*XERODERMA PIGMENTOSUM: A HUMAN DISEASE IN WHICH AN INITIAL STAGE OF DNA REPAIR IS DEFECTIVE**

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Abstract.—Homozygous xeroderma pigmentosum fibroblasts cannot repair damage to DNA bases, but can repair damage that involves chain breaks. In xeroderma pigmentosum, therefore, there is a defect in an early step in repair at which base damage is recognized and the polynucleotide chain broken enzymatically (by an endonuclease). Heterozygous fibroblasts repair base damage to normal extents. Carcinogenesis in xeroderma pigmentosum, and perhaps in some normal individuals, may be the result of somatic mutations caused by unrepaired damage.

Xeroderma pigmentosum is an autosomal recessive disease in which the skin is extremely sensitive to UV light and there is a high incidence of skin cancers.[1,2] There are two similar clinical forms: one has skin symptoms only and the other, the de Sanctis-Cacchione syndrome,[3,4] has neurological disorders in addition.

Skin fibroblasts from patients with homozygous xeroderma pigmentosum show reduced amounts of DNA repair replication *in vitro*.[5] Repair replication, which is the insertion of small regions of new bases into DNA strands to replace excised damaged regions,[6,7] is a final stage of DNA repair. Its absence in xeroderma pigmentosum cells could be due to a defect in any of the earlier stages. To delineate a possible defect in these early stages, we have studied repair replication after two kinds of radiation damage to DNA: (*a*) base damage without chain breakage (UV light[8]), (*b*) chain breakage (X-rays,[9,10] UV light after incorporation of bromouracil deoxyriboside into DNA).[11,12] The rationale for these experiments is that repair of damage to DNA bases requires enzymatic scission of the polynucleotide chain,[13] excision of damage, and repair replication.[7] Repair of strand breaks does not require the initial enzymatic chain scission, but may involve the later stages[14,15] (Fig. 1).

The results show that fibroblasts from homozygous xeroderma pigmentosum patients can perform repair replication after chain breakage but not after base damage; they may therefore lack some enzyme necessary for an initial stage of repair. In the heterozygote, normal amounts of repair replication occur after irradiation with UV light.

Materials and Methods.—Tissue culture: Sterile 1-mm punch biopsies were taken from the skin of a male patient (unrelated to the patients used for our first study[5]) suffering from xeroderma pigmentosum with neurological disorders, from a parent of another patient, and from normal individuals. Biopsies taken from nonmalignant skin of sun-exposed regions (forearm) and unexposed regions (back) gave similar results. Fibroblast cultures were developed from biopsies, using McCoy's medium[16] with 30 per cent fetal calf serum (Grand Island Biological Co.) and grown in glass flasks or plastic Petri dishes at 37°C.

Irradiation: Cultures were irradiated with UV light (predominantly 2537 Å) at an incident dose rate of 7 erg/mm²/sec, using the procedure described previously.[5, 17] The

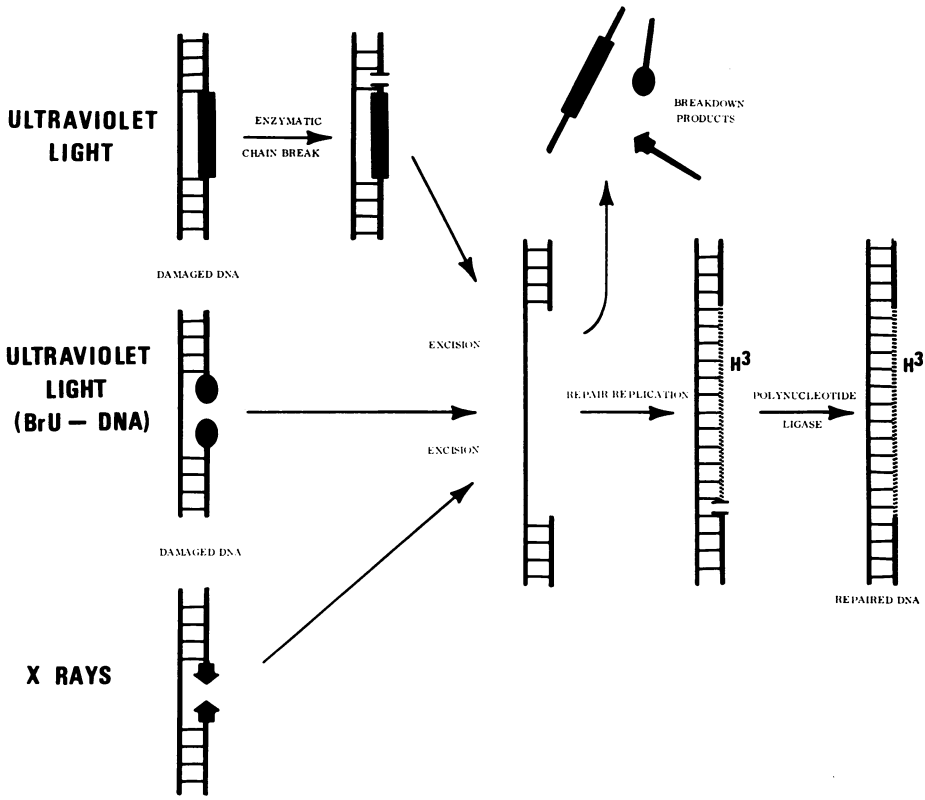

FIG. 1.—Possible scheme for DNA repair in mammalian cells indicating common pathways for repair of base damage and single strand breaks. This scheme illustrates merely the necessary stages of repair, and not every biochemical reaction, and is intended as a guide to the interpretation of results in this paper.

X-ray source was a 300 kVp G. E. Maxitron, which gave a dose rate of 3.4 kr/min with no external filtration.

Labeling methods: To label cells, the medium was replaced with freshly prepared McCoy's medium which contained H^3TdR (10 μCi/ml, 17 Ci/mmole) (New England Nuclear Corp.). After incubation, the radioactive medium was removed and the cells were washed twice with 0.15 *M* sodium chloride-0.015 *M* sodium citrate (saline-citrate) and processed for autoradiography.

Autoradiography: Cultures were treated for 5 min with 0.25% trypsin, and the resulting cell suspensions immediately centrifuged. The pellet was dispersed in 0.7% sodium citrate for 5 min at room temperature and centrifuged; cells were fixed with acetic acid: ethanol (1 : 3) for 10 min, and suspensions dropped onto wet slides and air-dried. Slides were dipped in Kodak NTB liquid emulsion and developed after 1–8 weeks' exposure. The average number of grains over nuclei was determined by counting 40 labeled cells, and the background was determined by counting the number of grains in 20 fields equal in size to a cell nucleus. Background was subtracted from grain number before expressing the results as an average number of grains per nucleus. The exposure was adjusted so that the actual average number of grains counted was between 20 and 50. The standard error of an average grain number per nucleus was 15%.

Results.—Unscheduled synthesis after UV-irradiation: After irradiation with

UV light, most cells incorporate H^3TdR into DNA during all stages of the cell cycle, instead of only during the *S* phase.[18-22] This phenomenon, "unscheduled synthesis,"[20] is correlated with repair replication, which is detected in cesium chloride density gradients in many cell types,[5, 21] and both processes respond in a similar manner to various inhibitors.[22] They therefore represent the same DNA repair process seen through quite different techniques.[23] Cells that are out of the *S* phase (i.e., those in G_1, G_2, and mitosis) and incorporate H^3TdR after UV irradiation are performing only unscheduled synthesis, since semiconservative replication is confined to the *S* phase. To ensure that *S*-phase cells could be unambiguously identified in autoradiographs, cultures were labeled for one hr with H^3TdR before irradiation to label *S*-phase cells heavily, and then were labeled for two hours more before fixation and autoradiography. Cells undergoing unscheduled synthesis were then clearly distinguishable as lightly labeled cells (Fig. 2, *c*, *d*). The amount of H^3TdR incorporated by unscheduled synthesis after various doses of UV was determined by counting the number of grains over nuclei of lightly labeled cells (Fig. 3).

In normal and heterozygous xeroderma pigmentosum fibroblasts, the unscheduled synthesis increased as a function of UV dose and approached a plateau after doses of 300 to 400 erg/mm^2. Previously, unscheduled synthesis has been shown to be a linear function of the log dose.[5, 19] It is plotted against a linear dose scale here to include zero dose points and to show the relatively rapid increase at low dose levels. In homozygous xeroderma pigmentosum fibroblasts there is no increase in unscheduled synthesis as a function of UV dose above the grain number recorded for zero dose.[24] Unscheduled synthesis, like repair replication,[23] is therefore absent in this case of the disease.

Unscheduled synthesis after BrUdR substitution of DNA: To replace DNA thymine with BrU, cultures of normal and homozygous xeroderma pigmentosum fibroblasts were grown in 5 μg/ml BrUdR for 25 hr.[16] They were then rinsed, grown for one hr in H^3TdR to label *S*-phase cells heavily, irradiated with UV light, and labeled for two hr in H^3TdR. The histogram of grain numbers for the two cell types is shown in Figure 4. Both types of fibroblasts show an increase in unscheduled synthesis as a function of dose, but the xeroderma pigmentosum fibroblast cultures also have a population of cells with very low grain numbers. The mean of the latter distribution is similar to that of irradiated xeroderma pigmentosum fibroblasts with no BrUdR (Fig. 3). In view of the qualitative observation that xeroderma pigmentosum fibroblasts grow more slowly than normal fibroblasts, those cells with near zero grain counts probably did not enter the *S* phase during the 25 hr of growth in BrUdR and consequently contain no BrUdR. The cells with near-zero grain numbers were therefore excluded before calculating average grain numbers; only lightly labeled cells with greater than 10 grains/week exposure (i.e., 20 grains for histograms of Fig. 4) were used. The average grain number as a function of UV dose is shown in Figure 5, where it can be seen that both normal and xeroderma pigmentosum fibroblasts have a similar dose response.

Unscheduled synthesis after irradiation with X rays: Unscheduled synthesis was studied after irradiation with X rays, at doses of 0.5 to 100 kr, with the same

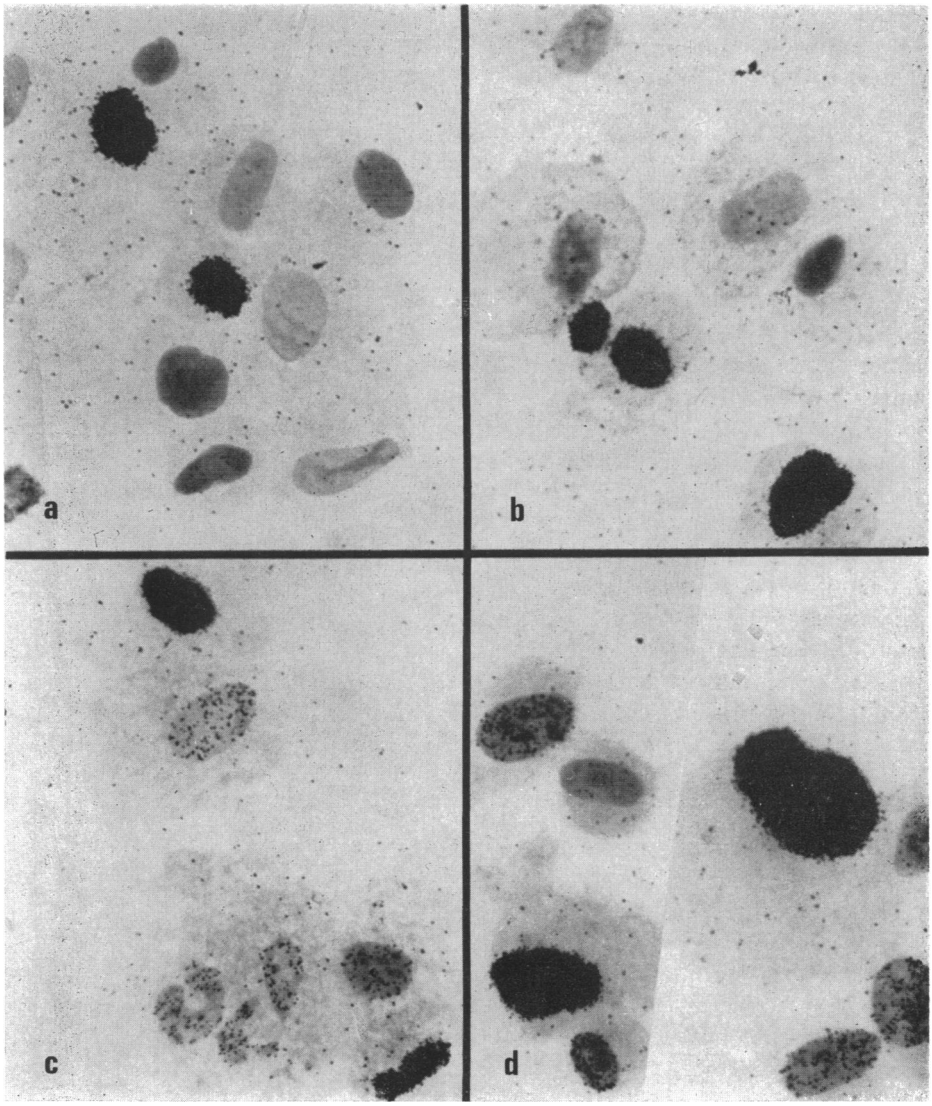

FIG. 2.—Autoradiographs of human fibroblasts irradiated with UV light and labeled for 2 hr in H^3TdR: heavily labeled *S*-phase cells, cells lightly labeled from unscheduled synthesis, and unlabeled cells. (*a*) homozygous xeroderma pigmentosum control, (*b*) homozygous xeroderma pigmentosum, 630 erg/mm^2, (*c*) normal fibroblasts, 630 erg/mm^2, (*d*) homozygous xeroderma pigmentosum after 25 hr in BrUdR, 700 erg/mm^2.

protocol as that used for irradiation with UV light. Both normal and xeroderma pigmentosum fibroblasts showed increases in unscheduled synthesis due to irradiation, but the amount is barely detectable after 0.5 and 1 kr (Table 1). Unlike the previous experiment with BrUdR-substituted cells, however, the xeroderma pigmentosum fibroblasts did not have a population of cells with near-zero grain count after irradiation.

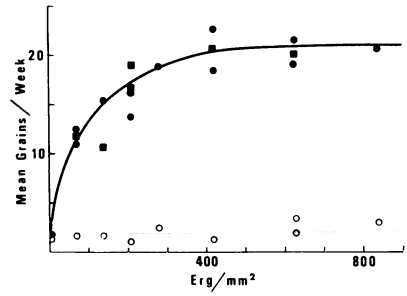

FIG. 3.—Average grain number per week exposure over lightly labeled cells in autoradiographs of human fibroblasts irradiated with UV light and labeled for 2 hr with H^3TdR. ●, Normal fibroblasts; ■, heterozygous xeroderma pigmentosum fibroblasts; ○, homozygous xeroderma pigmentosum fibroblasts.

Discussion.—These experiments demonstrate that fibroblasts, which are homozygous for the gene xeroderma pigmentosum, cannot perform unscheduled synthesis (i.e., DNA repair) after irradiation with UV light. These cells can perform normal amounts of repair after irradiation with X rays, or after irradiation with UV light when their DNA contains BrU. Xeroderma pigmentosum

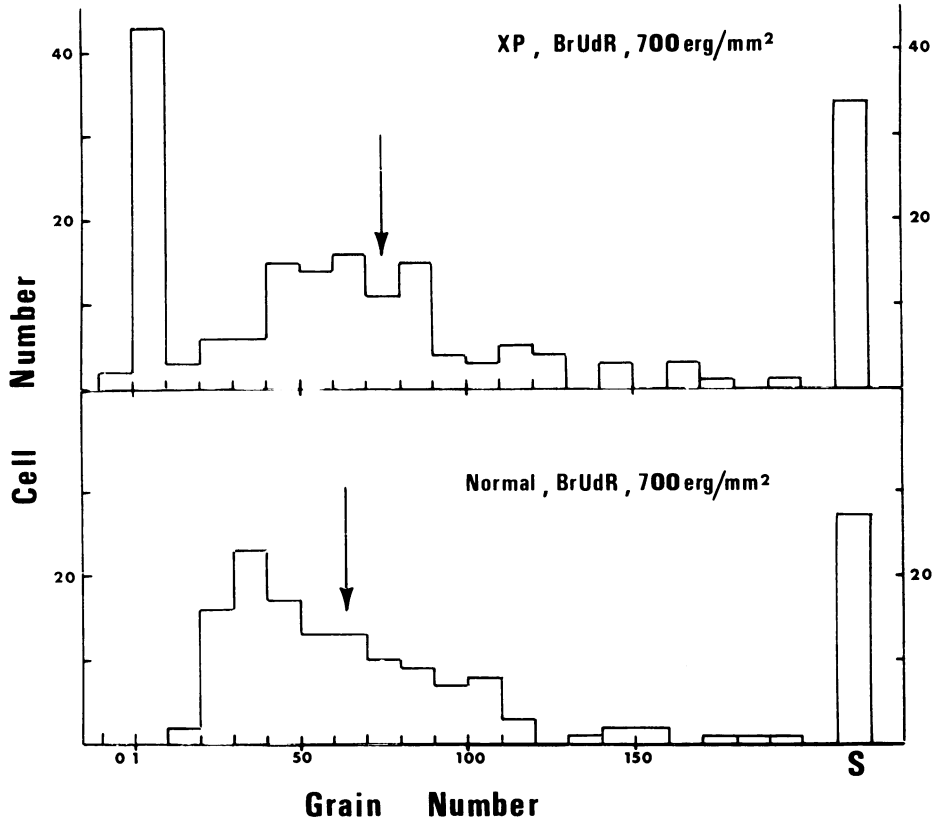

FIG. 4.—Histogram of grain numbers in autoradiographs (2 weeks' exposure) of normal and xeroderma pigmentosum fibroblasts grown for 25 hr in BrUdR and irradiated with 700 erg/mm² UV. Grain number classes 0, 1 to 9, 10 to 19, etc. *S* indicates cells with more than 200 grains/nucleus in the *S* phase of the cell cycle during labeling. Arrows indicate average grain numbers excluding *S*-phase cells.

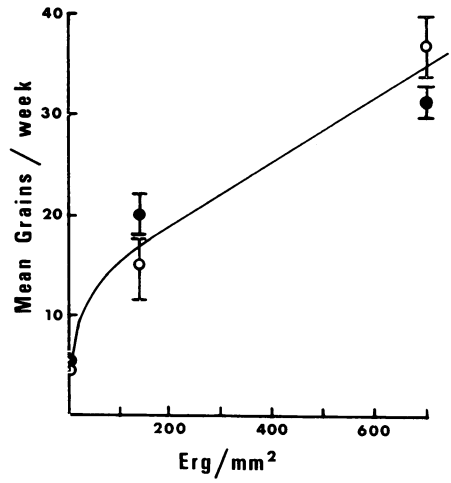

FIG. 5.—Average grain numbers over lightly labeled cells in autoradiographs of human fibroblasts grown for 25 hr in BrUdR, irradiated with UV light, and labeled with H^3TdR for 2 hr. ●, Normal fibroblasts; ○, homozygous xeroderma pigmentosum fibroblasts. Bars denote standard errors of mean.

cells therefore cannot repair damage from UV light,[8] but can repair the other forms of damage that involve chain breakage.[9-12] Heterozygotes perform repair after irradiation with UV light to the same extent as normal cells. Repair after each of these three kinds of radiation injury involves the insertion of small numbers of bases throughout the DNA,[5, 15, 17-22, 25] presumably to replace excised damaged regions. Repair of many different kinds of radiation injury may thus proceed at least in part by a common metabolic process (Fig. 1).

The breaks produced by X rays are not reparable by the rejoining enzyme alone.[26] This enzyme, polynucleotide ligase, requires juxtaposed 3'OH and 5'phosphate termini,[27, 28] and rejoining may occur after excision of a few bases on either side of the break and their replacement by repair replication (Fig. 1).[14] A similar process may also be required after the strand breaks formed in BrU-substituted DNA by UV light, but the amount of repair at relatively low doses (100 to 200 erg/mm², Fig. 5) is much larger than that after low doses of X rays (Table 1). The former damage may be much more extensive, requiring more repair than X-ray damage. The sensitizing effects of BrU substitution to radiation may be due to incomplete repair of such extensive damage.[17]

The deficiency in xeroderma pigmentosum may therefore be in an enzyme that breaks DNA (an endonuclease?) near damaged regions.[29] This enzyme

TABLE 1. *Average grain numbers over lightly labeled cells (unscheduled synthesis) after irradiation with X rays. (Labeled with 10 μCi/ml, 17 Ci/mmole H^3TdR in McCoy's medium, and autoradiographs exposed for 8 weeks.)*

Dose	Normal fibroblasts	Xeroderma pigmentosum
0.5 kr	7.9	—
1.0 kr	10.8	12.6
6.0 kr	26.6	25.1
100 kr*	25.5*	23.0*

* These experiments at this dose were done six months earlier than the others, and the autoradiographs were exposed for only one week. If we assume that the exposure and development conditions for autoradiography are similar in the two sets of experiments, the grain numbers can be corrected by assuming a linear increase of grain numbers with exposure time.[27] This correction gives 204 grains (normal) and 184 grains (xeroderma pigmentosum) for 8 weeks' exposure.

would not be necessary when the damage itself involves a chain break. The enzymes for later stages of repair may all be present in xeroderma pigmentosum, because normal amounts of repair occur after damage involving chain breakage. If pyrimidine dimers are responsible for the major part of the biological effect of UV light, then dimer excision should occur in normal, but not xeroderma pigmentosum, fibroblasts. Contrary to a previous report,[30] however, dimer excision has not been detected in preliminary experiments from either of these human cell types.[31]

An outstanding feature of xeroderma pigmentosum is a high level of carcinogenesis. This would be consistent with the theory of somatic gene mutation, resultant from defective repair, in the etiology of cancer.[32] But the occurrence of malignant cells, such as HeLa, which can perform repair replication of UV damage,[18-22, 25] shows that defective repair is not essential for carcinogenesis. Somatic mutation could perhaps occur in normal cells because of inefficient repair, since even normal human cells have only a limited capacity for repair of some forms of damage, e.g., pyrimidine dimers.[30, 31]

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Note added in proof: Dimer excision can now be detected in normal fibroblasts, but only at low doses below 200 erg/mm^2. At this dose, the dimer yield is low, and insufficient H^{3}TdR could be incorporated into xeroderma pigmentosum fibroblast to prove conclusively the absence of excision.

Abbreviations: DNA, deoxyribonucleic acid; UV, ultraviolet; H^{3}TdR, tritium-labeled thymidine; BrU, bromouracil; BrUdR, bromouracil deoxyriboside; BrU-DNA, double-strand DNA molecules with bromouracil replacing thymine in one strand.

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[16] Although not mentioned in the GIBCO catalog as a deliberate constituent, McCoy's medium contained significant amounts of TdR. On the basis of the density of DNA synthesized in McCoy's medium containing 5 μg/ml BrUdR and 10^{-6} *M* FUdR, the medium appeared to contain between 4 and 5 μg/ml of TdR. In the experiments using BrUdR, cultures were transferred to 199 medium with 15% fetal calf serum to avoid competition with TdR.

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[24] The grain numbers over non-*S*-phase cells in unirradiated normal and in homozygous xeroderma pigmentosum fibroblasts were slightly above background (Fig. 3). This did not appear to be due exclusively to nuclear labeling, but was distributed over the whole cell and may have been due to cytoplasmic labeling of protein or an infection such as mycoplasma. This artifact is therefore irrelevant to the study of unscheduled synthesis and does not interfere with the general conclusions based on the grain numbers in Fig. 3.

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