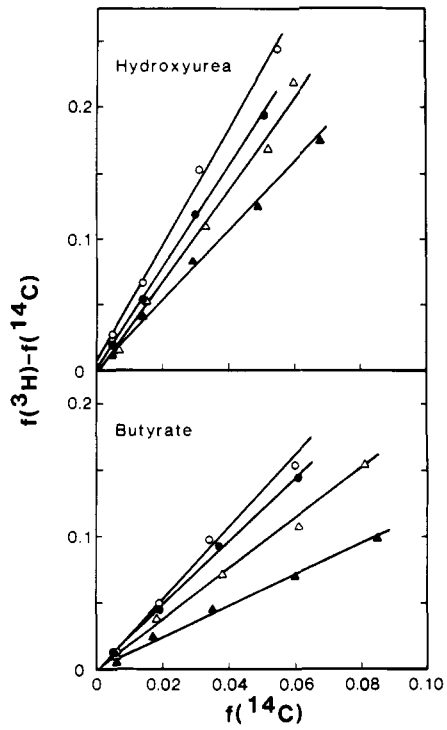

FIGURE 7: Initial slopes of difference curves for human fibroblasts treated with 2 mM hydroxyurea (upper panel) or 20 mM sodium butyrate (lower panel) following different labeling times. Confluent cells, prelabeled with [^{14}C]dThd, were treated with hydroxyurea or sodium butyrate (Materials and Methods), irradiated with 12 J/m^2 UV light at time zero, and labeled with [^{3}H]dThd immediately after irradiation for 10 (○), 15 (●), 20 (△, lower panel), 30 (△, upper panel), or 45 min (▲). Staphylococcal nuclease digestions were performed as described under Materials and Methods (also see Figure 4). The 100% values for ^{3}H varied from 7558 dpm (for hydroxyurea-treated cells; 10-min labeling time) to 44 853 dpm (for sodium butyrate treated cells; 45-min labeling time). The 100% values for ^{14}C varied from 3461 to 6114 dpm.

Table I: Measurement of the Extent of Rearrangement in Cells Treated with Sodium Butyrate or Hydroxyurea

chase time (h)	treatment[a]	^{3}H/^{14}C[b]	f_s/ξ	f_s[c]
0	HU	2.32	4.56	0.78
0	BU	4.26	2.54	0.51
0.5	HU	3.23	2.27	0.39
0.5	BU	5.51	1.68	0.34
3.5	HU	2.45	1.78	0.30
3.5	BU	4.51	1.33	0.27

[a] Cells, prelabeled with [^{14}C]dThd, were treated with either 2 mM hydroxyurea (HU) for 45 min or 20 mM sodium butyrate (BU) for 48 h prior to irradiation. Cells were labeled with [^{3}H]dThd for 30 min immediately after irradiation and chased for the times shown with conditioned medium containing 50 μM unlabeled dThd and hydroxyurea or sodium butyrate. [b] Data represent the ratio of total ^{3}H dpm to total ^{14}C dpm in the nuclei in each case. This ratio was 0.09 for hydroxyurea-treated cells and 0.04 for sodium butyrate treated cells when the irradiation step was omitted. [c] Calculated by using a value for ξ of 0.17 for hydroxyurea-treated cells and 0.20 for sodium butyrate treated cells (see text).

even the small changes in ξ that were measured for each set of cells.

The values for the fraction of repair-incorporated nucleotides that is staphylococcal nuclease sensitive (f_s), determined from the data in Figure 8A and the values of ξ given above, are shown in Figure 8B. These values are, at least in theory, corrected for any differences that exist between the average rates of digestion of newly repaired DNA (prior to nucleosome

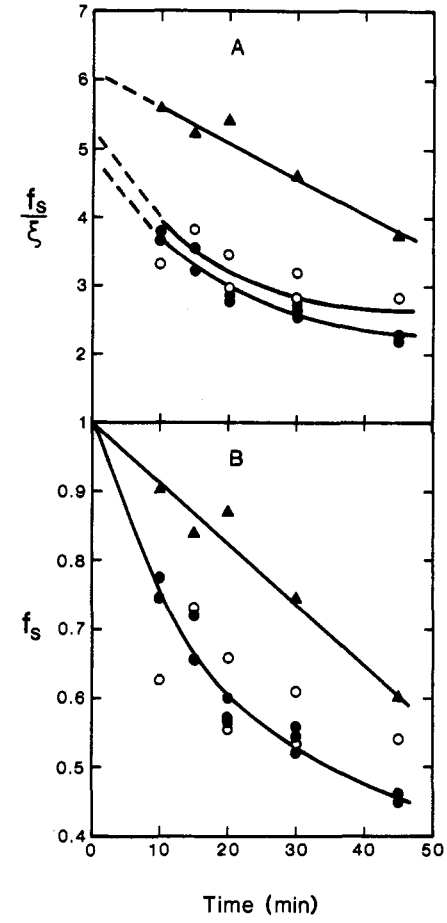

FIGURE 8: (A) Relative staphylococcal nuclease sensitivity of repair-incorporated nucleotides as a function of labeling time for untreated cells (○) and cells treated with 2 mM hydroxyurea (▲) or 20 mM sodium butyrate (●). Each set of confluent cells was treated as described under Materials and Methods (also see Figures 4 and 7). Cells were irridiated at time zero. Ordinate values represent the ratio of the fraction of repair-incorporated nucleotides that is staphylococcal nuclease sensitive (f_s) to the fraction of bulk DNA that is staphylococcal nuclease sensitive (ξ) determined from the initial slope of the difference curves obtained in each case (see text). Separate data points at one labeling time for a given set of cells represent values for different experiments. Dashed lines represent extrapolations of the data to the zero time value predicted by the measured values of ξ in each case (see text). (B) Fraction of repair-incorporated nucleotides that is staphylococcal nuclease sensitive as a function of labeling time. Data in panel A were multiplied by the corresponding value of ξ in each case. Symbols are the same as those in panel A.

rearrangement) in the nuclei from each set of cells. These data indicate that the rate of redistribution of repair-incorporated nucleotides in both sodium butyrate treated and untreated cells is initially (i.e., immediately after irradiation) ~2.5 times greater than that in cells treated with 2 mM hydroxyurea. Similar results were obtained for cells treated with 10 mM hydroxyurea (data not shown). (It is noted that, for untreated cells, there is more error in these measurements due to the higher background of replicative synthesis in these cells, as well as the variatioin in this background level.)

In a previous study, we showed that the extent of redistribution of repair-incorporated nucleotides in hydroxyurea-treated cells was the same as that in untreated cells (Smerdon et al., 1979). In the present study, I have examined the effect of sodium butyrate on the extent of this redistribution process. Table I shows the results for sodium butyrate treated cells pulse labeled for 30 min after irradiation and chased with unlabeled dThd for 0, 0.5, and 3.5 h. For comparison, I have included the results for cells treated with 2 mM hydroxyurea. As can

be seen, although the values of f_s for the two sets of cells are different immediately after the pulse period, these values are similar following the 3.5-h chase. Therefore, treatment with either sodium butyrate or hydroxyurea (at least under the conditions used here) does not alter the overall extent of randomization of repair-incorporated nucleotides involved in the redistribution process. [It is noted that the higher values of $^3H/^{14}C$ observed in the sodium butyrate treated cells (Table I) are the result of increased levels of repair synthesis during early times after irradiation in these cells (Smerdon et al., 1982b).]

Analysis of Nucleosome Core DNA. The analysis of nuclease-sensitive DNA examines the level of repair-incorporated nucleotides in acid-soluble DNA fragments released from nuclei by nuclease digestion. In using this analysis, one assumes a "two-state model" of chromatin structure when determining the fraction of repair-incorporated nucleotides that is nuclease sensitive (f_s) (Smerdon et al., 1978); the method does not differentiate between different "nuclease-sensitive states" that may exist and the potential redistribution between these states prior to attaining the final "nuclease-resistant state". Thus, it is important to complement such studies with an analysis of nuclease-resistant DNA. To this end, I have examined the levels of repair-incorporated nucleotides in nucleosome core DNA.

Staphylococcal nuclease digestion products from cells, prelabeled with [^{14}C]dThd and labeled during repair synthesis with [^{3}H]dThd as described earlier, were separated on agarose gels. The core DNA region of these gels was cut out, and the ^3H dpm and ^{14}C dpm profiles were determined. Examples are shown in Figure 9 for cells labeled for 30 min after irradiation. The data indicate that the levels of ^3H dpm in the core DNA of hydroxyurea-treated cells are considerably less than those in sodium butyrate treated cells or untreated cells. However, one cannot compare these data directly since they have not been normalized to the total ^3H dpm incorporated in each case. Therefore, the ratio of the total ^3H dpm to ^{14}C dpm in the core DNA region (called R_{core}) was determined from profiles such as those in Figure 9 and divided by the $H_{100\%}/C_{100\%}$ values for the corresponding nuclei (called R_{nuclei}). This ratio (i.e., R_{core}/R_{nuclei}) has been previously denoted as RS(core) (Tlsty & Lierberman, 1978). The values of RS(core) as a function of labeling time for each set of cells are shown in Figure 10A. As can be seen, over the time interval shown, the data for cells treated with 10 mM hydroxyurea are considerably lower than those for untreated cells; cells treated with sodium butyrate yielded values that are intermediate to those for the hydroxyurea-treated and untreated cells.

In the past, we have not corrected the values of RS(core) for contributions due to DNA replicative synthesis [e.g., see Smerdon & Lieberman (1978)]. However, such as correction is important in these studies, since only a small fraction of repair-incorporated nucleotides is initially located in nuclease-resistant DNA (Figure 8B) and, therefore, the contribution of nucleotides incorporated by DNA replicative synthesis is much greater in the DNA of nucleosome cores. Therefore, a "corrected" RS(core) was obtained by using the equation

$$\text{RS(core)} = \frac{R_{core} - 0.39R_{control}}{R_{nuclei} - 0.39R_{control}}$$

where $R_{control}$ is the $H_{100\%}/C_{100\%}$ value for nuclei from unirradiated cells labeled for the same time period as the irradiated cells. The constant 0.39 is the UV suppression factor described earlier. The results of this correction analysis are shown in

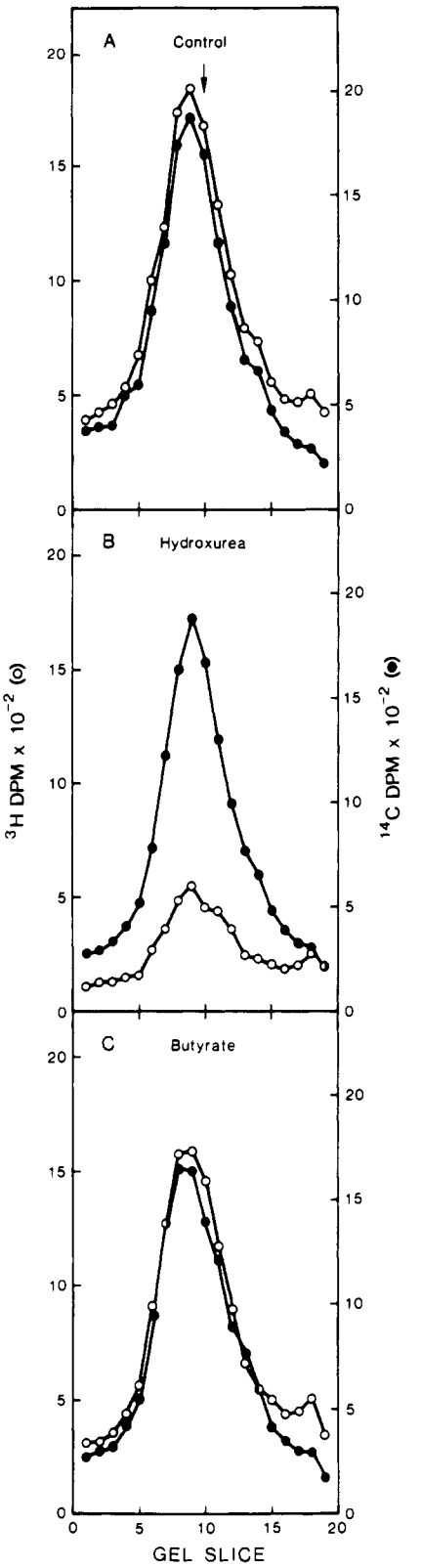

FIGURE 9: Electrophoretic profiles of nucleosome core DNA from untreated cells (A) and cells treated with 10 mM hydroxyurea (B) or 20 mM sodium butyrate (C). Confluent cells, prelabeled with [^{14}C]dThd, were treated with hydroxyurea or sodium butyrate as described under Materials and Methods and labeled for 30 min with [^{3}H]dThd following irradiation. Staphylococcal nuclease digestions of nuclei were carried out as in Figure 4 except that only one incubation time was used. The DNA was isolated and electrophoresed on 2.8% agarose gels. The fraction of total ^{14}C dpm rendered acid soluble was 0.38, 0.39, and 0.42 for panels A, B, and C, respectively. The arrow in panel A shows the position of the middle of the bromophenol blue marker dye which migrates at ~135 bp on these gels (Smerdon & Lieberman, 1980). Migration was from left to right.

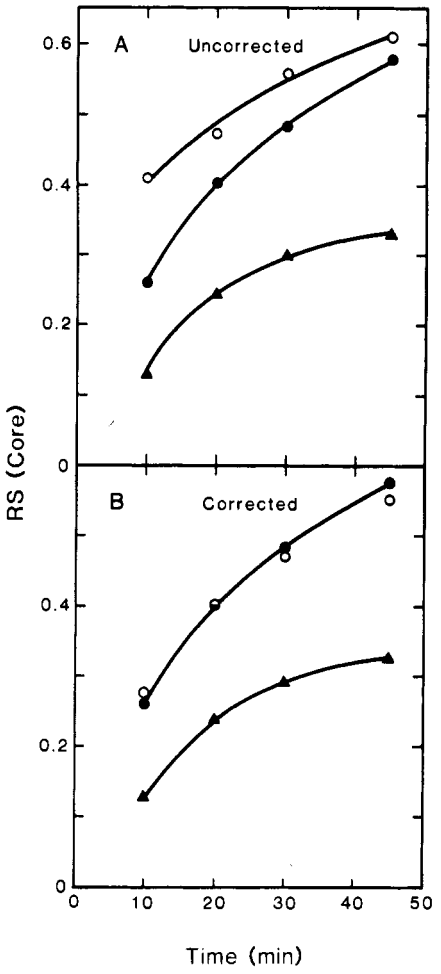

FIGURE 10: Fraction of repair-incorporated nucleotides in nucleosome core DNA as a function of labeling time for untreated cells (○) and cells treated with 10 mM hydroxyurea (▲) or 20 mM sodium butyrate (●). Values of RS(core) were determined from the electrophoretic profiles of nucleosome core DNA (e.g., Figure 9) where the range of the fraction of ^{14}C dpm rendered acid soluble by staphylococcal nuclease was 0.37–0.42, 0.34–0.39, and 0.36–0.42 for the untreated cells, hydroxyurea-treated cells, and sodium butyrate treated cells, respectively. Values were either not corrected (A) or corrected (B) for the contribution of replicative synthesis to the total ^{3}H dpm incorporated (see text for details).

Figure 10B. It is clear that the difference observed in Figure 10A between the data for sodium butyrate treated cells and untreated cells is due entirely to differences in the levels of replicative synthesis in these cells. On the other hand, these values are ~2-fold higher at each labeling time than those for the cells treated with 10 mM hydroxyurea. Thus, the rate of formation of nucleosome core structures in newly repaired regions of DNA decreased by ~50% in the cells treated with 10 mM hydroxyurea. A decrease of ~40% was obtained for cells treated with 2 mM hydroxyurea (data not shown). These results are in general agreement with those obtained from the analysis of nuclease-sensitive DNA.

Discussion

In this report, I have examined the effects of sodium butyrate and hydroxyurea on the rate and extent of redistribution of repair-incorporated nucleotides during very early times after UV irradiation in confluent, normal human fibroblasts. The following methods were used: (1) the analysis of staphylococcal nuclease sensitive DNA in nuclei (i.e., DNA that is rapidly digested to acid-soluble form); and (2) the analysis of nucleosome core DNA (i.e., 140–160-bp DNA protected from rapid digestion by staphylococcal nuclease). When these two methods were used, special care was taken to account for the contribution of DNA replicative synthesis to the total nucleotides incorporated during the repair labeling period. Correction of the data for this contribution was especially important for analyses involving untreated cells. When this correction was made, the results from each analysis were in good agreement with each other.

The results from each of these methods indicate that treatment of confluent human fibroblasts with 20 mM sodium butyrate for 48 h prior to UV irradiation has little or no effect on the rate of redistribution of repair-incorporated nucleotides during the time interval of 10–45 min immediately after irradiation. Furthermore, the extent of redistribution (i.e., the final amount of randomization of repair-incorporated nucleotides) is also unaffected by sodium butyrate treatment. Recently, we have reported that treatment of confluent human fibroblasts with sodium butyrate, under the conditions used here, results in (1) a marked decrease in DNA replicative synthesis, (2) a 1.5–2-fold stimulation in DNA repair synthesis during early times (i.e., 0–2 h) after UV irradiation, and (3) a maximal hyperacetylation of the core histones, where ~58% of the total histone H4 contains three or four acetyl residues per molecule (Smerdon et al., 1982b). The results of the present study indicate that the enhanced level of repair synthesis occurring at early times after irradiation in sodium butyrate treated cells is followed by rearrangements of chromatin structure that are similar to those in untreated cells. Furthermore, these results suggest that hyperacetylation of many of the core histones prior to the onset of excision repair has little or no effect on the rate at which these rearrangements occur. It has been proposed that the acetylation of core histones alters the interaction between these proteins and DNA [e.g., see McGhee & Felsenfeld (1980)]. If this is the case, however, the results obtained here suggest that such changes in histone–DNA interactions in a large fraction of the total chromatin prior to repair in human cells do not alter the rate or extent of structural rearrangements that occur in newly repaired regions of the genome.

The results obtained for hydroxyurea-treated cells differed from those obtained for cells treated with sodium butyrate. A 1.7–2.5-fold decrease in the rate of redistribution of repair-incorporated nucleotides was observed during the 10–45-min postirradiation time interval in confluent cells treated with 2 mM hydroxyurea. A 2–2.5-fold decrease in this rate was observed in cells treated with 10 mM hydroxyurea. Therefore, hydroxyurea concentrations at least as low as 2 mM yield a near-maximal inhibition of the rate of redistribution of repair-incorporated nucleotides. It was shown previously that treatment of confluent cells with 10 mM hydroxyurea has no effect on the extent of redistribution (Smerdon et al., 1979).

These results allow further insight into the process of nucleosome rearrangement in newly repaired regions of DNA. A number of investigators have now shown that millimolar concentrations of hydroxyurea increase the "persistence" of repair-induced DNA strand breaks (i.e., following incision) in mammalian cells during early times after UV irradiation (Ben-Hur & Ben-Ishai, 1971; Collins et al., 1977; Collins, 1977; Erixon & Ahnstrom, 1979; Collins & Johnson, 1981). The most likely explanation for these results is that in hydroxyurea-treated cells there is a decrease in the rate of final ligation of repair patches, whereas there is no change in the rate of incision. Since hydroxyurea is known to block the activity of ribonucleotide reductase (Reichard, 1972), it has been proposed that the decreased rate of ligation in hydroxyurea-treated cells is actually the result of partial inhibition

of repair synthesis due to a reduction in nucleotide pool concentrations [see Collins & Johnson (1981) for a detailed discussion of this topic]. Indeed, Collins et al. (1982) have recently reported that 10 mM hydroxyurea partially inhibits the incorporation of nucleotides during repair synthesis following UV irradiation in "quiescent", normal human fibroblasts. Therefore, it is reasonable to assume that any events that are "coupled" to the excision repair process, and occur after the repair synthesis step, will be partially inhibited by hydroxyurea. The results of the present study suggest that such a coupling exists between nucleosome rearrangement and the completion of the repair synthesis step. Additional evidence for this proposal was recently reported by Cleaver (1982). Using normal human fibroblasts exposed to 20 μM cytosine arabinoside (ara C) and pulse labeled immediately after UV irradiation, Cleaver observed a reduction in the rate at which the ratio of repair-incorporated nucleotides and bulk DNA rendered acid soluble by staphylococcal nuclease decreased during a subsequent chase period. Thus, the decreased rate of nucleosome rearrangement in newly repaired DNA observed in the present report appears to be a general feature of the partial inhibition of repair synthesis rather than some specific alteration of the chromatin structure in these regions by hydroxyurea.

It is important to note that the results of the present study raise the possibility that nucleosome rearrangement may occur very rapidly after the completion of a repair patch and that the rate-limiting step for the rearrangement process is the final ligation event. This concept is somewhat different from the way in which we have viewed this process in the past where it was thought that following the completion of the excision repair steps the newly repaired DNA remained in an "unrearranged state" for some time prior to the rearrangement that eventually occurs. This new possibility would require that many of the repair-incorporated nucleotides in nuclease-sensitive DNA are associated with *incomplete* repair patches. These repair patches, which would contain single-strand nicks or gaps in the DNA, may also have increased sensitivity to exogenous nucleases. Indeed, there is a small decrease in ξ (i.e., the fraction of bulk DNA that is nuclease sensitive) in hydroxyurea-treated cells (see text and Figure 8), even though there is no change in the rate of digestion of bulk DNA in these cells (Smerdon et al., 1978, 1979). Therefore, this change is most likely due to an increase in the overall staphylococcal nuclease sensitivity of repair-incorporated nucleotides prior to nucleosome rearrangement. Such an increase in nuclease sensitivity could be the result of a larger fraction of incomplete repair patches in cells treated with hydroxyurea. However, this increased nuclease sensitivity must involve more than simply an increase in the number of nicks or gaps in the DNA since these regions are not preferentially digested when naked DNA is used as a substrate (Smerdon et al., 1978).

Finally, the use of sodium butyrate as an effective inhibitor of DNA replicative synthesis in DNA repair studies deserves comment. To date, all other agents that effectively inhibit DNA replicative synthesis, either by interacting directly with the polymerase enzymes (e.g., ara C) or by interacting with enzymes involved in the production of DNA precursors (e.g., hydroxyurea), have been shown to partially inhibit DNA repair [see Collins & Johnson (1981)]. On the other hand, sodium butyrate, which reduces the levels of replicative synthesis by "arresting" cells in the G_1 stage of the cell cycle and blocking their entrance into S phase (D'Anna et al., 1980; Darzynkiewicz et al., 1981; Littlefield et al., 1982), enhances DNA repair synthesis during early times after UV irradiation (see above). This enhancement is short-lived, however, and both the total level of repair synthesis and the fraction of UV-induced endonuclease-sensitive sites removed at times $\geq$4 h are similar in cells with or without sodium butyrate treatment (Smerdon et al., 1982b). The nature of this enhancement of repair synthesis is currently under debate. Williams & Friedberg (1982) reported that in *growing* cultures of human fibroblasts treated with 10 mM sodium butyrate the enhanced repair synthesis that they observed correlated with an increased specific activity of labeled dThd in the nucleotide pools of these cells, whereas we observed that in *confluent* human fibroblasts treated with 20 mM sodium butyrate the enhanced repair synthesis during the first few hours after UV irradiation correlated with a stimulation in the rate of removal of UV-induced endonuclease-sensitive sites (Smerdon et al., 1982b). However, although the nature of the short-lived stimulation of repair synthesis by sodium butyrate remains to be clarified, the studies to date indicate that this agent does not *inhibit* DNA repair in UV-irradiated cells at concentrations where DNA replicative synthesis is reduced to background levels. The present study indicates that this is the case even for the final event associated with UV-induced excision repair, (i.e., rearrangements of chromatin structure in the newly repaired regions of DNA). Although from the discussion thus far one would think that sodium butyrate is an ideal agent to use in repair studies to selectively enhance the fraction of nucleotides incorporated by repair synthesis relative to replicative synthesis, the following considerations should be kept in mind. First of all, sodium butyrate treatment results in the "modulation" of expression of certain genes in mammalian cells (Reeves & Cserjesi, 1979), and changes in the expression of genes associated with DNA repair could alter the results of studies of the repair process. Second, as alluded to earlier, the hyperacetylation of histones in the chromatin of sodium butyrate treated cells could have a marked influence on specific steps in all repair pathways and/or specific steps in a given repair pathway. Thus, although sodium butyrate treatment does not appear to inhibit nucleotide excision repair in UV-irradiated, normal human fibroblasts, it may inhibit (or enhance) the repair of other DNA-damaging agents as well as have differing effects on different cell types. Clearly, caution should be used in utilizing this short-chained fatty acid in repair studies until further investigations can be made concerning its affects on repair processes in general.

Acknowledgments

I thank Suey Lan for her excellent technical assistance, Holly Schumacher for her help in the preparation of the manuscript, and Drs. Brinda Ramanathan and Raymond Reeves for their critical reviews of the manuscript.

Registry No. Sodium butyrate, 156-54-7; hydroxyurea, 127-07-1.

References

Ben-Hur, E., & Ben-Ishai, R. (1971) *Photochem. Photobiol. 13*, 337–345.

Bodell, W. J., & Cleaver, J. E. (1981) *Nucleic Acids Res. 9*, 203–213.

Cleaver, J. E. (1982) *Am. J. Hum. Genet. 34*, 566–575.

Collins, A. R. S. (1977) *Biochim. Biophys. Acta 478*, 461–473.

Collins, A. R. S., & Johnson, R. T. (1981) in *DNA Repair: A Laboratory Manual of Research Procedures* (Friedberg, E. C., & Hanawalt, P. C., Eds.) Vol. 1B, pp 341–360, Marcel Dekker, New York.

Collins, A. R. S., Schor, S. L., & Johnson, R. T. (1977) *Mutat. Res. 42*, 413–432.

Collins, A. R. S., Squires, S., & Johnson, R. T. (1982) *Nucleic Acids Res. 10*, 1203-1213.
D'Anna, J. A., Tobey, R. A., & Gurley, L. R. (1980) *Biochemistry 19*, 2656-2671.
Darzynkiewicz, Z., Traganos, F., Xue, S.-B., & Melamed, M. R. (1981) *Exp. Cell Res. 136*, 279-293.
Erixon, K., & Ahnstrom, G. (1979) *Mutat. Res. 59*, 257-271.
Jack, P. L., & Brookes, P. (1982) *Carcinogenesis (London) 3*, 341-344.
Kaneko, M., & Cerutti, P. A. (1980) *Cancer Res. 40*, 4313-4319.
Kitzis, A., Tichonicky, L., Defer, N., & Kruh, J. (1980) *Biochem. Biophys. Res. Commun. 93*, 833-841.
Lieberman, M. W., Smerdon, M. J., Tlsty, T. D., & Oleson, F. B. (1979) in *Environmental Carcinogenesis* (Emmelot, P., & Kriek, E., Eds.) pp 345-363, Elsevier/North-Holland Biomedical Press, Amsterdam.
Littlefield, B. A., Cidlowski, N. B., & Cidlowski, J. A. (1982) *Exp. Cell Res. 141*, 283-291.
McGhee, J. D., & Felsenfeld, G. (1980) *Annu. Rev. Biochem. 49*, 1115-1156.
Nelson, D. A., Perry, M., Sealy, L., & Chalkley, R. (1978) *Biochem. Biophys. Res. Commun. 82*, 1346-1353.
Oleson, F. B., Mitchell, B. L., Dipple, A., & Lieberman, M. W. (1979) *Nucleic Acids Res. 7*, 1343-1361.
Perry, M., & Chalkley, R. (1981) *J. Biol. Chem. 256*, 3313-3318.
Reeves, R., & Cserjesi, P. (1979) *J. Biol. Chem. 254*, 4283-4290.
Reichard, P. (1972) *Adv. Enzyme Regul. 10*, 3-16.
Simpson, R. (1978) *Cell (Cambridge, Mass.) 13*, 691-699.
Smerdon, M. J., & Lieberman, M. W. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4238-4241.
Smerdon, M. J., & Lieberman, M. W. (1980) *Biochemistry 19*, 2992-3000.
Smerdon, M. J., Tlsty, T. D., & Lieberman, M. W. (1978) *Biochemistry 17*, 2377-2386.
Smerdon, M. J., Kastan, M. B., & Lieberman, M. W. (1979) *Biochemistry 18*, 3732-3739.
Smerdon, M. J., Watkins, J. F., & Lieberman, M. W. (1982a) *Biochemistry 21*, 3879-3885.
Smerdon, M. J., Lan, S. Y., Calza, R. E., & Reeves, R. (1982b) *J. Biol. Chem. 257*, 13441-13447.
Tlsty, T. D., & Lieberman, M. W. (1978) *Nucleic Acids Res. 5*, 3261-3273.
Vidali, G., Boffa, L. C., Bradbury, E. M., & Allfrey, V. G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2239-2243.
Williams, J. I., & Friedberg, E. C. (1979) *Biochemistry 18*, 3965-3972.
Williams, J. I., & Friedberg, E. C. (1982) *Photochem. Photobiol. 36*, 423-427.
Zolan, M. E., Smith, C. A., Calvin, N. M., & Hanawalt, P. C. (1982) *Nature (London) 299*, 462-464.

recA Filaments in Solution†

Susan M. Cotterill and Alan R. Fersht*

ABSTRACT: recA protein has been shown previously by electron microscopy to form long filaments. The extent of oligomerization in solution can be followed by light scattering since the filaments scatter light far more strongly than the constituent units. Filament formation is highly sensitive to the presence of various nucleotides and ions: ATP, ADP, CTP, UTP, TTP, and GTP all disrupt the filaments at concentrations of 1 mM while AMP at the same concentration has no effect. The presence of Na^+, K^+, Ca^{2+}, and Mg^{2+} at higher concentrations also causes filament disruption but in a highly cooperative manner with the midpoints for the transitions at 17, 18, 50, and 30 mM, respectively. The disruption is extremely fast; stopped-flow measurements indicate a half-life of <1 ms on the addition of 40 mM NaCl, 1.5 ms for 1 mM ATP, and 7.5 ms for 1 mM ADP. The rate constant for formation of filaments increases with increasing protein concentration. Cross-linking experiments on the protein at pH 8.1 indicate the presence of an oligomer of at least a hexamer. This degree of association persists on the addition of nucleotides and salts at concentrations that disrupt the larger filaments.

The recA protein of *Escherichia coli* is a relatively small protein, M_r 37 800 (Horii et al., 1980; Sancar et al., 1980), which catalyzes a wide range of activities. It has a central role in the "SOS" response to cell damage (Radman, 1975; Witkin, 1976) and is also vital for recombination and post-replicative DNA repair (Clark, 1973; Radding, 1978).

The cloning of the recA gene has facilitated the production of large amounts of pure protein and has led to rapid progress in characterizing its activities in vitro (McEntee & Epstein, 1977; Sancar & Rupp, 1979; Ogawa et al., 1979). It has been shown to be a single-stranded (ss) and double-stranded (ds) DNA-dependent ATPase (Ogawa et al., 1979; Roberts et al., 1979; Weinstock et al., 1979; McEntee et al., 1979a,b; Shibata et al., 1979a,b; West et al., 1980) and to catalyze D-loop formation, branch migration, and strand exchange in the presence of a nucleotide (Cassuto et al., 1981; Cox & Lehman, 1981; DasGupta et al., 1981; West et al., 1981). It has also been shown to be a polynucleotide- and ATP-dependent protease (Roberts et al., 1979; Craig & Roberts, 1980), cleaving the repressors lex, λ, and p22 at a specific Ala–Gly bond (Pabo et al., 1979; Horii et al., 1981; Sauer et al., 1981).

Much of the recent research on the recA protein has centered on the nature of the complexes formed on the binding of the protein to DNA. Electron microscopy has revealed long chains of recA molecules forming smooth filaments with

† From the Department of Chemistry, Imperial College of Science and Technology, London SW7 2AY, U.K. *Received December 30, 1982.* This work was supported by SERC Grant GR/A94249. S.M.C. is the recipient of an SERC studentship.

0006-2960/83/0422-3525$01.50/0 © 1983 American Chemical Society