Significance of Repair of Human DNA: Evidence From Studies of Xeroderma Pigmentosum

XP is a rare autosomal recessive disease in which all patients have accelerated chronic actinic degeneration of sun-exposed skin, including development of numerous sunlight-induced neoplasms (1-3). Some XP patients also develop neurologic abnormalities that are due to premature death of nerve cells (1). A decade ago Cleaver discovered (4) that cells from XP patients were defective in repairing UV radiation-induced damage to their DNA. Since then, numerous additional studies have confirmed that XP patients have defective repair of DNA damaged by UV and certain mutagenic chemicals (1, 5-11). XP is currently the only human disease in which inherited defects in DNA repair have been conclusively shown to exist. It is the purpose of this editorial to describe recent studies relating certain clinical manifestations of XP to the patients' defective DNA repair and to illustrate how such studies have contributed to our understanding of the consequences of physical and chemical damage to human DNA.

Despite the low incidence of XP, estimated to occur with a frequency of 1 (12) to 4 (1) per million births, established cell repositories3 can provide cells from all 18 XP patients who were evaluated clinically at the National Institutes of Health (1, 13) as well as from several of the most important and unusual XP patients from Europe and the Middle East whose cells were studied in detail at Erasmus University, Rotterdam, The Netherlands. Published studies of approximately 70 Japanese patients with XP (14, 15), together with numerous published reports on other XP cells, suggest that the literature may already contain data on as many as 150 XP strains. Photobiologic and cell-fusion studies show that there are at least the following seven confirmed genetic forms of XP: six excision-deficient forms designated complementation groups A through F (1, 15, 16) and the excision-proficient form (17) designated the "XP variant" (18-21). To understand the basic aspects of XP and to choose appropriate cell strains for study, one must understand the principles of this classification and some of the photobiologic and clinical features of each genetic form. Of particular importance is the heterogeneity of DNA repair-dependent processes recently found (13, 22-24) among strains within each of the most common excision-deficient groups (i.e., groups A and C). Furthermore, certain clinical manifestations are now known to be related to the overall capacity of a cell strain to repair its damaged DNA to a biologically functional state (13, 22). There is now substantial evidence (13, 22, 25, 26) for the hypothesis that the normal functioning of DNA repair processes prevents not only sunlight-induced neoplasia but also premature death of neurons in all healthy humans.

RELEVANT CLINICAL ABNORMALITIES ON SUN-EXPOSED SKIN

XP patients have excessive freckling on sun-exposed skin usually before 2 years of age (fig. 1) [for color photographs, cf. (1)]. There is evidence (1, 27, 28) that each XP freckle, as might be the case for freckles in normal persons, is a clone of melanocytes derived from a single melanin-producing cell that has undergone a UV-induced mutation. Sun-exposed XP skin also has hypopigmented areas that might also arise from mutations making the melanocytes unable to produce normal pigmentation. Many children with XP develop actinic keratoses and frank cutaneous cancers before they reach 10 years of age (1). Actinic keratoses are the most frequent, followed by basal and squamous cell carcinomas and malignant melanomas (1). These tumors in XP patients occur with the same relative frequencies as, and are clinically and pathologically indistinguishable from, those in persons without XP (1, 29). However, XP patients can develop hundreds of such tumors before they are 30 years old (1). The sunlight-exposed portions of the eyes of all XP patients develop conjunctivitis, and many patients develop neoplasms of the conjunctiva or cornea (30). All these XP pigmentation abnormalities and neoplasms might arise from somatic mutations induced by the UV radiation in sunlight that induces photochemical damage in the DNA of human cells (31). The mutation frequency per unit dose of UV is higher in XP cells than in normal cells (32, 33). Sun-exposed XP skin also shows marked atrophy, and in vitro studies of XP cells have shown them to be more readily killed by UV radiation than normal skin (34). Sun-exposed XP skin also shows marked atrophy, and in vitro studies of XP cells have shown them to be more readily killed by UV.

Abbreviations Used: SV40=simian virus 40; UDS=unscheduled DNA synthesis; XP=xeroderma pigmentosum.

Editor's note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.

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ABBREVIATIONS USED: SV40=simian virus 40; UDS=unscheduled DNA synthesis; XP=xeroderma pigmentosum.
radiation than normal cells (13, 14, 22, 32). Although all these XP abnormalities may be the result of photochemical damage in DNA, the mechanisms leading to the clinical manifestations are not fully understood.

DEFECTIVE UV-INDUCED UDS

Although UV radiation causes several types of damage to DNA (34, 35), the pyrimidine dimer is probably one of the most biologically significant types of UV damage (36). When DNA is exposed to UV radiation, covalently linked cyclobutane dimers form between adjacent pyrimidines on the same DNA strand (1, 31, 34, 37-40). In excision repair of DNA, the dimer-containing region is removed (probably by several sequential reactions involving at least an endonuclease and an exonuclease), the resulting space is then filled in by repair synthesis by a DNA polymerase with the use of the intact opposing strand as a template, and the newly synthesized portion is joined to the remaining part of its strand by a polynucleotide ligase. The steps through repair synthesis can be evaluated autoradiographically (fig. 2) by the measurement of the radioactive thymidine incorporated during UDS. UDS is DNA synthesis that occurs during phases of the cell cycle other than the scheduled S-phase (1, 11). Cultured dermal fibroblasts from all excision repair-deficient XP cells show a reduced rate of such UV-induced UDS. The excision defect is probably present in all XP cells capable of division and has been demonstrated with epidermal cells (42-45), conjunctival cells (30), peripheral blood lymphocytes (17, 45), and Epstein-Barr virus-transformed lymphocyte lines (46).

COMPLEMENTATION GROUP ANALYSIS

The autoradiographic technique for the determination of UV-induced UDS was first utilized in Bootsmans laboratory at Erasmus University (47, 48) to show by cell-fusion studies that at least three complementation groups were among excision-deficient XP strains. Subsequently, our laboratory found four complementation groups among the National Institutes of Health XP patients (1, 49). In a collaborative study between our laboratories (16) we had established by 1975 the existence of five complementation groups among the excision-deficient XP patients, and these groups were designated A, B, C, D, and E. More recently, the existence of another group, designated F, has been confirmed (15). When a fibroblast from one complementation group is fused with an inactive Sendai virus to a fibroblast from another group, each cell in the resulting binucleate cell (heterokaryon) provides the other with its missing gene product so that both nuclei perform more UV-induced UDS than either could perform when unfused. Thus the nuclei “complement” each other and acquire normal rates of UV-induced UDS (1, 16, 49) (fig. 3; binuclear cell e). Fusions between fibroblasts in the same complementation group show no complementation (1, 16, 49, 50) (fig. 3; binuclear cell f). Most likely each of the six complementation groups has a different deoxyribonucleotide sequence defective and all members of the same complementation group have the same nucleotide sequence defective. However, the defects within the nucleotide sequence of any given complementation group can differ except in the case of strains from the same kindred. This interpretation predicts heterogeneity of the repair defect (i.e., genetic polymorphism and multiple alleles) among strains from unrelated patients within a complementation group, and results from experiments of post-UV colony-forming ability (13) and from UV-induced sister chromatid exchanges (23, 24) are in accord with this hypothesis (see below).

Text-figure 1 shows results from one of the experiments in which we demonstrated for the first time that an XP patient with some neurologic abnormalities was in complementation group C. The control donor’s irradiated mononuclear cells (text-fig. 1a) had a mean grain count of UV-induced UDS of 79.3 grains/nucleus (arrow), whereas the unirradiated cells (data not presented) had a mean grain count of only 0.85 grains/nucleus. When strain CRL 1333 was fused with a known group A (text-fig. 1b) or group D strain (text-fig. 1d) and the cells were irradiated, a population of binuclear cells (heterokaryons) was present whose nuclei had essentially the same frequency of grain distribution and mean grain count (81.0 and 84.0, respectively) as those of the control donor’s irradiated mononuclear cells (text-fig. 1a), which indicated that complete complementation had occurred. No complementation occurred when strain CRL 1333 was fused to a known group C strain (text-fig. 1c). Thus strain CRL 1333 is assigned to complementation group C. The binuclear cells that gave rise to the populations of labeled nuclei having mean grain counts of 6.6 (text-fig. 1b) and 18.6 (text-fig. 1d) are homokaryons of the strains used in the particular fusions, and each homokaryon contains two nuclei from the same strain. The binuclear cells that gave rise to the population of labeled nuclei having a mean grain count of 10.6 (text-fig. 1c) are homokaryons of strain CRL 1333 or of strain XP9BE as well as heterokaryons formed by the fusion of a cell from one strain with a cell from the other strain. These heterokaryons show no complementation, inasmuch as their nuclei are derived from strains in the same complementation group. The mean grain count of 10.6 (text-fig. 1c) was similar to the mean grain count (data not presented) for strain CRL 1333’s irradiated mononuclear cells (10.1) and for its binuclear homokaryons (10.9) obtained on slides in this experiment in which cells from strain CRL 1333 were fused to each other. The data show that strain CRL 1333 has approximately 15% of the control donor’s UDS rate and is, therefore, well within the range (10-20% of normal).

In partial accordance with an international effort to standardize nomenclature of cell strains from patients with hereditary diseases, designations such as XP9BE will signify both the patient and fibroblast strains derived from the patient: XP, xeroderma pigmentosum; BE, Bethesda; LO, London; RO, Rotterdam; OS, Osaka. The number is that assigned to the particular patient in the city’s XP patient series.
FEATURES OF THE XP COMPLEMENTATION GROUPS

In the United States (1) and Europe (5) the most frequently found complementation group is group C. Only one group C patient appears to have the neurologic abnormalities of XP (25) (see below). Interestingly, no group C patient has yet been found among the 70 patients studied in Japan (15). Group A patients are the most common excision-deficient patients in Japan and are probably the second most common group in the United States (1) and in Europe (5). Most group A patients have very severe neurologic abnormalities that become manifest before 7 years of age, whereas other group A patients have few or no known neurologic abnormalities (1, 13). The typical group A patient, with or without neurologic abnormalities, has from 0.4 to 1.3% of the normal rate of UV-induced UDS (51), but one patient, XP8LO, has 0.8% of the normal rate (50, 52). XP patients in all five confirmed group D kindreds have neurologic abnormalities that generally become well manifested between 7 and 12 years of age (13). We (1, 16, 49, 53) and others (50, 54) have found these group D patients to have more UV-induced UDS than group C strains (i.e., 25-50% of the normal rate), although the Rotterdam investigators obtain a lower rate with one of our group D kindreds (16). Each of complementation groups B, E, and F is represented by a single kindred. Group B is of unusual interest in that the sole patient in this group has another rare autosomal recessive disease, Cockayne’s syndrome (1, 55), which is characterized by acute sun sensitivity, cachectic dwarfism, and neurologic and skeletal abnormalities (55–57). Although fibroblasts from patients with only Cockayne’s syndrome have decreased post-UV colony-forming ability (58, 59) and decreased host-cell reactivation of UV-irradiated adenovirus (60), their UV-induced UDS is normal (59), and they have no evidence of sunlight-induced skin cancers or pigmentation abnormalities (56, 57, 59, 61, 62).

BIOCHEMICAL AND PHYSICOCHEMICAL ABNORMALITIES IN XP CELLS

The primary inherited DNA repair defect has not been established in any form of XP. Probably such knowledge will be obtained only after purification to homogeneity of the appropriate enzymes and other factors involved in DNA repair in XP and normal human cells. The biochemical and physical abnormalities reported in XP cells are extremely complex for at least the following reasons: 1) Each agent that damages DNA, whether it is a physical (X-ray, UV) or chemical agent, produces more than one type of damage, and not all these types of damage are known; 2) abnormalities found in one XP cell strain may differ from those found in another strain, whether or not the strains are in the same complementation group.

The excision proficiency of the variant form of XP was first described in strain XP4BE (17) and was found in all his nucleated cells studied, i.e., peripheral blood small lymphocytes (17), dermal fibroblasts (17, 18), epidermal cells (42, 43), and even his tumor cells (63). Normal rates of excision repair have been confirmed in XP variants by studies of UV-induced UDS (14, 18, 64),

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**Text-Figure 1.—** Histograms of autoradiographs showing that strain CRL 1333 is in group C. Methods as in legends to figs. 2 and 3; explanation and discussion are in text. (Data are from Robbins JH, Moshell AN, Andrews AD, et al; Unpublished data.)

Previously reported for group C patients (1, 16, 49). The significance of this patient’s complementation group assignment will be discussed.
the bromodeoxyuridine photolysis test (1, 65), removal of thymine dimers (10), and disappearance of endonuclease-sensitive sites (66). However, the variant had decreased host-cell reactivation of UV-irradiated adenovirus 2 (19). Such host-cell reactivation (i.e., repair) of UV-damaged, double-stranded DNA viruses that replicate in the cell’s nucleus (9, 15, 67-69) and of UV-damaged, purified DNA of SV40 (70) has been shown to be defective in all XP patients whether excision-proficient or excision-deficient. These studies showed conclusively that the defective phenomena previously observed in XP cells were not due to abnormally increased UV damage to the XP cells (compared with the damage in normal cells), for only the virus was irradiated. The XP cells’ inability to repair the UV-irradiated, purified SV40 DNA (70) and the low UV dose required to prevent reactivation of adenovirus in the most defective cells (69) indicate that the UV-induced damage which XP cells cannot repair normally is most likely pyrimidine dimers rather than DNA-protein cross-links.

XP variant fibroblasts were characterized further and found to have a severe defect in “postreplication repair” (20, 71), i.e., a defect in the ability to synthesize new daughter DNA immediately after UV irradiation. Such synthesis in the variant cells is sensitive to caffeine, but in normal cells is insensitive to caffeine. An interesting finding (71) was that the XP strains tested from groups A, B, C, and D have an intermediate defect in postreplication repair and some sensitivity to caffeine, whereas the group E cells show no detectable postreplication repair defect (71).

XP cells appear to repair single strand breaks (72) and probably most of the other biologically significant DNA damage induced by X-ray as well as normal cells (73, 74). However, one component of X-ray damage produced under anoxic conditions and reported to be repaired abnormally by XP cells (75) may resemble a form of UV-type damage that XP cells do not repair normally.

Other techniques have been used to study abnormalities related to DNA repair primarily in excision-deficient cells. Thymine dimers have been shown to be removed from the acid-insoluble fraction at a lower rate than normal (76, 77); repair replication has been shown to be deficient by centrifugation methods (4, 34, 78-80) and by the bromodeoxyuridine photolysis method (65, 81); the cells lose UV-endonuclease sites at a slower rate than normal cells (66); the cells show no detectable evidence of single-strand DNA breaks in the alkaline elution technique of Fornace et al. (82). It has been reported (83) that human cells have a light-dependent enzymatic repair process that monomerizes the thymine dimer, i.e., splits it so that the bases are restored to their preirradiation configuration without being excised. It has been further reported that XP patients (84) and their parents (84) are defective in such photoreactivation, but the significance, and even the existence, of this process in human cells have been questioned (85).

It has been reported (86) that group A and group D cells also have defects in another mode of excision repair than that described above, i.e., a defect in base excision repair. In base excision repair (87), damaged bases in the DNA are cleaved from the sugar in the DNA backbone, and the DNA strand is then incised by an apurinic-apyrimidinic site endonuclease. Other biochemical studies (88) have shown that extracts from some, but not all, XP strains are unable to excise pyrimidine dimers from chromatin but can excise them from purified DNA. Therefore, the repair enzymes of some XP strains may be unable to reach the DNA damage in their native chromatin.

As previously mentioned, XP cells are deficient in repairing DNA damaged by certain chemical mutagens-carcinogens (1, 6, 7, 9, 11). The N-acetoxy ester derivatives of aromatic amide carcinogens and the K-region epoxides of carcinogenic hydrocarbons kill and mutagenize XP fibroblasts more readily than normal fibroblasts (32, 33). Evidence indicates that different modes of DNA repair are required to repair the different types of damage caused by a single chemical carcinogen and to repair the different types of damage caused by different carcinogens (6, 11, 87, 89).

Although the chemical and other DNA repair-dependent abnormalities in XP are multiple, complex, and not currently understood, one may draw certain useful conclusions concerning the defects in XP and the patients’ excessive UV-induced neoplasms and pigmentation abnormalities. All XP patients’ skin fibroblasts have an abnormal susceptibility to the killing effects of UV radiation as demonstrated by their decreased ability to form colonies after UV irradiation in vitro (13-15, 22). In addition, all XP strains so far tested also have impaired abilities to reactiviate UV-irradiated double-stranded DNA viruses (9, 15, 69) and purified viral DNA (70). Cockayne’s syndrome patients who do not have coincident XP also have decreased host-cell reactivation of UV-irradiated adenovirus (60) but do not have the tumors or pigmentation abnormalities of XP. Therefore, the defect(s) leading to increased sensitivity to the killing effects of UV and to decreased host-cell reactivation, as these parameters are currently measured, appear necessary but not sufficient phenomena to account for UV-induced neoplasia and pigmentation abnormalities. But the cutaneous abnormalities of sun-exposed XP skin develop whenever these defective parameters are present together with either decreased UV-induced UDS (e.g., XP group E and group F kindreds) or with abnormal “postreplication” repair (e.g., XP variants) or with both decreased UV-induced UDS and abnormal postreplication repair (XP groups A-D). Inasmuch as no human without such clinical evidence of premature, chronic solar skin damage has yet been shown to have one of the aforesaid combinations of defective DNA repair-dependent processes found in XP cells, probably the normal functioning of these processes protects normal humans from such premature solar damage.

Before concluding the discussion of DNA repair-dependent abnormalities in XP, I should emphasize...
that the processes by which photochemical and other damage to DNA leads to mutations (e.g., neoplasms and freckles) are still unknown. On the one hand, an "error-free" DNA repair process should not lead to mutations that result in cancer or freckles unless it is overwhelmed with DNA damage. An "error-prone" DNA repair process, on the other hand, would more likely result in heritable somatic mutations in the damaged cells' progeny. The normal human nucleotide excision repair process may be essentially error-free. In fact, there is evidence that in the excision-deficient XP cells, the residual UV-induced UDS of the excision repair process is error-free (32, 33, 90). Nevertheless, if the cells synthesize daughter DNA from parental DNA in which the excision repair has not been completed, heritable somatic mutations may result. Slow excision repair may also result in the presentation of photochemically damaged DNA to error-prone repair processes that would result in somatic mutations in either the parent or the daughter DNA. There are inducible error-prone DNA repair processes in bacteria (91, 92), and experiments are being performed (93) to determine whether they also exist in mammalian cells.

INCREASED UV-INDUCED SISTER CHROMATID EXCHANGES IN XP CELLS

Sister chromatid exchanges have recently become amenable to appropriate study with the development of improved and accurate methods for their detection (94, 95). There are abnormalities in the number of sister chromatid exchanges in several diseases, all associated with the development of various cancers. For example, high frequencies of "spontaneous" sister chromatid exchanges are found (96) in phytohemagglutinin-stimulated cultured leukocytes from patients with Bloom's syndrome (97) and from skin cancer patients with histories of inorganic trivalent arsenic ingestion (98). Furthermore, the number of sister chromatid exchanges has been reported to be abnormally increased in untreated cells (99) from patients with the inherited disease dyskeratosis congenita and also in their cells (100) treated with trimethylpsoralen followed by 365 nm UV radiation, a combination believed sufficient to produce psoralen-DNA cross-linking photoadducts. Untreated XP cells show no abnormal number of spontaneous sister chromatid exchanges (23, 24, 101-103). However, after exposure of XP cells to 254 nm UV radiation, certain XP strains show much greater numbers of UV-induced sister chromatid exchanges than do normal cells (23, 24, 101, 103). In studies of this phenomenon in XP fibroblasts (23) and in XP lymphocyte lines (24) from excision-deficient XP patients and from XP variants, the following results were obtained (23, 24): Cells from all XP patients were capable of performing at least a normal number of UV-induced sister chromatid exchanges; cells from patients in groups A, C, D, and probably B showed abnormally large numbers of UV-induced sister chromatid exchanges; different strains within a group had significant differences in their abnormal number of UV-induced sister chromatid exchanges, but cells from XP siblings had identical increases. Although the precise molecular events involved in sister chromatid exchange formation are unknown (104), unrepaired DNA damage may cause the excessive UV-induced sister chromatid exchanges observed in XP cells. Of further interest is the recent finding (105, 106) that abnormally increased numbers of sister chromatid exchanges occur in XP fibroblasts exposed in culture to chemical carcinogens including those previously believed to be handled normally by XP cells, for example, methyl methanesulfonate or ethyl methanesulfonate, methyl-nitro-nitrosoguanidine, and ethylnitrosourea. Such sister chromatid exchange formation in XP cells constitutes a sensitive system for the detection of possible genetic effects of such chemicals on human cells (105, 106).

RELATIONSHIP BETWEEN POST-UV COLONY-FORMING ABILITY AND NEUROLOGIC ABNORMALITIES OF XP

Some XP patients develop neurologic abnormalities that first appear in infancy, childhood, or as late as the third decade of life, that usually worsen, and that are due to the premature death of central nervous system neurons in the absence of recognizable and specific histopathology (1). Therefore, as early as 1974, we have considered these neurologic abnormalities of XP to be the result of an abnormal aging of the human nervous system (1). The abnormalities include mental deterioration, microcephaly, sensorineural deafness, areflexia, choreoathetosis, ataxia, extensor plantar reflexes, spasticity, and a neuropathic electromyogram and muscle biopsy (1). Sufficient numbers of these neurologic abnormalities have been found in each of several group A patients (representing four kindreds) and in patients from all five confirmed group D kindreds to indicate that the patients' inherited deoxyribonucleotide sequences defining groups A and D, respectively, determine the occurrence of the neurologic abnormalities in these patients (1, 25, 61). Young children with XP who have all or most of these neurologic abnormalities, together with retarded growth and sexual development, are properly classified as having the De Sanctis-Cacchione syndrome (1).

However, our purported relationship between DNA repair and the premature death of nerve cells in XP patients had certain conceptual problems: Although patients in all five of the group D kindreds generally had a similar time of onset and type of neurologic abnormalities, some of the group A kindreds had severe neurologic abnormalities at an early age whereas others had few or no known neurologic abnormalities even as late as their fourth decade of life (1); no laboratory test had distinguished group A cell strains from patients with neurologic abnormalities from group A cell strains from patients with no known neurologic abnormalities. A second conceptual problem arose from our recent finding (25, 61) that one of the 13 known group C kindreds had a few neurologic abnormalities, where-
as the other 12 had no evidence of XP-type neurologic abnormalities. We have recently performed post-UV colony-forming ability experiments (13, 25, 61, 62) that resolved these questions and showed for the first time a relationship between premature death of neurons and the capacity to restore damaged DNA.

Log-phase fibroblasts were irradiated with 254 nm UV light from a germicidal lamp, trypsinized, and replated at known densities. After 2-4 weeks’ incubation the cells were fixed and stained in the dishes and then scored for colony formation. The colony-forming efficiency, defined as the number of colonies obtained divided by the number of cells plated, was determined for each UV dose. The post-UV colony-forming ability was calculated by the division of the colony-forming efficiency of the irradiated cells at a given dose by the colony-forming efficiency of that strain’s unirradiated cells in the same experiment (13). Twenty-seven XP strains and five control donor strains were studied in over 100 experiments. From two to seven experiments were done with each strain.

Text-figure 2 shows the results of these studies (13, 25, 61). The five group A strains with the lowest post-UV colony-forming ability are all from patients who had severe XP-associated neurologic abnormalities such as progressive mental deterioration, sensorineural hearing loss, ataxia, spasticity, choreoathetosis, and areflexia and in whom these abnormalities became clinically well manifested before the age of 7 years. The early onset of these numerous abnormalities is indicated in text-figure 2 as ++++. The group D strains tested, all of which have greater post-UV colony-forming ability than the aforementioned group A strains, are all from patients who had the same XP-associated neurologic abnormalities as the group A patients but in whom these abnormalities usually became clinically well manifested between 7 and 12 years of age and are, therefore, designated ++. The post-UV colony-forming ability curve for strain XP12BE of group A (1) is below that of the lowest non-neurologic group C curve (bottom line of group C zone) but for most of its long exponential portion is above the group D curves.

This strain was obtained from a patient, now 12 years old, when she was 7 years old. She had only two neurologic abnormalities, absent deep tendon reflexes and an abnormal electroencephalogram (1). Although the age of onset of these abnormalities is unknown, she has not developed any other neurologic abnormalities over the past 2 years. Her neurologic abnormalities are, therefore, among the mildest (+) of the patients with XP-associated neurologic abnormalities. The post-UV colony-forming ability curve for strain CRL 1333, from group C (text-fig. 1), is located close to that of strain XP12BE. Strain CRL 1333 was from a 17-year-old woman with XP who was reported to us to have only a few neurologic abnormalities, viz., mental retardation and microcephaly. Her post-UV colony-forming ability curve, like the curve for XP12BE, is significantly below the lowest group C curve that forms the lower border of the non-neurologic group C zone but significantly above the curve forming the uppermost border of the group D zone. Thus the post-UV colony-forming ability curves for these 2 patients traverse what has been called the post-UV “transition zone” (61) and is situated between the neurologic group D zone and the non-neurologic group C zone. Within this zone we might expect, and apparently have found, post-UV colony-forming ability curves from XP patients with only a few clinically apparent neurologic abnormalities of XP in their second decade of life.

Almost all of the post-UV colony-forming ability curve of group A strain, XP1LO, is within the zone of the group C curves, whereas most of the curve for the highest group A strain, XP8LO, is above that of any group C strain tested. Neither XP1LO (currently 39 yr old) (107) nor patient XP8LO (currently 6 yr old) (50) has any known XP-associated neurologic abnormalities. Similarly, none of the eight group C patients (representing seven kindreds), the two XP variants, or the group E patient (XP2RO), most of whom are already adults and whose curves are represented in text-figure 2, has any known XP-associated neurologic abnormalities.
The differences in post-UV colony-forming ability among the XP strains and between the XP and control donor strains reflect the different capacities of the cells to repair their UV-damaged DNA to the biologically functional level required to complete repeated cycles of cell division. That differences between XP strains' post-UV colony-forming ability result only from differences in their inherited mutations at the DNA repair loci is supported by the findings that, among the four XP sibling pairs studied, post-UV colony-forming ability was identical among strains from siblings (13).

It was important to determine whether the same relative sensitivities to UV shown by fibroblasts in our post-UV colony-forming ability experiments could be demonstrated with a slightly different test of cell killing (staining with trypan blue) and with a different cell type. Accordingly, we prepared lymphocyte lines from many of our XP patients by transforming their peripheral blood B-lymphocytes with Epstein-Barr virus. We had previously shown that XP patients' lymphocyte lines manifested the same DNA repair-dependent abnormalities as their fibroblasts, i.e., increased sensitivity to UV radiation (108), decreased rates of UV-induced UDS (46), and increased UV-induced sister chromatid exchanges (24). Results with this system have so far indicated the same relative sensitivities to UV radiation as those obtained with the post-UV colony-forming ability of the XP patients' fibroblasts as the following relative survivals of the lymphocyte lines indicate: normal control donors > group E (XP3RO) > XP variant (XPPHBE) > high group C (XP2BE; XP8BE; XP9BE) > low group C (XP1BE; XP3BE) > group A patient with only a few neurologic abnormalities (XP12BE) > group D (XP17BE) > severe neurologic group A (XP20S) (Andrews AD, Moshell AN, Robbins JH; Moshell AN, Newfield SA, Andrews AD, et al: Unpublished observations).

The fibroblast and lymphocyte survival results suggest that different kindreds within a single complementation group (e.g., A or C) display genetic polymorphism, i.e., have different inherited mutations in their DNA repair gene loci. The results also suggest that XP patients with neurologic abnormalities have DNA repair processes so defective that neuronal DNA, perhaps damaged "spontaneously" or by endogenous chemicals, is not repaired to the extent required for neuron survival. Such damaged neuronal DNA apparently requires the same process(es) for its repair as DNA damaged by UV radiation. At least three discrete deoxyribonucleotide sequences, those defining DNA excision repair complementation groups A, C, and D, determine the occurrence of the neurologic abnormalities in XP patients. Considerable evidence suggests that the nucleotide sequence defined by the group A defects and that defined by the group D defect(s) are distinct and do not overlap, inasmuch as fusing a group A fibroblast with a group D fibroblast results in a heterokaryon with fully restored UV-induced UDS (1, 16, 49) and host-cell reactivation (109). Furthermore, the relevant group C nucleotide sequence appears discrete from the sequences defining groups A and D, inasmuch as fusing the neurologic group C patient's strain (CRL 1339) with a group A (text-fig. 1b) or group D strain (text-fig. 1d) results in heterokaryons with fully restored UV-induced UDS.

Our XP data can be extended logically to the following hypotheses (13, 25, 61, 62). The damage induced in the XP cells by the UV radiation requires the same process(es) for its repair as the currently unknown damage we postulate must occur in the DNA of the central nervous system of both XP and normal individuals during embryonic and postnatal life (1). The XP neurons are not repaired as efficiently as normal neurons. As further damage occurs, the XP neurons die prematurely when their genetic information is reduced below the critical level required for biologic function. Even though human neuronal DNA does not replicate after the second postnatal year, the functional integrity of the DNA of these post-mitotic neurons is essential for the survival of the cells. Therefore, damaged neuronal DNA could be involved in the pathogenesis of other degenerative diseases of the human nervous system as well as in "normal aging" of that system. The loss of functional integrity of neuronal DNA can be the result of inherited defects in DNA repair processes such as in XP. Other inherited diseases, such as Cockayne's syndrome (55-57), ataxia telangiectasia (8, 110, 111), and Fanconi's anemia (112), can also have degeneration and/or other abnormalities of the nervous and other organ systems, and cells from these patients have been shown to be abnormally sensitive to certain physical and/or chemical mutagens. Thus Cockayne's syndrome cells are abnormally sensitive to UV radiation (58, 59), ataxia telangiectasia fibroblasts ((113, 114); Andrews AD, Barrett SF, Robbins JH: Unpublished observations] and lymphocyte lines ([115]; Moshell AN, Newfield SA, Robbins JH: Unpublished observations] to γ-rays and possibly to certain chemicals (8, 11, 116), and Fanconi's anemia cells to the DNA interstrand cross-linking properties of certain chemicals (117, 118). Whether or not these diseases are ultimately convincingly shown to be caused by inherited primary defects in DNA repair, it is possible that their nervous system and other clinical abnormalities are due to DNA damage caused by endogenous chemicals or physicochemical events to which their cells are abnormally sensitive in a manner analogous to the postulations concerning death of neurons in XP (1, 13, 61, 62). It is of interest that patients who have ataxia telangiectasia (8, 12, 110, 111) or Fanconi's anemia (12, 112) have increased incidences of malignant neoplasms in various organs.

RELATIONSHIP BETWEEN POST-UV COLONY-FORMING ABILITY AND ACUTE SUN SENSITIVITY IN XP PATIENTS

Among the 18 XP patients we have evaluated clinically (1), we have been unable to relate the degree of the patients' accelerated solar skin degenerations to any in vitro tests of DNA repair. Any such correlation that
might exist would be obscured by the absence of accurate quantitation of the patients' previous sun exposures. However, we have found a correlation between the patients' post-UV colony-forming abilities and the presence or absence of a history of acute sun sensitivity (skin blistering and/or severe erythema after a sun exposure, which would not produce such results in normal individuals). XP patients whose cells had the lowest post-UV colony-forming abilities had histories of acute sun sensitivity, whereas patients with higher post-UV colony-forming abilities had never experienced such acute reactions (13, 26). Thus the acute sun sensitivity experienced by some XP patients apparently results from excessive killing or damage of cells in their skin as a result of inadequate DNA repair.

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Figure 1.—Areas of skin protected from sunlight have not developed pigmentation abnormalities, keratoses, or cancers.
FIGURE 2.—Autoradiographs of normal and XP fibroblasts. A: Normal donor’s nonirradiated cells after 3-hr incubation with tritiated thymidine. The cell, which is “heavily labeled” (arrowhead), was in scheduled (S-phase) DNA synthesis. The other cells are unlabeled. B: The normal donor’s UV-irradiated cells after a 3-hr postirradiation incubation with tritiated thymidine. All cells (except for heavily labeled S-phase cells not shown) are “lightly labeled” owing to the UV-induced incorporation of tritiated thymidine during DNA repair synthesis. C: Irradiated cells of strain XP2BE after a 3-hr postirradiation incubation with tritiated thymidine. All cells other than the heavily labeled cell in S-phase synthesis (arrowhead) are lightly labeled, owing to repair synthesis, but the amount of labeling and, therefore, of repair synthesis is considerably less than that of the normal donor’s cells. Fibroblasts irradiated in saline with 150 ergs/mm² of UV light from a General Electric germicidal lamp (No. G15T8), emitting predominantly 254 nm UV light. The cells were incubated for 3 hr with tritiated thymidine in 37°C air:CO₂ (95%:5%) incubator. Then they were washed with phosphate-buffered saline, fixed in a glutaraldehyde-cacodylate solution, and prepared for autoradiography (emulsion, NTB-3; exposed time, 1 wk). [Figs. are from (1, 46). They are reproduced with permission from the Annals of Internal Medicine.)

Acid hematoxylin. X 300

FIGURE 3.—Autoradiograph of unfused mononuclear fibroblasts (a to d) and of binuclear fibroblasts (e to g) in a Sendai virus-treated culture containing cells from patients XP1BE (group C) and XP12BE (group A). Cells were exposed to 150 ergs/mm² of UV light and treated as described in the legend of fig. 2. The binuclear cell with numerous grains over its nuclei (e) is a heterokaryon whose nuclei have complemented each other, inasmuch as both nuclei have a normal or nearly normal amount of UV-induced tritiated thymidine incorporation, compared with the incorporation in the normal cells in fig. 2B. The other binuclear cells (f and g) are homokaryons, and their nuclei have no more incorporation than the nuclei of the unfused mononuclear cells (a to d). [Fig. is from (1). It is reproduced with permission from the Annals of Internal Medicine.) Acid hematoxylin. X 420