Karn, J., Brenner, S., Barnett, L., & Cesareni, G. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5172–5176.
Lunberg, N., & Gilbert, W. (1985) *Cell (Cambridge, Mass.) 40*, 81–91.
MacGillivray, R. T. A., & Davie, E. W. (1984) *Biochemistry 23*, 1626–1634.
Magnusson, S., Peterson, T. E., Sottrup-Jensen, L., & Claeys, H. (1975) in *Proteases and Biological Control* (Reich, E., Rifkin, D. B., & Shaw, E., Eds.) pp 123–149, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
Maniatis, T., Jeffrey, A., & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1184–1188.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning. A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.
McMullen, B., & Fujikawa, K. (1984) *Circulation 70*, II–351.
Messing, J. (1983) *Methods Enzymol. 101*, 20–78.
Neurath, H. (1984) *Science (Washington, D.C.) 224*, 350–357.
Neurath, H., & Walsh, K. A. (1976) in *Proteolysis and Physiological Regulation* (Ribbons, D. W., & Brew, K., Eds.) pp 29–40, Academic Press, New York.
Olafson, R. W., Jurasek, L., Carpenter, M. R., & Smillie, L. B. (1975) *Biochemistry 14*, 1168–1177.
Olson, M. O. J., Nagabhushan, N., Dzwiniel, M., Smillie, L. B., & Whitaker, D. R. (1970) *Nature (London) 228*, 438–442.
Owen, W. G., Esmon, C. T., & Jackson, C. M. (1974) *J. Biol. Chem. 249*, 594–605.
Pennica, D., Holmes, W. F., Kohr, W. J., Harkins, R. N., Vehar, G. A., Ward, C. A., Bennet, W. F., Yelverton, E., Seeburg, P. H., Heyneker, H. L., Goeddel, D. V., & Collen, D. (1983) *Nature (London) 301*, 214–221.
Perry, R. P. (1976) *Annu. Rev. Biochem. 45*, 605–629.
Sanger, F., Nicklen, S., & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5467.
Sottrup-Jensen, L., Claeys, H., Zajdel, M., Petersen, T. E., & Magnusson, S. (1978) in *Progress in Chemical Fibrinolysis and Thrombolysis* (Davidson, J. F., Rowan, R. M., Samana, M. M., & Desnoyers, P. C., Eds.) Vol. 3, pp 191–209, Raven Press, New York.
Southern, E. M. (1975) *J. Mol. Biol. 98*, 503–517.
Stenflo, J., & Suttie, J. W. (1977) *Annu. Rev. Biochem. 46*, 157–172.
Sternberg, N., Tiemeier, D., & Enquist, L. (1977) *Gene 1*, 255–280.
Swift, G. H., Craik, C. S., Stary, S. J., Quinto, C., Lahaie, R. G., Rutter, W. J., & McDonald, R. J. (1984) *J. Biol. Chem. 259*, 14271–14278.
Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5201–5205.
Verde, P., Stoppelli, M. P., Galeffi, P., Di Nocera, P., & Blasi, F. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4727–4731.
Vieira, J., & Messing, J. (1982) *Gene 19*, 259–268.
Walz, D. A., Hewett-Emmett, D., & Seegers, W. H. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1969–1972.
Watanabe, Y., Tsukada, T., Notake, M., Nakanishi, S., & Numa, S. (1982) *Nucleic Acids Res. 10*, 1459–1469.
Wieringa, N., Hofer, E., & Weissmann, C. (1984) *Cell (Cambridge, Mass.) 37*, 915–925.
Woo, S. L. C. (1980) *Methods Enzymol. 68*, 389–395.

Stimulation of Deoxyribonucleic Acid Excision Repair in Human Fibroblasts Pretreated with Sodium Butyrate†

Steven L. Dresler
Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110
Received April 3, 1985

ABSTRACT: The effect of pretreatment with sodium butyrate on DNA excision repair was studied in intact and permeable confluent (i.e., growth-inhibited) diploid human fibroblasts. Exposure to 20 mM sodium butyrate for 48 h increased subsequent ultraviolet (UV)-induced [*methyl*-^{3}H]thymidine incorporation by intact AG1518 fibroblasts by 1.8-fold and by intact IMR-90 fibroblasts by 1.2–1.3-fold. UV-induced incorporation of deoxy[5-^{3}H]cytidine, deoxy[6-^{3}H]cytidine, and deoxy[6-^{3}H]uridine, however, showed lesser degrees of either stimulation or inhibition in butyrate-pretreated cells. This result suggested that measurements of butyrate's effect on DNA repair synthesis in intact cells are confounded by simultaneous changes in nucleotide metabolism. The effect of butyrate on excision repair was also studied in permeable human fibroblasts in which excision repair is dependent on exogenous nucleotides. Butyrate pretreatment stimulated UV-induced repair synthesis by 1.3–1.7-fold in permeable AG1518 cells and by 1.5–2-fold in permeable IMR-90 cells. This stimulation of repair synthesis was not due to changes in repair patch size or composition or in the efficiency of DNA damage production but rather resulted from a butyrate-induced increase in the rate of damage-specific incision of DNA. The increased rate of incision in butyrate-pretreated cells could be due either to increased levels of enzymes mediating steps in excision repair at or before incision or to alterations in chromatin structure making damage sites in DNA more accessible to repair enzymes.

Chromatin structure has a significant influence on mammalian DNA excision repair, as it does on other cellular processes which involve nuclear DNA [see, for instance, DePamphilis & Wassarman (1980) and Mathis et al. (1980)]. Transient changes in chromatin structure at the nucleosome level, which render newly synthesized repair patches highly susceptible to digestion by exogenous nucleases, are a consistent feature of excision repair of DNA damage in mammalian cells (Smerdon & Lieberman, 1978, 1980; Tlsty & Lieberman,

† Supported by Grant IN-36 from the American Cancer Society, by Grant CA 20513 from the National Institutes of Health, and by Brown and Williamson Tobacco Corp., Phillip Morris Inc., and the U.S. Tobacco Co.

0006-2960/85/0424-6861$01.50/0

1978; Oleson et al., 1979; Williams & Friedberg, 1979; Bodell & Cleaver, 1981; Zolan et al., 1982b; Sidik & Smerdon, 1984). It has been suggested that these perturbations of nucleosome structure are necessary for repair enzymes to gain access to DNA damage sites in chromatin (Williams & Friedberg, 1979). Supranucleosomal chromatin structure also affects excision repair. For example, furocoumarin adducts in α-satellite DNA of African green monkey cells are repaired more slowly than furocoumarin adducts in total cellular DNA (Zolan et al., 1982a, 1984). Also, during the repair of ultraviolet (UV)[1] damage in human fibroblasts, repair patches are nonrandomly distributed in the genome, possibly due to preferential DNA repair in certain domains of chromatin (Cohn & Lieberman, 1984). Recent data suggest that actively transcribed DNA sequences may be repaired more rapidly than total DNA (Mayne, 1984; Bohr et al., 1985).

One way to analyze the relation between chromatin structure and excision repair is to study the effects on repair of agents which are known to alter the structure of chromatin. One such agent is sodium butyrate, which inhibits histone deacetylase (Boffa et al., 1978; Candido et al., 1978; Sealy & Chalkley, 1978), leading to hyperacetylation of nuclear histones. The effect of sodium butyrate pretreatment on UV-induced DNA excision repair has been investigated in intact human fibroblasts. Smerdon et al. (1982) found that pretreatment with sodium butyrate stimulated the initial rates of both damage-induced [^{3}H]dThd incorporation and removal of UV endonuclease sensitive sites in confluent cells. Williams & Friedberg (1982), using growing cells, also found that treatment with butyrate stimulated damage-induced [^{3}H]dThd incorporation, but they attributed the stimulation to decreases in cellular TTP pools in butyrate-treated cells. Because of this controversy, I have studied the effects of sodium butyrate on UV-induced DNA excision repair both in intact confluent (i.e., growth-inhibited) human fibroblasts and in permeable fibroblasts in which excision repair is dependent on exogenous nucleotides (Dresler et al., 1982; Dresler, 1984). My data indicate that pretreatment with sodium butyrate does produce changes in cellular nucleotide metabolism but that it also stimulates excision repair of UV damage by increasing the rate of damage-specific incision of DNA.

Experimental Procedures

Cell Culture. Human diploid fibroblasts (AG1518 and IMR-90; Institute for Medical Research) were passed into plastic culture dishes or glass roller bottles, prelabeled with either [*methyl*-^{14}C]dThd (Amersham Corp.; 50–60 mCi/mmol) or [*methyl*-^{3}H]dThd (Amersham Corp.; 40–50 Ci/mmol), and grown to confluence as described (Dresler et al., 1982). RAJI cells (ATCC CCL 86) were grown as suspension cultures and prelabeled with [*methyl*-^{3}H]dThd as described (Dresler & Lieberman, 1983b).

Pretreatment with Sodium Butyrate. A stock solution of sodium butyrate was prepared by titration of 1 M butyric acid (Sigma) with NaOH to pH 7. Where indicated, sodium butyrate (20 mM) was added to the medium of fibroblast cultures 48 h prior to analysis of DNA excision repair.

Measurement of UV-Induced Repair Synthesis in Intact Fibroblasts Using BrdUrd Density Shift. Confluent fibroblasts, grown in plastic culture dishes, and, where indicated, pretreated with sodium butyrate as described above, were incubated with 50 μM BrdUrd for 60 min prior to UV irradiation. Medium was then removed, and the cells were exposed to radiation from a G15T8 germicidal lamp (which emits primarily at 254 nm) at a flux of 0.5 or 1.0 W/m^2. UV flux was measured with an International Light IL770A radiometer and a 254-nm probe. The medium, containing BrdUrd and sodium butyrate, as indicated, was supplemented with 50 μCi/mL [*methyl*-^{3}H]dThd and returned to the cells, which were incubated for 60 min at 37 °C. DNA was then isolated and analyzed by alkaline CsCl density gradient centrifugation, as described (Dresler & Lieberman, 1983a). Gradients were fractionated, the A_{260} of each fraction was measured, and the radioactivity of a 0.15-mL portion of each fraction was determined after addition of 1 mL of 25 mM HCl and 4 mL of RIA-Solve II (Research Products International Corp.). The DNA concentration of each fraction was calculated by using an extinction coefficient of 26.7 mL/(mg·cm). Repair synthesis was taken to be the difference between specific incorporation (^{3}H cpm per microgram of DNA) in the parental DNA peaks of corresponding damaged and undamaged samples.

Measurement of UV-Induced DNA Repair Synthesis in Intact Fibroblasts Using High-Performance Liquid Chromatography. Human fibroblasts were grown to a high degree of confluence (46 days in culture with a single medium change at day 9). Some culture plates were then treated with sodium butyrate as described above. For measurement of repair synthesis, medium was removed, and the cells were irradiated with UV radiation as described above. The medium, supplemented with 5 μCi/mL [*methyl*-^{3}H]dThd, 5 μCi/mL [5-^{3}H]dCyd (Moravek Biochemicals; 25 Ci/mmol), 5 μCi/mL [6-^{3}H]dCyd (Moravek Biochemicals; 6 Ci/mmol), or 5 μCi/mL [6-^{3}H]dUrd (Amersham Corp., 17.5 Ci/mmol), was replaced, and the cells were incubated for 60 min at 37 °C. DNA was isolated and digested with DNase I, nuclease P1, and alkaline phosphatase, and the nucleosides were separated by high-performance liquid chromatography, as described (Kastan et al., 1982). The A_{280} of the eluate was monitored continuously. The eluate was fractionated, and radioactivity was determined as described (Kastan et al., 1982). For each sample, the dCyd absorbance peak was cut out and weighed, and the weight was converted to the mass of dCyd present by comparison with standard chromatograms. The mass of dCyd was converted to the corresponding mass of DNA, assuming a molar abundance for dCyd in human DNA of 0.2 (Chargaff & Lipshitz, 1953) and a weighted mean mononucleotide residue mass of 309, and the specific radioactivities (^{3}H cpm per microgram of DNA) of the dCyd and dThd peaks were calculated.

Measurement of UV-Induced DNA Repair Synthesis in Permeable Fibroblasts. Confluent fibroblasts, prelabeled with [^{14}C]dThd and pretreated with sodium butyrate where indicated, were incubated for 60 min with 50 μM BrdUrd, washed, collected, and made permeable as described (Dresler et al., 1982). Where indicated, permeable cells were washed twice with the permeabilization solution prior to UV irradiation. For cells pretreated with sodium butyrate, the washing solution contained 5 mM sodium butyrate, and the permeabilization solution contained 7.5 mM sodium butyrate. Permeable cells were damaged in suspension at 4 °C with UV radiation from a G15T8 lamp at a flux of 3–4 W/m^2 (Dresler et al., 1982). Portions of permeable cell suspension (0.1–0.2 mL), either damaged or undamaged, were mixed at 4 °C with 0.5 volume of concentrated reaction mix to give the following final con-

[1] Abbreviations: UV, ultraviolet; dThd, thymidine; BrdUrd, 5-bromo-2′-deoxyuridine; dCyd, 2′-deoxycytidine; dUrd, 2′-deoxyuridine; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; BrdUTP, 5-bromo-2′-deoxyuridine 5′-triphosphate; ESS, UV endonuclease sensitive site(s); HMG, high-mobility group.

centrations: 40 mM Tris (pH 7.6 at 37 °C), 8 mM $MgCl_2$, 15 mM KCl, 5 mM ATP, 3 μM BrdUTP, 3 μM dCTP, 3 μM dATP, 3 μM dGTP, 40 μCi/mL [α-^{32}P]dCTP (Amersham Corp., 410 Ci/mmol), 167 mM sucrose, 2 mM dithiothreitol, and 0.67 mM EDTA. In samples containing butyrate-pretreated cells, the final sodium butyrate concentration was 5 mM. Samples were incubated at 37 °C for the time indicated and washed 3 times with 10 mM Tris (pH 7.6 at 37 °C), 1 mM $CaCl_2$, 250 mM sucrose, and 0.5% Triton X-100, and DNA was isolated and analyzed by alkaline CsCl density gradient centrifugation (Dresler & Lieberman, 1983a). Gradients were fractionated, and the radioactivity in each fraction was determined as described above. The $^{32}P/^{14}C$ ratio of the DNA of parental density was converted to specific dCMP incorporation (femtomoles of dCMP per microgram of DNA) as described (Dresler et al., 1982). Repair synthesis was taken to be the difference between specific incorporation in corresponding damaged and undamaged samples.

Measurement of Repair Patch Size. Permeable fibroblasts, prelabeled with [^{14}C]dThd, were irradiated with 100 J/m^2 UV radiation and incubated with the reaction mixture for 15 min at 37 °C, and DNA was isolated as described previously. Following addition of 200 μg of calf thymus DNA as carrier, each sample was precipitated with ethanol, dissolved in 1 mL of 10 mM Tris (pH 7.6 at 37 °C) and 1 mM EDTA, placed in a 12 × 75 mm plastic tube in an ice–H_2O bath, and subjected to 10 30-s bursts from a Branson 200 sonicator using a microtip and a power setting of 4. Each burst was followed by a 30-s pause, and the fifth burst was followed by a 5-min pause during which the sample was mixed well. A portion of each sample was precipitated with ethanol, dissolved in CsCl solution (ρ = 1.777 g/cm^3) containing 0.1 M KOH, and centrifuged at 50 000 rpm for 18 h at 20 °C in a Sorvall TV865 rotor. The gradients were fractionated, and radioactivity was determined as described previously. An identical gradient, centrifuged with the experimental samples but not containing DNA, was fractionated, and the refractive index of every second fraction was determined with a Bausch and Lomb 334610 refractometer and converted to density by using a standard formula (Vinograd & Hearst, 1962). To determine the sizes of the sheared fragments, another portion of each sample was precipitated with ethanol, dissolved in 98% formamide, placed in a boiling water bath for 2 min, and analyzed by electrophoresis on a 5% polyacrylamide gel containing 98% formamide (Maniatis et al., 1975). The gel was cut into 2-mm slices which were dissolved in H_2O_2–NH_4OH (Goodman & Matzura, 1971), and radioactivity was determined as described (Smerdon & Lieberman, 1980). Sizes of the sheared fragments were determined by comparison with the *Hae*III restriction fragments of φX174 and pBR322, which were run on the same gel and visualized by staining with ethidium bromide, and the number-average molecular weight of each sample was calculated (Dean et al., 1969).

To provide a density standard, permeable growth-phase cells were incubated for 15 min at 37 °C with a reaction mixture modified to contain 75 mM KCl, 5 μM [α-^{32}P]dCTP, 50 μM BrdUTP, 50 μM dATP, and 50 μM dGTP. These conditions are well suited to replicative synthesis (Dresler, 1984). DNA was isolated as described above, sheared with four 30-s bursts from the Branson 200 sonicator, ethanol-precipitated, and dissolved in CsCl solution (ρ = 1.82 g/cm^3) containing 0.1 M KOH. The sample was centrifuged, the gradient was fractionated, and the radioactivity was determined as described previously. An identical gradient was fractionated, and the refractive indexes of the fractions were measured and used to generate a density profile, as described previously.

Measurement of UV-Induced DNA Damage in Permeable Fibroblasts. UV-induced damage was measured by using the UV endonuclease sensitive site assay (Carrier & Setlow, 1970; van Zeeland et al., 1981; Ganesan et al., 1981; Paterson et al., 1981). Confluent fibroblasts, either prelabeled with 20 nCi/mL [^{3}H]dThd and pretreated with sodium butyrate as indicated or prelabeled with 20 nCi/mL [^{14}C]dThd and not pretreated with butyrate, were collected, made permeable, and washed twice with permeabilization solution as described above. Permeable cells were damaged in suspension at 4 °C with UV radiation at a flux of 3 W/m^2 (Dresler et al., 1982). For each UV dose, butyrate-treated (^{3}H-labeled) and control (^{14}C-labeled) cells were combined and treated to increase their permeability as described (van Zeeland et al., 1981; Ganesan et al., 1981), including exposure to a high concentration of NaCl. Portions of each sample were incubated with and without addition of a crude preparation of *Micrococcus luteus* UV endonuclease (Carrier & Setlow, 1970; Paterson et al., 1981) at 37 °C for 60 min. (This incubation time was shown in preliminary experiments to yield maximal incision of UV-damaged DNA; data not shown.) A 0.1-mL portion of each incubation mixture was added to 0.1 mL of 1 N NaOH and 10 mM EDTA which previously had been layered over a 5–20% sucrose gradient containing 0.3 N NaOH, 2 M NaCl, and 20 mM EDTA. The samples were allowed to remain in this lysis layer for 6 h at 20 °C and then were subjected to centrifugation for 16 h at 20 °C in Beckman SW 50.1 rotors. All samples incubated without UV endonuclease and the unirradiated sample incubated with UV endonuclease were centrifuged at 11 500 rpm in a Beckman L3-50 ultracentrifuge. UV-irradiated samples incubated with UV endonuclease were centrifuged at 19 000 rpm in a Beckman L5-75 ultracentrifuge. The gradients were fractionated, and the radioactivity of each fraction (~0.15 mL) was determined after addition of 1 mL of 0.1 N HCl and 3 mL of RIA-Solve. The mean molecular weight of each fraction was calculated by using equations developed by R. B. Setlow and co-workers (personal communication), and number-average molecular weights for each sample were determined by using the method of Dean et al. (1969) as elaborated by Brash & Hart (1983). The single-strand break frequency was calculated from the reciprocal of the number-average molecular weight.

Measurement of Damage-Specific Incision Using Alkaline Elution. Confluent fibroblasts, prelabeled with [^{14}C]dThd and pretreated with sodium butyrate as indicated, were collected, made permeable, and washed twice with permeabilization solution as described above. Portions of the cell suspension containing (1.0–1.5) × 10^6 cells, either unirradiated or irradiated with the indicated dose of UV, were mixed with 0.5 volume of a reaction mixture to give the following final concentrations: 40 mM Tris (pH 7.6 at 37 °C), 15 mM KCl, 8 mM $MgCl_2$, and 5 mM ATP. No deoxyribonucleoside triphosphates were added. The samples were incubated at 37 °C for 10 min and suspended in 5 mL of ice-cold 2.7 mM NaH_2PO_4, 13.1 mM Na_2HPO_4, 135 mM NaCl, and 4.9 mM KCl. About 5 × 10^5 RAJI cells, prelabeled with [^{3}H]dThd and irradiated with 350 rad of X-ray, were added, and the samples were analyzed by alkaline elution as described (Kohn et al., 1976; Dresler & Lieberman, 1983b).

Results

Effect of Butyrate Pretreatment on UV-Induced Repair Synthesis in Intact Human Fibroblasts. Control and butyrate-pretreated AG1518 human fibroblasts, unirradiated and irradiated with 12 J/m^2 UV radiation, were incubated with

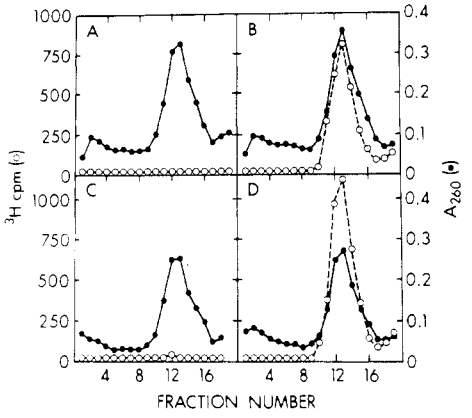

FIGURE 1: UV-induced incorporation of [^{3}H]dThd into DNA of intact control and butyrate-pretreated AG1518 fibroblasts. Confluent cells, control (A and B) or butyrate pretreated (C and D), were incubated for 60 min with 50 μM BrdUrd, irradiated with 0 J/m^2 (A and C) or 12 J/m^2 (B and D) UV radiation, and incubated for 60 min with 50 μM BrdUrd and 50 μCi/mL [^{3}H]dThd. DNA was isolated and analyzed by isopycnic centrifugation in alkaline CsCl. Gradients were fractionated, and A_{260} (●) and ^{3}H radioactivity (○) were measured. Density increases from right to left. These data were obtained in the same experiment as the data in Table I.

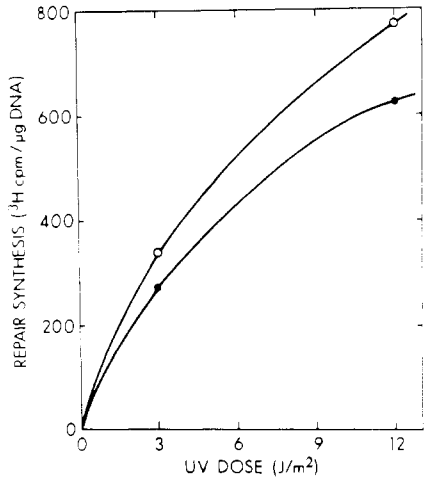

FIGURE 2: UV-induced repair synthesis in intact control (●) and butyrate-pretreated (○) IMR-90 fibroblasts. Confluent cells were incubated for 60 min with 50 μM BrdUrd, irradiated with the indicated dose of UV radiation, and incubated for 60 min with 50 μM BrdUrd and 50 μCi/mL [^{3}H]dThd. DNA was isolated and analyzed by isopycnic centrifugation in alkaline CsCl. Repair synthesis was determined as described under Experimental Procedures. These data were obtained in the same experiment as the data in Figure 4B.

[^{3}H]dThd and BrdUrd, and DNA was isolated and analyzed by isopycnic centrifugation in alkaline CsCl (Figure 1). Consistent with previous reports (Smerdon et al., 1982; Smerdon, 1983), butyrate pretreatment stimulated repair synthesis (determined as [^{3}H]dThd incorporation into DNA of parental density) by about 1.8-fold. Butyrate pretreatment also stimulated UV-induced [^{3}H]dThd incorporation in intact IMR-90 human fibroblasts, but the magnitude of the effect was smaller (1.2–1.3-fold; Figure 2).

To explore the possibility that the changes in UV-induced [^{3}H]dThd incorporation induced by butyrate pretreatment might be merely a result of changes in cellular nucleotide metabolism [as suggested by Williams & Friedberg (1982)], the effect of butyrate on UV-induced incorporation was studied by using several different exogenous labeled nucleosides (Table I). This study was performed simultaneously with that shown in Figure 1, using cells from the same passage group. As seen in Figure 1, unirradiated cells, whether butyrate pretreated

Table I: Incorporation of Exogenous Labeled Nucleosides during UV-Induced DNA Repair Synthesis in Intact Fibroblasts[a]

	incorporation (cpm/μg of DNA)			
	control cells		butyrate-pretreated cells	
labeled nucleoside	dCyd peak	dThd peak	dCyd peak	dThd peak
[*methyl*-^{3}H]dThd	0	4777	0	9377 (2.0)[b]
[5-^{3}H]dCyd	1986	0	2246 (1.1)	0
[6-^{3}H]dCyd	279	349	338 (1.2)	199 (0.6)
[6-^{3}H]dUrd	0	1328	0	240 (0.2)

[a] Confluent AG1518 cells, control or butyrate pretreated, were irradiated with 12 J/m^2 UV radiation and incubated for 60 min with the indicated labeled nucleosides at 5 μCi/mL. DNA was isolated and digested enzymatically to produce nucleosides which were separated by high-performance liquid chromatography (Kastan et al., 1982). The A_{280} of the column eluate was recorded continuously, and the area of the dCyd peak was used to calculate the mass of DNA represented by each sample. The eluate was also fractionated, radioactivity was determined, and specific incorporation into the dCyd and dThd peaks was calculated for each sample. These data were obtained in the same experiment as the data in Figure 1. [b] Numbers in parentheses indicate the ratio of incorporation in butyrate-pretreated cells to incorporation in control cells.

or not, incorporated essentially no labeled nucleoside, and incorporation in UV-irradiated cells was confined to the parental density DNA peak. This permitted the UV-induced incorporation data in Table I to be obtained simply by measuring total nucleoside incorporation in irradiated cells without using BrdUrd, avoiding artifactual changes in nucleotide metabolism which might be produced by high levels of exogenous nucleoside. For this study, AG1518 fibroblasts, control and butyrate pretreated, were irradiated with UV radiation and incubated with the indicated labeled nucleosides, following which DNA was isolated and digested enzymatically to yield nucleosides which were analyzed by high-performance liquid chromatography (Table I). With [*methyl*-^{3}H]dThd as the labeled nucleoside, butyrate-pretreated cells showed a 2-fold stimulation of UV-induced incorporation, consistent with the data in Figure 1. When [5-^{3}H]dCyd was used, however, only a 1.1-fold stimulation of UV-induced incorporation was seen. When the labeled nucleoside was [6-^{3}H]dUrd (which is incorporated into DNA as dThd), UV-induced incorporation in butyrate-pretreated cells was reduced to one-fifth the level seen in control cells. When labeled with [6-^{3}H]dCyd (which results in ^{3}H incorporation into DNA both as dCyd and as dThd), butyrate-pretreated cells showed a slight stimulation (1.2-fold) in UV-induced ^{3}H incorporation into dCyd but a decrease (to 0.6 times the control level) in ^{3}H incorporation into dThd. These data indicate that butyrate pretreatment produces significant changes in the DNA precursor metabolism of confluent fibroblasts. As a result, changes in the incorporation of exogenous labeled nucleosides into the DNA of intact cells may not be reliable indicators of the effects of butyrate pretreatment on DNA excision repair.

Effect of Butyrate Pretreatment on UV-Induced Repair Synthesis in Permeable Human Fibroblasts. The effects of altered cellular nucleotide pools on measurement of excision repair can be avoided by using a permeable cell excision repair system in which repair synthesis is dependent on exogenous nucleotides supplied in the reaction mixture (Dresler et al., 1982; Dresler, 1984). Confluent AG1518 fibroblasts, pretreated with sodium butyrate and then made permeable, irradiated with 100 J/m^2 UV radiation, and incubated with a reaction mixture permitting DNA excision repair, showed a 1.3–1.5-fold stimulation in the initial rate of repair synthesis as compared with control cells (Figure 3). This result indicates that the stimulation of repair synthesis produced by

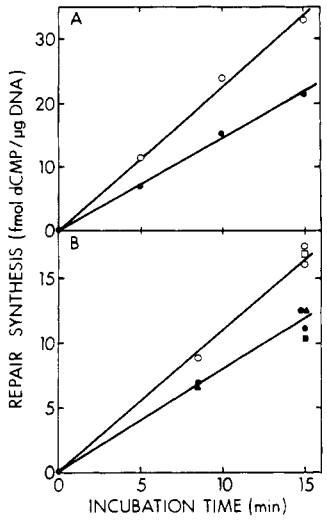

FIGURE 3: UV-induced repair synthesis in permeable control and butyrate-pretreated AG1518 fibroblasts. Confluent cells, control (●, ■, ▲) or butyrate pretreated (○, □), were made permeable, irradiated with 100 J/m^2 UV radiation, and incubated with repair synthesis reaction mixture for the indicated time. Repair synthesis was determined as described under Experimental Procedures. Some permeable cells (■, □) were washed twice with permeabilization solution prior to irradiation. Some permeable cells not pretreated with butyrate were incubated in repair synthesis reaction mixture containing 5 mM sodium butyrate (▲). The data in panels A and B are from two different experiments.

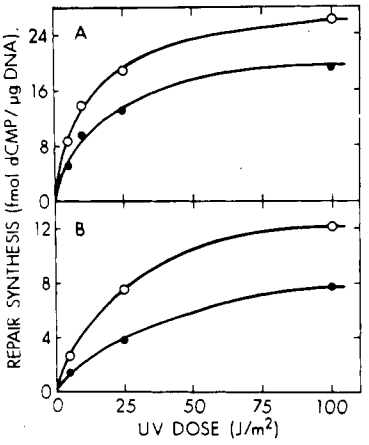

FIGURE 4: Repair synthesis in permeable control and butyrate-pretreated fibroblasts after irradiation with various doses of UV radiation. Confluent AG1518 (A) and IMR-90 (B) cells, control (●) and butyrate pretreated (○), were made permeable, irradiated with the indicated dose of UV radiation, and incubated for 15 min with repair synthesis reaction mixture, and repair synthesis was determined as described under Experimental Procedures. The data in Figure 4B were obtained in the same experiment as the data in Figure 2.

pretreatment with sodium butyrate is not primarily due to an alteration in nucleotide metabolism. This conclusion is strengthened by the fact that washing of the permeable cells prior to the repair synthesis incubation, using a protocol which has been shown to reduce endogenous nucleotide pools to negligible levels (Dresler & Lieberman, 1983b; Dresler, 1984), did not alter the butyrate-induced stimulation of repair synthesis (Figure 3B). Addition of sodium butyrate to the reaction mixture of cells not pretreated with butyrate did not alter the level of repair synthesis (Figure 3B), indicating that butyrate does not directly affect the repair process but alters the cells during the pretreatment period in a way which subsequently leads to changes in excision repair.

As seen in Figure 4A, butyrate pretreatment produced a similar stimulation of repair synthesis (1.4–1.7-fold) in

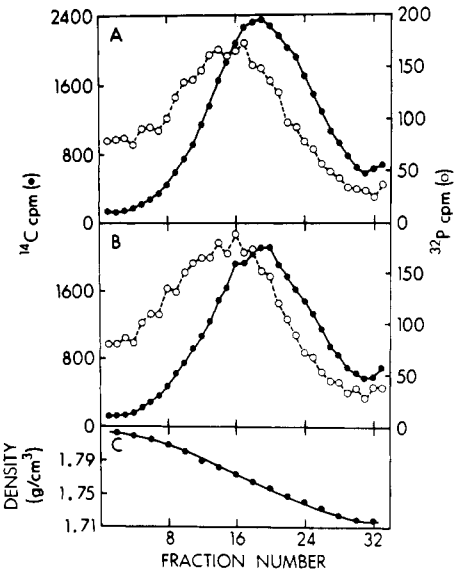

FIGURE 5: Alkaline CsCl density gradient centrifugation of sheared repair patch containing DNA from permeable control (A) and butyrate-pretreated (B) AG1518 fibroblasts. Confluent cells, prelabeled with [^{14}C]dThd, were made permeable, irradiated with 100 J/m^2 UV radiation, and incubated with repair synthesis reaction mixture containing [α-^{32}P]dCTP and BrdUTP. DNA was isolated, sheared by sonication to number-average molecular weights of 125 bases (control) and 115 bases (butyrate pretreated), and centrifuged to equilibrium in alkaline CsCl. Gradients were fractionated, and ^{14}C (●) and ^{32}P (○) radioactivities were determined. Centers of mass (in terms of fraction number) were as follows: control ^{14}C, 19.4, ^{32}P, 15.1; butyrate pretreated ^{14}C, 19.2, ^{32}P, 14.8. Panel C shows the density profile of a gradient containing no DNA which was centrifuged along with the experimental samples.

permeable AG1518 cells at all doses of UV radiation examined, from 5 to 100 J/m^2. Butyrate pretreatment also led to stimulation of repair synthesis in permeable IMR-90 fibroblasts at all UV doses studied (Figure 4B). The degree of butyrate-induced stimulation of repair synthesis seen in permeable IMR-90 cells (1.5–2-fold; Figure 4B) was much higher than the stimulation of UV-induced [^{3}H]dThd incorporation seen in intact IMR-90 cells of the same passage group analyzed in the same experiment (1.2–1.3-fold; Figure 2). This result suggests that, in intact IMR-90 cells, changes in nucleotide metabolism may partially mask the stimulation of repair synthesis induced by sodium butyrate, even when the labeled nucleoside used is [^{3}H]dThd.

Characterization of Repair Patches Synthesized in Butyrate-Pretreated Permeable Cells. Increased incorporation of specific nucleotides into repair patches could result from increased repair patch size or from alterations in repair patch composition and might not reflect an increase in the number of damage sites repaired. Mean repair patch size was determined for butyrate-pretreated and control cells by using the BrdUrd density shift technique (Edenberg & Hanawalt, 1972). Permeable cells, prelabeled with [^{14}C]dThd, were damaged with UV radiation and incubated with repair synthesis reaction mixture containing [^{32}P]dCTP and BrdUTP, and DNA was isolated. Analysis of this high molecular weight DNA by isopycnic centrifugation in alkaline CsCl revealed that in both control and butyrate-pretreated cells, all ^{32}P incorporation was due to repair synthesis (data not shown). Thus, without further purification, the remainder of each DNA sample was sheared by sonication. The single-strand densities of these sheared DNA samples were determined by isopycnic centrifugation in alkaline CsCl (Figure 5). For both control and butyrate-pretreated samples, the centers of mass of sheared DNA fragments containing repair-incorporated nucleotides

(^{32}P labeled) were 0.024 g/cm^3 greater than the centers of mass of the parental DNA fragments (^{14}C labeled). In this density gradient system, fully BrdUMP-substituted DNA (produced by semiconservative replication in permeable growing cells) is shifted to a density 0.12 g/cm^3 greater than that of unsubstituted, parental DNA (data not shown). Thus, for both control and butyrate-treated samples, the fractional BrdUMP substitution in DNA fragments containing repair patches was 0.2. The number-average lengths of the sheared fragments, determined by polyacrylamide gel electrophoresis in the presence of 98% formamide, were 125 and 115 bases for the control and butyrate-pretreated samples, respectively (data not shown). The estimated patch sizes were thus 25 bases for permeable control cells and 23 bases for permeable butyrate-pretreated cells. These sizes are not significantly different and are very similar to the UV-induced repair patch size (~30 bases) found in intact human fibroblasts using the density shift technique (Smith, 1978).

Repair patch compositions were compared by incubating UV-irradiated permeable control and butyrate-pretreated cells with a reaction mixture containing [^{32}P]dCTP at 9.2 Ci/mmol and [^3H]TTP at 13.3 Ci/mmol. DNA was isolated and purified by isopycnic centrifugation in neutral CsCl. (Parallel assays in which TTP was replaced by BrdUTP and DNA was analyzed on alkaline CsCl density gradients showed that incorporation into UV-irradiated control and butyrate-pretreated cells was entirely due to repair synthesis; data not shown.) The ratios of ^3H to ^{32}P incorporation in the two purified DNA samples were identical, 0.36. Taking into account the counting efficiencies of ^3H (0.18) and ^{32}P (0.86) and the specific activities of the two labeled compounds, the molar incorporation ratio (TMP/dCMP) was calculated to be 1.2. This ratio is lower than the molar ratio of TMP to dCMP in total human DNA (~1.5; Chargaff & Lipshitz, 1953), suggesting that UV-induced repair patches are not randomly distributed in the genome. Nonrandom distribution of UV-induced repair patches has also been demonstrated in intact human fibroblasts (Cohn & Lieberman, 1984).

Effect of Butyrate Pretreatment on Efficiency of DNA Damage Production by UV Radiation. The data presented above indicate that pretreatment of confluent fibroblasts with sodium butyrate leads to the synthesis of an increased number of repair patches following a given dose of UV radiation. This phenomenon could result from an increased efficiency of UV photoproduct formation in butyrate-treated cells. To assess this possibility, permeable AG1518 cells, control and butyrate pretreated, were irradiated with UV radiation, and the production of pyrimidine dimers, the principal form of UV photoproduct, was measured by the UV endonuclease sensitive site assay. This technique relies on the ability of *M. luteus* UV endonuclease to specifically cleave DNA at sites of pyrimidine dimers (see Experimental Procedures for details). As shown in Table II, butyrate pretreatment did not alter the efficiency of production of endonuclease-sensitive sites at either 25 or 100 J/m^2 UV radiation.

Effect of Butyrate Pretreatment on Damage-Specific Incision in Permeable Fibroblasts. The data presented above lead to the conclusion that pretreatment with sodium butyrate increases the number of repair patches synthesized per unit time in response to a given amount of DNA damage. This result suggests that the incision of DNA at sites of damage, the earliest known step in the excision repair process, may also proceed at an increased rate following butyrate pretreatment. Rates of damage-specific incision in permeable cells can be measured by using the technique of alkaline elution, which

Table II: Measurement of UV-Induced DNA Damage in Permeable Fibroblasts by the UV Endonuclease Sensitive Site Assay[a]

UV dose (J/m^2)	butyrate treatment	single-strand break frequency[b] (per 10^8 daltons) +UV endonuclease	single-strand break frequency[b] (per 10^8 daltons) −UV endonuclease	ESS frequency[c] (per 10^8 daltons)
0	−	1.2	0.7	0.5
0	+	1.2	0.8	0.4
25	−	17.2	1.0	16.2
25	+	17.2	1.0	16.2
100	−	37.0	1.3	35.7
100	+	37.0	1.4	35.6

[a] Permeable AG1518 fibroblasts, control or pretreated with sodium butyrate, were irradiated with the indicated dose of UV radiation and incubated with or without addition of a crude preparation of *M. luteus* UV endonuclease, and the number-average molecular weight of the single-stranded DNA fragments was measured by centrifugation in alkaline sucrose gradients, as described under Experimental Procedures. [b] Single-strand break frequencies were calculated from the reciprocal of the number-average molecular weight for each sample. [c] UV endonuclease sensitive site (ESS) frequency is the difference between the single-strand break frequencies of samples incubated with and without UV endonuclease.

is based on the finding that the rate at which DNA is eluted from a poly(vinyl chloride) filter by pH 12 buffer is a function of the frequency of single-strand breaks in the DNA (Kohn et al., 1976). For the incision assay, damaged and undamaged permeable human fibroblasts, prelabeled with [^{14}C]dThd, were incubated for 10 min in a reaction mixture containing ATP but lacking deoxyribonucleoside triphosphates and were deposited on filters along with reference RAJI cells which had been prelabeled with [^3H]dThd and irradiated with 350 rad of X-rays. The cells were then lysed, and the DNA was eluted (Dresler & Lieberman, 1983b). For each sample, the elution of experimental cell DNA (^{14}C labeled) was plotted against the elution of reference cell DNA (^3H labeled), permitting the relative rates of elution of various experimental samples to be compared directly. This technique was used to compare incision rates in butyrate-pretreated and control AG1518 fibroblasts damaged with 100 J/m^2 UV radiation (Figure 6). The DNA of unirradiated cells, both control and butyrate pretreated, eluted at the same slow rate, indicative of the presence of small numbers of nonspecific single-strand breaks. The DNA of UV-irradiated control cells eluted more rapidly as a result of the formation of damage-specific strand breaks during the incubation period. The DNA of UV-irradiated butyrate-pretreated cells eluted even more rapidly, indicating increased formation of damage-specific strand breaks during the incubation. Analysis of the elution data by the method of Erickson et al. (1977) indicates that, during the 10-min incubation period, UV-irradiated butyrate-pretreated AG1518 cells generated 1.5 times as many damage-specific strand breaks as were generated by UV-irradiated control cells. In parallel incubations with a complete repair synthesis reaction mixture, it was found that repair synthesis in these cells was stimulated 1.6-fold by the butyrate pretreatment (data not shown). Thus, the butyrate-induced stimulation of damage-specific incision was large enough to account for essentially all of the butyrate-induced stimulation of repair synthesis in these cells.

Incision rates were also compared in butyrate-pretreated and control IMR-90 fibroblasts irradiated with 10 J/m^2 UV radiation (Figure 7). In this experiment as well, butyrate-pretreated cells showed a dramatic increase in the rate of damage-specific strand break formation.

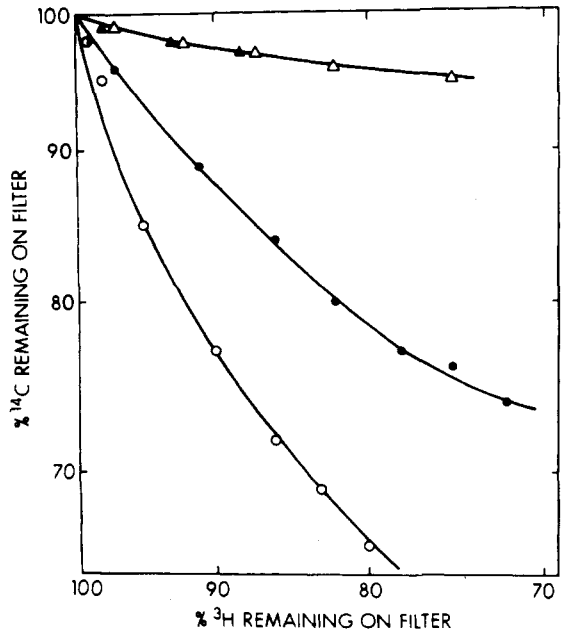

FIGURE 6: Comparison by alkaline elution of damage-specific incision in UV-irradiated permeable control and butyrate-pretreated AG1518 cells. Confluent cells, control (▲, ●) and butyrate-pretreated (△, ○), prelabeled with [^{14}C]dThd, were irradiated with 0 (▲, △) or 100 J/m^2 (●, ○) UV radiation, incubated for 10 min with reaction mixture lacking deoxyribonucleoside triphosphates, mixed with [^{3}H]dThd-labeled RAJI cells which had been irradiated with 350 rad of X-rays, and analyzed by alkaline elution. For each sample, the elution of experimental sample DNA (^{14}C labeled) is plotted against the elution of internal standard DNA (^{3}H labeled).

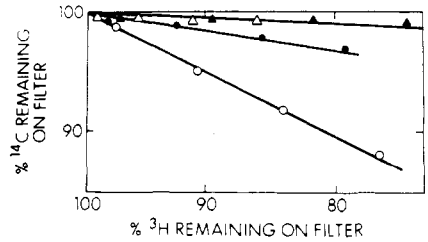

FIGURE 7: Comparison by alkaline elution of damage-specific incision in UV-irradiated permeable control and butyrate-pretreated IMR-90 cells. Cells were treated and analyzed as described in the legend to Figure 6, except that the dose of UV radiation was 10 J/m^2.

Effect of Butyrate Pretreatment on the ATP Requirement of UV-Induced Excision Repair. It was shown previously that ATP is required for UV-induced excision repair at or before the incision step (Dresler & Lieberman, 1983b). The stimulation of damage-specific incision by butyrate pretreatment suggests that butyrate might alter this ATP requirement. In fact, the ATP requirement for UV-induced excision repair (using repair synthesis as an end point) was found to be very similar in control and butyrate-pretreated cells (Figure 8). When the data in Figure 8 were plotted in double-reciprocal fashion, straight lines were generated, and the apparent K_m values for ATP for excision repair in both control and butyrate-pretreated cells were found to be 1 mM (data not shown).

Discussion

The data presented above indicate that pretreatment of confluent human fibroblasts with sodium butyrate results in an increased rate of specific incision of UV-damaged DNA. Control experiments demonstrate that this stimulation is not due to increased efficiency of DNA damage production by UV radiation in butyrate-pretreated cells. The increased incision rate is reflected by an increased rate of DNA repair synthesis, without alteration of repair patch size or composition. These

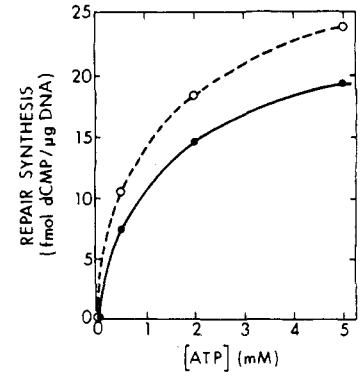

FIGURE 8: Dependence of UV-induced repair synthesis on ATP concentration in permeable control (●) and butyrate-pretreated (○) AG1518 fibroblasts. Confluent cells were made permeable, irradiated with 100 J/m^2 UV radiation, and incubated for 15 min with repair synthesis reaction mixture containing the indicated concentration of ATP. The concentration of $MgCl_2$ was also varied so that it was always 3 mM greater than the ATP concentration (Dresler & Lieberman, 1983b). Repair synthesis was determined as described under Experimental Procedures.

results are entirely consistent with those of Smerdon et al. (1982). The data reported here also indicate that pretreatment with sodium butyrate alters cellular nucleotide metabolism, confounding attempts to analyze changes in repair synthesis by measuring incorporation of exogenous labeled nucleotides into the DNA of intact cells. The data, however, do not support the conclusion (Williams & Friedberg, 1982) that changes in UV-induced DNA repair synthesis following sodium butyrate pretreatment are entirely an artifact resulting from changes in nucleotide metabolism.

Sodium butyrate could influence the rate of incision of damaged DNA through its known effects on chromatin structure. Butyrate inhibits nuclear histone deacetylation (Boffa et al., 1978; Candido et al., 1978; Sealy & Chalkley, 1978), leading to hyperacetylation of nucleosome core histones, primarily H3 and H4. Hyperacetylated chromatin is more sensitive to digestion by DNase I (Simpson, 1978; Vidali et al., 1978; Nelson et al., 1978; Perry & Chalkley, 1981), hyperacetylated chromatin oligomers show increased solubility in Mg^{2+}-containing buffers (Perry & Chalkley, 1981), and histone H3 in hyperacetylated chromatin is more accessible to a Ca^{2+}-dependent nuclear protein kinase (Whitlock et al., 1983). McGhee et al. (1983), using electric dichroism and analytical ultracentrifugation, found no gross disruption of the higher order structure of hyperacetylated chromatin from butyrate-treated HeLa cells but did detect a 10–20% increase in the lengths of thick (30 nm) chromatin fibers produced from hyperacetylated chromatin by the cation-induced filament–solenoid transition. Hyperacetylated chromatin cofractionates with transcriptionally active chromatin during DNase I digestion (Weintraub & Groudine, 1976), DNase II digestion (Davie & Candido, 1978), micrococcal nuclease digestion (Levy-Wilson et al., 1979), and HMG14–HMG17 affinity chromatography (Weisbrod, 1982), suggesting that histone acetylation is one of the factors involved in making specific DNA sequences accessible to the transcriptional apparatus. Histone hyperacetylation has also been implicated as a factor in the assembly of newly replicated DNA into chromatin (Candido & Dixon, 1972; Louie & Dixon, 1972; Ruiz-Carrillo et al., 1975; Jackson et al., 1976) and in the substitution of protamines for histones during spermatogenesis (Sung & Dixon, 1970; Candido & Dixon, 1972; Christensen & Dixon, 1982; Oliva & Mezquita, 1982). In the latter case, hyperacetylation apparently produces a highly relaxed chromatin

structure which facilitates binding of protamines to the DNA (Christensen et al., 1984). On the basis of findings such as these, it has been suggested that the DNA in chromatin containing hyperacetylated histones is generally more accessible to enzymes and other proteins, both endogenous and exogenous [see, for example, Mathis et al. (1980) and Oliva & Mezquita (1982)]. In this way, butyrate-induced histone hyperacetylation could facilitate access of DNA repair enzymes to sites of damage. Sodium butyrate has also been shown to affect a small, rapidly turned over fraction of histone acetylation (Plesko et al., 1983), histone phosphorylation (D'Anna et al., 1980; Boffa et al., 1981; Whitlock et al., 1983), and histone methylation (Boffa et al., 1981). The structural and functional consequences of these histone modifications are too poorly understood to speculate on their possible effects on excision repair.

Sodium butyrate pretreatment also might affect excision repair through changes in gene expression. In some cell types, butyrate has been shown to stimulate the expression of specific genes (Reeves & Cserjesi, 1979; McCue et al., 1984). In other cell lines, the drug has been found to inhibit the expression of small numbers of genes (Plesko et al., 1983; Truscello et al., 1983). Butyrate pretreatment might stimulate the synthesis of a DNA repair factor active at or before the incision step, or butyrate might suppress the production of a factor which retards excision repair. In preliminary studies, sodium butyrate pretreatment of xeroderma pigmentosum fibroblasts (group G) has resulted in stimulated of subsequent T4 UV endonuclease complemented excision repair (unpublished data). This finding suggests that the effect of sodium butyrate on UV-induced excision repair is due to a factor other than stimulation of the synthesis of the endogenous UV endonuclease.

Recent demonstrations that UV-induced DNA repair is nonrandomly distributed in genomes of mammalian cells (Cohn & Lieberman, 1984; Bohr et al., 1985) make studies of the effects of chromatin structure on DNA repair particularly interesting. The finding that butyrate pretreatment, which leads to histone hyperacetylation, also stimulates DNA excision repair suggests that in unperturbed cells more highly acetylated regions of chromatin may be repaired more rapidly than other chromatin domains. Such an effect could lead to a nonrandom distribution of repair at early times after DNA damage. Consistent with this concept, Mayne (1984) has recently suggested, on the basis of studies of the recovery of transcription after DNA damage, that actively transcribed genes (which are present in more highly acetylated chromatin; see above) are repaired preferentially. The effects of chromatin structure on the distribution of DNA repair could be of great significance in determining the effects of DNA damage on gene structure and expression.

ACKNOWLEDGMENTS

The helpful suggestions of Dr. Michael J. Smerdon and the assistance of Mark G. Frattini and Frederic G. Barr are much appreciated.

Registry No. Thymidine, 50-89-5; deoxycytidine, 951-77-9; deoxyuridine, 951-78-0; $HO_2C(CH_2)_2CH_3$, 107-92-6.

REFERENCES

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

Boffa, L. C., Vidali, G., Mann, R. S., & Allfrey, V. G. (1978) *J. Biol. Chem. 253*, 3364–3366.

Boffa, L. C., Gruss, R. J., & Allfrey, V. G. (1981) *J. Biol. Chem. 256*, 9612–9621.

Bohr, V. A., Smith, C. A., Okumoto, D. S., & Hanawalt, P. C. (1985) *Cell (Cambridge, Mass.) 40*, 359–369.

Brash, D. E., & Hart, R. W. (1983) in *DNA Repair: A Laboratory Manual of Research Procedures* (Friedberg, E. C., & Hanawalt, P. C., Eds.) Vol. 2, pp 173–183, Marcel Dekker, New York.

Candido, E. P. M., & Dixon, G. H. (1972) *J. Biol. Chem. 247*, 5506–5510.

Candido, E. P. M., Reeves, R., & Davie, J. R. (1978) *Cell (Cambridge, Mass.) 14*, 105–113.

Carrier, W. L., & Setlow, R. B. (1970) *J. Bacteriol. 102*, 178–186.

Chargaff, E., & Lipshitz, R. (1953) *J. Am. Chem. Soc. 75*, 3658–3661.

Christensen, M. E., & Dixon, G. H. (1982) *Dev. Biol. 93*, 404–415.

Christensen, M. E., Rattner, J. B., & Dixon, G. H. (1984) *Nucleic Acids Res. 12*, 4575–4592.

Cohn, S. M., & Lieberman, M. W. (1984) *J. Biol. Chem. 259*, 2463–2469.

D'Anna, J. A., Tobey, R. A., & Gurley, L. R. (1980) *Biochemistry 19*, 2656–2671.

Davie, J. R., & Candido, E. P. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3574–3577.

Dean, C. J., Ormerod, M. G., Serianni, R. W., & Alexander, P. (1969) *Nature (London) 222*, 1042–1044.

DePamphilis, M. L., & Wassarman, P. M. (1980) *Annu. Rev. Biochem. 49*, 627–666.

Dresler, S. L. (1984) *J. Biol. Chem. 259*, 13947–13952.

Dresler, S. L., & Lieberman, M. W. (1983a) *J. Biol. Chem. 258*, 9990–9994.

Dresler, S. L., & Lieberman, M. W. (1983b) *J. Biol. Chem. 258*, 12269–12273.

Dresler, S. L., Roberts, J. D., & Lieberman, M. W. (1982) *Biochemistry 21*, 2557–2564.

Edenberg, H., & Hanawalt, P. (1972) *Biochim. Biophys. Acta 272*, 361–372.

Erickson, L. C., Bradley, M. O., & Kohn, K. W. (1977) *Cancer Res. 37*, 3744–3750.

Ganesan, A. K., Smith, C. A., & van Zeeland, A. A. (1981) in *DNA Repair: A Laboratory Manual of Research Procedures* (Friedberg, E. C., & Hanawalt, P. C., Eds.) Vol. 1, Part A, pp 89–97, Marcel Dekker, New York.

Goodman, D., & Matzura, H. (1971) *Anal. Biochem. 42*, 481–486.

Jackson, V., Shires, A., Tanphaichitr, N., & Chalkley, R. (1976) *J. Mol. Biol. 104*, 471–483.

Kastan, M. B., Gowans, B. J., & Lieberman, M. W. (1982) *Cell (Cambridge, Mass.) 30*, 509–516.

Kohn, K. W., Erickson, L. C., Ewig, R. A. G., & Friedman, C. A. (1976) *Biochemistry 15*, 4629–4637.

Levy-Wilson, B., Watson, D. C., & Dixon, G. H. (1979) *Nucleic Acids Res. 6*, 259–274.

Louis, A. J., & Dixon, G. H. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1975–1979.

Maniatis, T., Jeffrey, A., & van de Sande, H. (1975) *Biochemistry 14*, 3787–3794.

Mathis, D., Oudet, P., & Chambon, P. (1980) *Prog. Nucleic Acid Res. Mol. Biol. 24*, 1–55.

Mayne, L. V. (1984) *Mutat. Res. 131*, 187–191.

McCue, P. A., Gubler, M. L., Sherman, M. I., & Cohen, B. N. (1984) *J. Cell Biol. 98*, 602–608.

McGhee, J. D., Nickol, J. M., Felsenfeld, G., & Rau, D. C. (1983) *Nucleic Acids Res. 11*, 4065–4075.

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.
Oliva, R., & Mezquita, C. (1982) *Nucleic Acids Res. 10*, 8049–8059.
Paterson, M. C., Smith, B. P., & Smith, P. J. (1981) in *DNA Repair: A Laboratory Manual of Research Procedures* (Friedberg, E. C., & Hanawalt, P. C., Eds.) Vol. 1, Part A, pp 99–111, Marcel Dekker, New York.
Perry, M., & Chalkley, R. (1981) *J. Biol. Chem. 256*, 3313–3318.
Plesko, M. M., Hargrove, J. L., Granner, D. K., & Chalkley, R. (1983) *J. Biol. Chem. 258*, 13738–13744.
Reeves, R., & Cserjesi, P. (1979) *J. Biol. Chem. 254*, 4283–4290.
Ruiz-Carrillo, A., Wangh, L. J., & Allfrey, V. G. (1975) *Science (Washington, D.C.) 190*, 117–128.
Sealy, L., & Chalkley, R. (1978) *Cell (Cambridge, Mass.) 14*, 115–121.
Sidik, K., & Smerdon, M. J. (1984) *Carcinogenesis (London) 5*, 245–253.
Simpson, R. T. (1978) *Cell (Cambridge, Mass.) 13*, 691–699.
Smerdon, M. J. (1983) *Biochemistry 22*, 3516–3525.
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., Lan, S. Y., Calza, R. E., & Reeves, R. (1982) *J. Biol. Chem. 257*, 13441–13447.
Smith, C. A. (1978) in *DNA Repair Mechanisms* (Hanawalt, P. C., Friedberg, E. C., & Fox, C. F., Eds.) pp 311–314, Academic Press, New York.
Sung, M. T., & Dixon, G. H. (1970) *Proc. Natl. Acad. Sci. U.S.A. 67*, 1616–1623.
Tlsty, T. D., & Lieberman, M. W. (1978) *Nucleic Acids Res. 5*, 3261–3273.
Truscello, A., Geering, K., Gaggeler, H. P., & Rossier, B. C. (1983) *J. Biol. Chem. 258*, 3388–3395.
van Zeeland, A. A., Smith, C. A., & Hanawalt, P. C. (1981) *Mutat. Res. 82*, 173–189.
Vidali, G., Boffa, L. C., Bradbury, E. M., & Allfrey, V. G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2239–2243.
Vinograd, J., & Hearst, J. E. (1962) *Prog. Chem. Org. Nat. Prod. 20*, 417.
Weintraub, H., & Groudine, M. (1976) *Science (Washington, D.C.) 193*, 848–856.
Weisbrod, S. T. (1982) *Nucleic Acids Res. 10*, 2017–2042.
Whitlock, J. P., Galeazzi, D., & Schulman, H. (1983) *J. Biol. Chem. 258*, 1299–1304.
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., Cortopassi, G. A., Smith, C. A., & Hanawalt, P. C. (1982a) *Cell (Cambridge, Mass.) 28*, 613–619.
Zolan, M. E., Smith, C. A., Calvin, N. M., & Hanawalt, P. C. (1982b) *Nature (London) 299*, 462–464.
Zolan, M. E., Smith, C. A., & Hanawalt, P. C. (1984) *Biochemistry 23*, 63–69.

Molecular Structure of the β-Adrenergic Receptor

Robert G. L. Shorr,*,‡ Darrell R. McCaslin,§ Mark W. Strohsacker,‡ Gary Alianell,∥ Richard Rebar,‡ Jeffrey M. Stadel,‡ and Stanley T. Crooke‡

Department of Molecular Pharmacology, Smith Kline and French Laboratories, Philadelphia, Pennsylvania 19101, Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710, and Beckman Instruments, King of Prussia, Pennsylvania 19406

Received January 29, 1985

ABSTRACT: The β-adrenergic receptor from several tissues has been purified to homogeneity or photoaffinity radiolabeled and its subunit molecular weight determined by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis. In this study we have examined the oligomeric structure of nondenatured β_1- and β_2-adrenergic receptor proteins, as solubilized with the detergent digitonin. Model systems used were frog and turkey red blood cell as well as rat, rabbit, and bovine lung plasma membrane preparations. To correct for the effects of detergent binding, sedimentation equilibrium analysis in various solvents, as adapted for the air-driven ultracentrifuge, was used. With this approach an estimate of 6 g of digitonin/g of protein binding was determined, corresponding to a ratio of 180 mol of digitonin/mol of protein. Protein molecular weights estimated by this method were 43 500 for the turkey red blood cell β_1 receptor and 54 000 for the frog red blood cell β_2 receptor. Molecular weights of 60 000–65 000 were estimated for β_1 and β_2 receptors present in mammalian lungs. These values agree with estimates of subunit molecular weight obtained by SDS gel electrophoresis of purified or photoradiolabeled preparations and suggest β-adrenergic receptors to be digitonin solubilized from the membrane as single polypeptide chains.

The β-adrenergic receptor recognizes epinephrine and norepinephrine and modulates the production of cyclic AMP by the enzyme adenylate cyclase. On the basis of pharmacological interactions, β receptors can be divided into two subtypes termed β_1 and β_2 (Lands et al., 1967). Both receptor subtypes stimulate adenylate cyclase activity. An early step in the mechanism by which this is believed to occur is the formation of a complex between receptor and a GTP binding regulatory component of the enzyme (Stadel et al., 1980). While the subunit molecular weight of the β-adrenergic receptor has been determined in several model systems (Shorr et al., 1981, 1982a,b; Benovic et al., 1983; Stiles et al., 1983), the nature

‡Smith Kline and French Laboratories.
§Duke University Medical Center.
∥Beckman Instruments.

0006-2960/85/0424-6869$01.50/0