PHOTOREACTIVATION AND EXCISION REPAIR OF ULTRAVIOLET RADIATION-INJURED DNA IN PRIMARY EMBRYONIC CHICK CELLS

M. C. PATERSON, P. H. M. LOHMAN,
E. A. DE WEERD-KASTELEIN, *and* A. WESTERVELD

From the Laboratory for Molecular Genetics, Leiden, the Medical Biological Laboratory TNO, Rijswijk 2100, and the Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands. Dr. Paterson's present address is the Division of Biology and Health Physics, Chalk River Nuclear Laboratories, Atomic Energy of Canada Limited, Chalk River, Ontario KOJ 1J0, Canada.

ABSTRACT Primary embryonic chick cells have been evaluated on the basis of their capacity to repair photochemical lesions produced in the deoxyribonucleic acid (DNA) by ultraviolet (UV) radiation. The fate of one prominent class of UV photoproducts, cyclobutane pyrimidine dimers, was monitored by an in vitro enzymatic assay. UV-irradiated cultures were incubated for prescribed times after which their damaged, radioactive-labeled DNA was extracted and exposed to a purified UV endonuclease selectively active toward sites altered by dimer formation. Single-strand scissions specifically introduced by the enzyme treatment and, therefore, the dimer-containing sites remaining in the DNA were quantified retrospectively by velocity sedimentation in alkaline sucrose. When the chick fibroblasts were incubated in black light, essentially all nuclease-susceptible sites rapidly disappeared from the UV-damaged DNA. In sharp contrast, incubation of the irradiated cultures in total darkness severely impeded the metabolic machinery responsible for site elimination. A substantial amount of UV-stimulated DNA repair synthesis was also detected in the chick cells by conventional techniques involving isopyknic centrifugation and autoradiography. However, the UV photoproducts triggering this indicator of excision repair were probably not dimers since incubation of the irradiated cultures in the light rather than in the dark did not lead to a diminution in the extent of repair synthesis. By these criteria of DNA repair, it appears that embryonic chick cells primarily rely on a highly proficient, light-requiring mechanism, presumably enzymatic photoreactivation, for dimer elimination but also possess a light-independent, excision-type process to cope with other, as yet unidentified, photochemical defects.

INTRODUCTION

Far ultraviolet (UV) radiation ($\lambda < 320$ nm) induces the formation of cyclobutane dimers between adjacent intrastrand pyrimidines within the deoxyribonucleic acid

(DNA) of biological systems. Fortunately, many living organisms encompassing a wide phylogenetic distribution possess efficient enzymatic mechanisms to cope with these lethal UV photoproducts (recently reviewed by Setlow and Setlow, 1972). During enzymatic photoreactivation (PR), a repair process catalyzed by the photoreactivating enzyme (PRE) upon illumination with light of wavelength 320–500 nm, pyrimidine dimers are directly monomerized to individual bases *in situ* (reviewed by Cook, 1970). In a second process, excision repair, UV-damaged intrastrand sites are released from the DNA and accurately replaced; this occurs by a series of light-independent events involving two single-strand scissions to excise the damage, resynthesis to fill in the gap and strand rejoining (Setlow and Setlow, 1972).

Aside from the unequivocal demonstration of enzymatic PR in embryonic chick cells as demonstrated by the detection of PRE activity in cellular extracts (Cook and McGrath, 1967) and of a photorepair mechanism to eradicate pyrimidine dimers from the endogeneous DNA (Pfefferkorn and Coady, 1968), there is little information on the DNA repair processes at the disposal of these avian cells when confronted with radiation damage. Thus, to further our understanding of photoenzymatic repair in the chick fetus and to gain some insight into other, as yet unidentified, repair processes, we have performed a series of experiments on primary fibroblasts established from chick embryonic tissue. Our results confirm the existence of a proficient, light-induced mechanism in the chick cultures which swiftly removes most, and probably all, pyrimidine dimers from UV-injured DNA. When held in the dark, however, the chick cells are severely retarded in dimer elimination despite the apparent presence of an excision-type mechanism to overcome other photochemical defects whose precise chemical structure remains unknown.

MATERIALS AND METHODS

Fibroblast Cultures

Primary embryonic strains of the White Leghorn chick were established from tissue biopsies of 10-day old embryos by a standard isolation procedure (Paul, 1970). Under sterile conditions, the chick embryo was carefully removed from the egg, decapitated, and eviscerated, and the wings and legs were then excised. The embryonic "carcass" was thoroughly washed in Hanks' balanced salt solution (Hanks and Wallace, 1949), chopped up finely, and trypsinized to free the cells. The cells were collected and cultivated in Roux flasks at 37°C. In this and all subsequent cultivations the basic growth medium was thymidine-free F12 medium (Ham, 1965) supplemented with 15% (vol/vol) fetal calf serum (Gibco, Grand Island Biological Co., Grand Island, N.Y.) (referred to as the F12 growth medium). The fibroblast preparations were normally subcultured three times before experimental use. The doubling time of the five strains independently isolated for usage during the course of the investigation varied from 16 to 18 h.

In certain experiments we also utilized a normal primary human strain, designated AH, previously established from a healthy volunteer (Kleijer and Bootsma, 1971). This strain was subjected to exactly the same manipulations as the embryonic chick ones.

Experimental cultures were routinely grown in Falcon plastic petri dishes (diam 9 cm)

(Falcon Plastics, Div. of B-D Industries, Inc., Los Angeles, Calif.) containing 4 ml of the F12 growth medium and, when destined for autoradiographic analysis, a sterile coverslip. Each dish was seeded with either $\sim 2 \cdot 10^6$ chick cells or $\sim 5 \cdot 10^5$ human cells and subsequently incubated for 48 h in a 37°C incubator continuously aerated with a humidified mixture of 95% air and 5% carbon dioxide. Under these conditions, the chick and human cultures were at a comparable phase of growth (i.e. late exponential) at the end of this cultivation period. Certain experiments (i.e. those employing the in vitro enzymatic assay) required the DNA within the chick fibroblasts to be prelabeled with radionuclides; this was achieved by incubating the cultures for 48 h in the F12 growth medium containing either 0.5 μCi/ml [^{3}H-methyl]dThd (2 Ci/mmol) or 1.0 μCi/ml [2-^{14}C]dThd (59 mCi/mmol) (both purchased from Radiochemical Centre, Amersham, Great Britain).

UV Irradiation

Upon discarding the incubation medium, the monolayer cultures were washed twice with Hanks' balanced salt solution followed by exhaustive withdrawal of the washing solution. The fibroblasts were immediately exposed in open petri dishes to doses of predominantly 254 nm radiation administered by a Philips TUV low pressure mercury tube (15 watt) (Philips Electronic Instruments, Mount Vernon, N.Y.) at an incident exposure rate of 8 ergs/mm^2 per s, as measured by a photovoltaic dosimeter (Jagger, 1961). To obviate undesired enzymatic PR, the radiation treatment as well as all subsequent manipulative procedures with the cultures were conducted under dim yellow light emitted from Philips "gold" fluorescent lamps ($>$480 nm).

Postirradiation Incubation

Prewarmed growth medium was immediately added to the UV-damaged avian cultures. In many instances one set of duplicate samples was placed in the dark (i.e. the cultivation incubator) while the other was set in a cabinet illuminated with photoreactivating light. In both cases the samples were incubated for specified times at 37°C in a water-saturated atmosphere of 5% carbon dioxide in air. The light source was a horizontal bank of four fluorescent black-light tubes (Philips TL 40 W/08 RS-F40 BLB) mounted directly below the incubation cabinet. The bottom of the cabinet consisted of 13-mm thick plate glass to filter out radiation having wavelengths shorter than 320 nm. The wavelength distribution of the light directly impinging on the cultures ranged from 320 to 420 nm with an emission maximum at 380 nm. In a control experiment the incident intensity of the total radiation spectrum, $\sim$80 ergs/mm^2 per s, (measured by a Hewlett-Packard 8330A Radiant Flux Meter; Hewlett-Packard Co., Palo Alto, Calif.) was found to be considerably higher than that needed to saturate the photorepair reaction in the chick cells exposed to 500 ergs/mm^2 of 254 nm radiation.

Endonucleolytic Detection of UV-Damaged Sites

We have recently described a sensitive enzymatic assay which exploits the ability of a UV endonuclease purified from the bacterium *Micrococcus luteus* to attack UV-damaged DNA at specific sites distorted by the presence of pyrimidine dimers (Paterson et al., 1973, 1974). The same assay was employed here to generate in vivo kinetics of the disappearance of UV-injured sites (arbitrarily denoted as nuclease-susceptible sites) from the DNA of chick cells during postirradiation incubation. In brief, paired cultures of ^{3}H-labeled, UV-irradiated and ^{14}C-labeled, unirradiated control cells were simultaneously subjected to identical incubation

conditions, collected, and mixed in equal amounts. After cell lysis, the radioactive DNA was isolated, and samples were incubated with and, as an internal control to evaluate any inherent size difference in the two DNA populations, without the UV endonuclease from *M. luteus*. The DNA products resulting from the endonucleolytic attack were then sedimented through alkaline sucrose (5–20%, wt/vol) gradients at 40,000 rpm (21°C) for 120 min in a Beckman SW 50.1 rotor (Beckman Instruments, Inc., Fullerton, Calif.). After gradient fractionation followed by the counting of the radioactivity in the fractions to produce DNA distribution profiles, weight average molecular weight (MW) values for the two sedimented populations of macromolecules were computed on a Digital PDP 8/1 computer (Digital Equipment Corp., Maynard, Mass.). This analysis yielded the number of single-strand breaks in the irradiated DNA attributable to incubation with the UV endonuclease and, in retrospect, the incidence of UV-altered sites sensitive to the endonucleolytic action of the purified enzyme.

DNA Repair Synthesis

Measured by Isopyknic Centrifugation in Sodium Iodide. The extent to which UV radiation stimulates repair replication in the embryonic chick strain compared with that in the human strain was quantified by the technique of Lohman and co-workers (1973). For both chick and human (AH) cultures, sets of duplicate preparations were cultivated in BrdUrd (2 μg/ml) and FdUrd (10^{-6} M) for 2 h prior to the administration of 254 nm light. Following the addition of F12 growth medium supplemented with BrdUrd (2 μg/ml), [^{3}H-methyl]-dThd (10 μCi/ml; 2 Ci/mmol), FdUrd (10^{-6} M), and hydroxyurea (10^{-3} M) one set of duplicates was returned to the dark while the second was illuminated with black light; all samples were then incubated for 3 h. Details pertaining to all subsequent treatments including extraction of intracellular DNA, centrifugation of the extracted samples to equilibrium in neutral NaI gradients, and analysis of the resulting density profiles to ascertain the magnitude of DNA repair replication (expressed as counts per minute per microgram DNA of normal density) were as described elsewhere (Lohman et al., 1973).

Measured by Autoradiography. Monolayer cultures of both chick and human strains were treated in a manner similar to that detailed above for the isopyknic centrifugation analysis. To ensure unequivocal identification of cells in the DNA synthesis (S) phase of the division cycle at the time of UV irradiation the preparations were prelabeled in the dark for 1 h in F12 medium containing 10 μCi/ml [^{3}H-methyl]dThd (2 Ci/mmol). After momentarily removing the medium for exposure to UV radiation, fresh radioactive medium was added, and then one set of duplicates was returned to the dark while the second was illuminated with black light; all samples were then incubated for 3 h. Finally, the coverslips were removed from the dishes, rinsed in saline, and fixed with Bouin's solution.

Autoradiograms, prepared with Kodak AR10 stripping film, were exposed for 1 wk before development and straining with hemotoxylin and eosin. For each preparation the level of UV-induced DNA synthesis (UDS), expressed as mean grain number per nucleus, was estimated by counting grains over 50 nuclei in the G1 or G2 phase of the cell cycle.

RESULTS

Quantification of Nuclease-Susceptible Sites in UV-Damaged DNA

The sedimentation profiles, depicted in Fig. 1, typify the results of routine experiments in which DNA was coextracted from UV-damaged and undamaged primary embryonic chick cells, incubated with and without the UV endonuclease purified

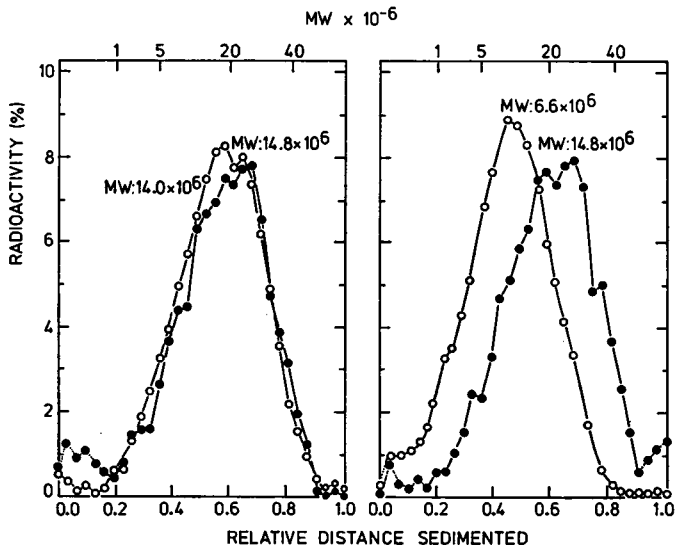

FIGURE 1 Sedimentation patterns of radioactive DNA from an embryonic chick strain illustrating the application of the in vitro enzymatic assay. The DNA was coextracted *in toto* from ^{3}H-labeled, UV-irradiated (○) (incident dose, 100 ergs/mm^2) and ^{14}C-labeled, unirradiated (●) primary chick fibroblasts, after which samples were incubated in the absence (left panel) or presence (right panel) of the purified UV endonuclease and finally centrifuged in alkaline sucrose gradients as detailed in Materials and Methods. The regions of each profile depicted with a dotted line were arbitrarily judged to be divorced from the main body of sedimented DNA and consequently were excluded from the calculation of MW.

from *M. luteus*, and finally analyzed by velocity sedimentation in alkaline sucrose. When the DNA extract was incubated alone, the intrinsic difference in the size distribution of the irradiated and the unirradiated DNA corresponded to only 0.1 breaks per 10^7 daltons, number average molecular weight (MN) (for details concerning this calculation, see Paterson et al., 1973). Incubation with the *M. luteus* endonuclease, however, resulted in a diminution in the sedimentation coefficient of the UV-irradiated species to the extent that this DNA, relative to the control one, now contained 1.7 breaks per 10^7 daltons (MN). We conclude therefore that the avian DNA preexposed in vivo to 100 ergs/mm^2 of UV light sustained, on the average, 1.6 single-strand scissions per 10^7 daltons (MN) as a direct consequence of incubation with the purified enzyme. Once more, the endonucleolytic attack was directed in a highly selective fashion toward radiation-damaged DNA as the MW of the unirradiated control DNA was not significantly altered by the enzyme treatment. Hence, the purified preparation of UV endonuclease was free of any detectable contamination by nonspecific endonucleases.

The dose-response curve, plotted in Fig. 2, illustrates the incidence of strand breaks produced by the purified enzyme in vitro as a function of the UV irradiation preadministered to the chick DNA in vivo. Assuming that the induction of photo-

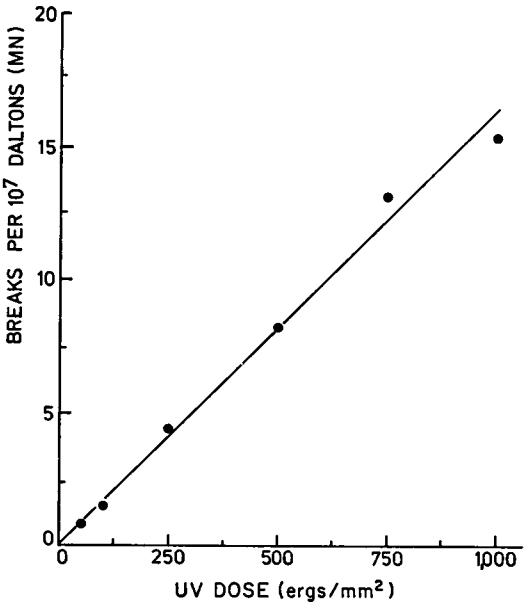

FIGURE 2 Correlation between UV irradiation impinging on the chick monolayer cultures and frequency of intrastrand scissions subsequently introduced in the extracted (radiation-damaged) DNA by the purified UV endonuclease. For each dose delivered in vivo, the corresponding strand-breakage data were obtained by performing the in vitro enzymatic assay followed by computer analysis of two parallel sedimentation profiles analogous to those shown in Fig. 1. Each point is the arithmetic mean (standard deviation $\leq 10\%$) of at least three independent determinations. MN, number average molecular weight.

products was linear with dosage over the UV exposure range used here (Setlow, 1968), the observed linearity in the curve confirms the prediction that incubation with the UV endonuclease systematically produced one intrastrand scission at each of the nuclease-susceptible damaged sites in the extracted chick DNA.

Fate of Nuclease-Susceptible Sites in Irradiated Chick Cells

By incubating UV-irradiated cultures for appropriate times before DNA extraction and UV endonucleolytic analysis the in vitro enzymatic assay may be gainfully employed to establish a time course of the in vivo disappearance of nuclease-susceptible sites from the damaged DNA of chick fibroblasts. Such curves, shown in Fig. 3, indicate that the chick cultures were severely defective in the elimination of sites when the post-UV incubation was conducted in complete darkness. For example, in cultures receiving an incident dose of 250 ergs/mm^2, few, if any, of the sites induced by 254 nm radiation disappeared from the DNA of the cells within 24 h. Even after exposure to only 50 ergs/mm^2 $\sim$80% of the sites still remained in the endogeneous DNA after 24 h.

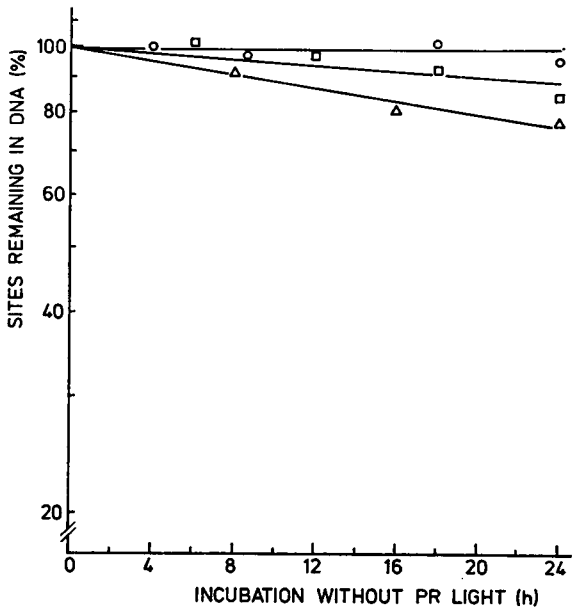

FIGURE 3 Disappearance of nuclease-susceptible sites from the DNA of UV-irradiated embryonic chick cells during subsequent incubation in total darkness. The individual curves correspond to the different UV doses administered to the cells. Following incubation, the cultures were lysed and the incidence of sites remaining in the purified DNA was measured as outlined in Fig. 1. The data were normalized by expressing the number of sites in the incubated samples as a percentage of the number initially present in the corresponding nonincubated ones. The absolute number of sites initially produced by each dose (the amount found in the nonincubated cultures and taken as 100%) may be determined from Fig. 2. Each point is the arithmetic mean (standard deviation $\leq 12\%$) of at least two independent determinations. Incident UV dose (ergs/mm^2): △, 50; ○, 100; □, 250.

In sharp contrast to the pronounced incompetency observed in the dark. fibroblasts incubated with PR light rapidly eliminated virtually all of the nuclease-susceptible sites from their UV-damaged DNA (see Fig. 4). As expected, the rate of site removal during exposure to light varied inversely with the UV irradiation preadministered to the cells. For example, essentially all the sites introduced by 50 ergs/mm^2 of 254 nm radiation disappeared within 2 h, whereas only about 20% of those produced by 1,000 ergs/mm^2 were eliminated during the same interval. The data in Fig. 4 may also be expressed as absolute rates of substrate turnover; that is, for each UV dose, the total number of nuclease-susceptible sites eliminated from the irradiated DNA per cell during 2 h of exposure to black light. As indicated by the results in Table I, at lower UV doses (50–250 ergs/mm^2) the in vivo rate of site removal increased with the number of available sites in a manner approximating first-order kinetics. By 500 ergs/mm^2, however, site removal proceeded at maximal velocity and became independent of substrate concentration.

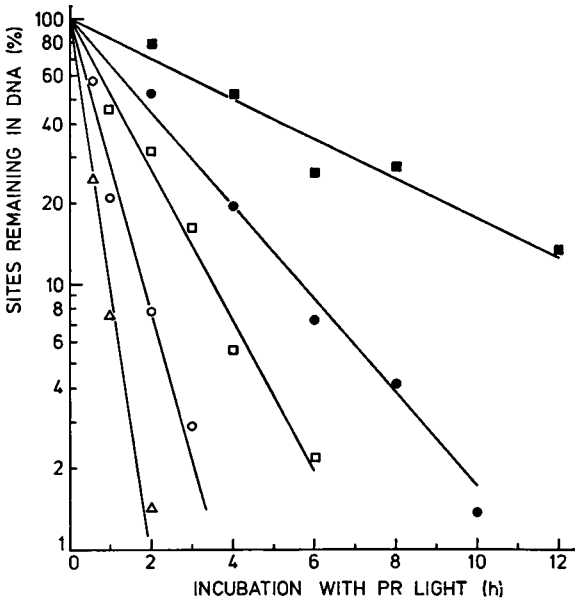

FIGURE 4 Disappearance of nuclease-susceptible sites from the DNA of UV-damaged embryonic chick cells during subsequent exposure to photoreactivating light. The data were generated exactly as specified in Fig. 3 except that during postirradiation incubation, the cell samples, rather than being held in the dark, were illuminated with black light. Each point is the arithmetic mean (standard deviation $\leq 14\%$) of two independent determinations. Incident UV dose (ergs/mm^2): △, 50; ○, 100; □, 250; ●, 500; ■, 1,000.

TABLE I

ESTIMATION OF THE ABSOLUTE NUMBER OF UV ENDONUCLEASE-SUSCEPTIBLE SITES DISAPPEARING FROM UV-DAMAGED DNA PER CHICK CELL DURING 2 h OF POSTIRRADIATION INCUBATION

UV dose	Number of sites initially induced* ($\times 10^{-4}$)	Percent sites disappearing‡	Number of sites disappearing ($\times 10^{-4}$)
ergs/mm²			
50	25	99	25
100	50	92	46
250	125	74	93
500	250	57	143
1,000	500	31	155

* Based on Sober (1970) and assumes that nuclease-susceptible sites exclusively contain pyrimidine dimers.
‡ From Fig. 4.

Capacity to Perform DNA Repair Synthesis

To complement the in vitro enzymatic assay, experiments were conducted to quantify the levels of DNA repair replication and unscheduled DNA synthesis occurring in the chick fibroblasts in response to graded doses of UV radiation.

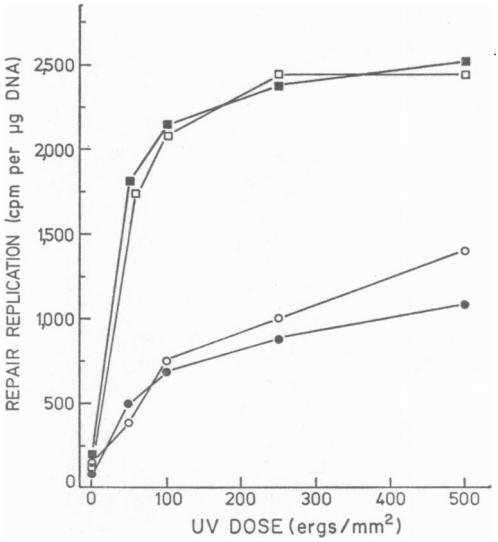

FIGURE 5 The relative amounts of UV-stimulated repair replication occurring in the UV-damaged DNA of embryonic chick (circles) and AH human (squares) cells during 3 h of incubation in total darkness (closed symbols) or in the presence of black light (open symbols). The observed levels of repair replication, measured by isopyknic centrifugation in neutral NaI gradients, are plotted as a function of UV dose incident on the cell samples.

Assayed as UV-Induced Repair Replication. The data summarized in Fig. 5 demonstrate the occurrence of repair replication not only in the human (AH) strain, as expected (Cleaver, 1973), but also in the one established from chick embryonic tissue. The human and avian cultures displayed very similar general kinetics for this nonconservative type of DNA synthesis. In both strains, the rate at which radioactive precursors were incorporated into damaged DNA increased rapidly with incident UV dose up to 100 ergs/mm². At higher UV doses the rate of increase was much less and, particularly in the case of human cells, the curves appeared to reach a plateau value by 500 ergs/mm². The magnitude of repair replication displayed by the AH cells in Fig. 5 is in quantitative agreement with that established in earlier studies on the same human strain (Paterson et al., 1974). The human fibroblasts clearly performed more repair replication than did the chick cells. Irrespective of the presence or absence of black light during postirradiation incubation, the level of repair attained in the chick cells approximated only 40% of that achieved in the human cells.

Despite the presence of a highly proficient PR mechanism in the embryonic chick cells (Fig. 4), exposure to black light during 3 h of post-UV incubation did not significantly alter the kinetics of repair replication in these avian cells. A similar indifference to black-light illumination was also observed for the human cells; this

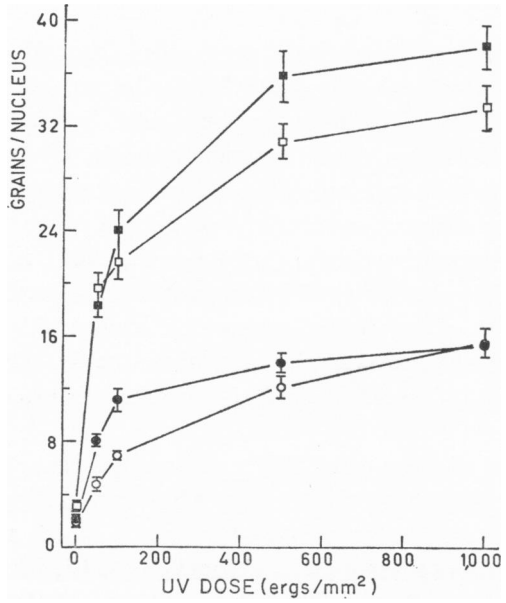

FIGURE 6 Comparative levels of UV-induced unscheduled DNA synthesis occurring in G1 and G2 cells of embryonic chick (circles) and AH human (squares) strains during 3 h of postirradiation incubation in total darkness (closed symbols) or in the presence of black light (open symbols). The magnitude of UDS (i.e., unscheduled incorporation of [^{3}H- methyl]-dThd into UV-damaged DNA in vivo, determined according to the autoradiographic protocol described in Materials and Methods) is evaluated in relation to the incident UV dose delivered to the cultures. Each UDS value (mean grain counts per nucleus ± standard error of the mean) is based on a random analysis of 50 nuclei in autoradiograms exposed for 7 days.

was expected, however, since such cells apparently lack an enzymatic PR process (Rasmussen and Painter, 1964; Cleaver, 1966)[1].

Assayed as UV-Induced Unscheduled DNA Synthesis. The overall kinetics with which UV light stimulated UDS in the human and chick strains (Fig. 6) closely resemble the corresponding patterns for repair replication (Fig. 5). Once more, it can be seen that during post-UV incubation in the dark the level of UDS in the avian cells reached at most 40% of that obtained in human cells, a relative value in good agreement with that found above for repair replication. And again, even in the PR-proficient embryonic chick cells, about the same amount of DNA repair synthesis was found in both strains after exposure to black light as after incubation in the dark.

[1] Paterson, M. C., P. H. M. Lohman, A. Westerveld, and M. L. Sluyter. 1974. Submitted to *Proc. Natl. Acad. Sci. U. S. A.*

DISCUSSION

In this communication a UV endonuclease purified from *M. luteus* has been employed as an in vitro biochemical probe to follow the metabolic removal of UV-injured sites from the DNA of embryonic chick cells. Exposure to the PRE from *Streptomyces griseus*, however, effectively transforms the DNA extracted from UV-irradiated cultures into a form totally insensitive to subsequent endonucleolytic attack by the *M. luteus* enzyme (unpublished data). And since the only known substrate for a PRE is pyrimidine dimers (Setlow et al., 1965), it directly follows that the nuclease-susceptible sites detected in the avian DNA contain only these UV photoproducts. Hence, as concluded earlier for human DNA (Paterson et al., 1973), it appears beyond reasonable doubt that the in vitro enzymatic assay specifically monitors UV-injured chick DNA for sites distorted by the presence of pyrimidine dimers.

Although the actual elimination of UV photoproducts per se has not been demonstrated here, our PR studies on the removal of the nuclease-susceptible sites from chick DNA in vivo are consistent with earlier ones in which analogous embryonic chick strains were shown to possess a functional enzymatic PR mechanism catalyzing the remonomerization of pyrimidine dimers (Cook and McGrath, 1967; Pfefferkorn and Coady, 1968). It seems reasonable to assume then that the light-triggered mechanism mediating site removal and the photoenzymatic repair process are one and the same.

Regardless of the precise mechanism involved, our data indicate that the photorepair process detected in the embryonic chick cells attacks nuclease-susceptible sites more than an order of magnitude faster than does, for instance, the excision-repair mechanism operative in human cells. For example, after an incident UV dose of 50 ergs/mm^2, the incubation time necessary to reduce the incidence of sites by 63% requires about 12 h in the latter (extrapolated from data in Fig. 5 of Paterson et al., 1973) as compared with less than 1 h in the former (Fig. 4). Once more, virtually all of the sites initially induced by 254 nm radiation disappear from the DNA of the chick fibroblasts, whereas in the excision-proficient human cells the enzymatic machinery catalyzing site removal stalls with about 25% of the sites still remaining in the irradiated DNA. Finally, the UV irradiation necessary to saturate the cellular constituents mediating photorepair (presumably the PRE molecules whose activity has been detected by Cook and McGrath, 1967) in the chick cultures approximates 500 ergs/mm^2 (Table I), an exposure about five times greater than that needed to tie up the endogeneous pool of enzymes governing site removal in the human strain (Paterson et al., 1973). It might be envisaged from these observations that if any pyrimidine dimers were induced in the DNA of embryonic chick cells such photoproducts would be immediately subject to photoreversal, in which case the cells could manage without a backup mechanism for the correction of these photochemical lesions. In fact, our results are consistent with this notion, as we were

unable to obtain unequivocal evidence for the existence of a light-independent repair process in chick cells which could eradicte dimer-containing sites from endogeneous DNA. In view of the known absence of photoenzymatic repair in the adult fowl (Cook and McGrath, 1967), it should be of interest to elucidate the mechanism by which cells derived from this source cope with dimers in their DNA.

We have repeatedly observed that the in vitro enzymatic assay detects ~30% fewer nuclease-susceptible sites in the DNA extracted from chick compared with that from human fibroblasts following exposures of the cell cultures to identical doses of UV radiation. A fraction of this discrepancy in site yield may be explained by differences in the base ratios of the two eukaryotic DNAs. Adjacent pairs of thymine are about sixfold more prone to dimer formation than are paired cytosines (Setlow and Carrier, 1966); therefore, as a first approximation the incidence of sites is dictated by the thymine content of the DNA. In this instance thymine comprises ~30.5 mol % in human but only ~26.5 in embryonic chick DNA (values averaged from data compiled by Sober, 1970). The remaining portion of the discrepancy can be readily accounted for by (*a*) slight differences in the UV irradiation actually impinging on the DNA within the cells of both species and (*b*) a 10% uncertainty in the assay. Thus, as reported elsewhere for human DNA (Paterson et al., 1973), incubation of the extracted chick DNA with the purified UV endonuclease most likely results in the quantitative conversion of dimer-containing sites into nuclease-susceptible ones.

Based largely upon extensive photochemical studies on bacteria the pyrimidine dimer is generally regarded as the principal UV photoproduct subject to excision repair (Setlow and Setlow, 1972). Two recent reports provide evidence that this generalization also holds true for mammalian cells. (*a*) In various human and rodent fibroblasts a good correlation exists between the relative levels of DNA repair synthesis and relative extents of dimer excision (Setlow et al., 1972). (*b*) The amount of repair replication in photoreactivable marsupial (rat kangaroo) cells, but not in nonphotoreactivable rodent (mouse L) cells, is much diminished if the postirradiation incubation is conducted under visible light rather than in the dark (Krishnan and Painter, 1973). In stark contrast to these observations we have found here that while the level of DNA repair synthesis detected in the embryonic chick cells approximates 40–50% of that found in human (AH) cells, dimers are probably not the primary photoproducts involved because (*a*) relatively few, if any, nuclease-susceptible sites are removed from UV-injured chick DNA during incubation of the cultures in the dark, and (*b*) the magnitude of repair synthesis in the chick cultures is practically the same regardless of the presence or absence of black light during post-UV incubation. The limited repair data presently available for *Xenopus* fibroblasts suggest that a similar situation may also exist in these amphibian cells (Cook, 1972).

The most reasonable explanation for our results is that there exists a specific class of photochemical lesions, other than pyrimidine dimers, in the DNA of irradiated embryonic chick cells which is subject to excision repair. Even if the repaired regions

in human and chick DNA are of identical size, the incidence of the avian photoproducts cannot be estimated with any degree of certainty solely on the basis of the relative levels of repair synthesis detected in the two strains and the known number of dimers in human DNA (Cleaver, 1973). Therefore, the frequency as well as the precise chemical structure of the unidentified photoproducts in the chick DNA must await further experimentation.

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