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Defect in DNA Synthesis in Skin of Patients with Xeroderma Pigmentosum Demonstrated in vivo

Abstract. *Exposure of normal human skin in vivo to ultraviolet irradiation at wavelengths shorter than 320 nanometers stimulated an unscheduled DNA synthesis in all of the cell layers of the epidermis and in the upper dermal fibrocytes. The skin of patients with xeroderma pigmentosum did not show this response. Correlation of these findings with previous tissue culture studies suggests that the defect in repair of the damaged DNA in xeroderma pigmentosum cells occurs in vivo as well as in vitro.*

Stimulation of an unscheduled DNA synthesis by ultraviolet irradiation has been demonstrated by autoradiography in a number of mammalian cell types, including human skin cells in vivo and human fibroblasts in vitro with [^{3}H] thymidine (TdR) as the radioactive tracer (*1–3*). The unscheduled synthesis is represented by a uniform, sparse, nuclear labeling of cells not in the DNA replication phase of the cell cycle, whereas cells synthesizing DNA in preparation for division show a dense accumulation of silver grains. This unscheduled synthesis induced by ultraviolet irradiation is apparently missing or is greatly reduced in cultured fibroblasts from patients with the genetic disease xeroderma pigmentosum (XP) (*2*, *3*). This is a rare, inherited, autosomal, recessive disorder characterized by a peculiar sensitivity to ultraviolet radiation which results in multiple cutaneous malignancies within the first few years of life (*4*). We attempted to determine whether the defect in response to ultraviolet radiation noted in cultured XP fibroblasts could be demonstrated in the skin of these patients in vivo. Patients with the two clinical varieties of XP were studied; one individual was affected with skin changes only and two patients were affected with neurological and skin abnormalities (the de Santis-Cacchione syndrome). These were the same patients whose fibroblasts Cleaver studied initially (*2*). Eight normal subjects served as controls.

The clinically undamaged skin of each subject was irradiated with 13.6 $\times$ 10^6 erg/cm^2 of ultraviolet energy at wavelengths shorter than 320 nm (Hanovia hot quartz contact lamp). Fifteen minutes later, [^{3}H]TdR (10 μc; 11 c/mmole) was injected intradermally into the irradiated and adjacent nonirradiated skin of each subject. Biopsies of the injected sites were obtained 1 hour later, and the tissue was fixed in a mixture of formalin, acetic acid, and alcohol. After the specimens were dehydrated, they were embedded in paraplast and sectioned at 4 μm. The sections were then coated with Kodak nuclear track emulsion (type NTB-2). The tissue was exposed for

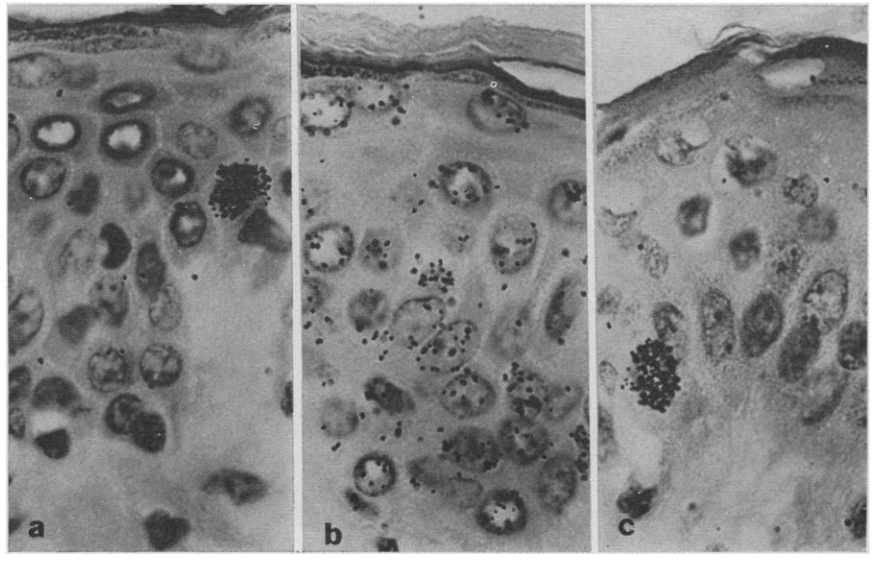

Fig. 1. (a) Normal human epidermis not subjected to ultraviolet irradiation. The autoradiograph shows the dense labeling of one basal cell in the premitotic DNA synthesis phase of the cell cycle. (b) Normal human epidermis subjected to ultraviolet irradiation. The autoradiograph shows sparse labeling of cells throughout the epidermis. (c) Epidermis of a patient with XP subjected to ultraviolet irradiation. Autoradiograph shows one densely labeled basal cell, but no sparse labeling.

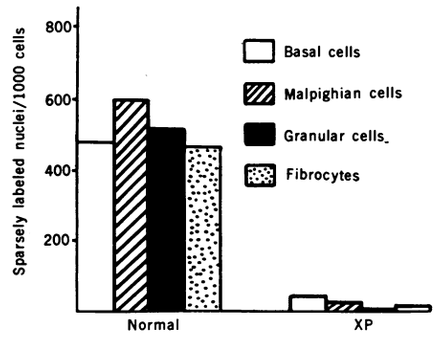

Fig. 2. The mean values for sparsely labeled nuclei (3 to 15 silver grains) in normal skin subjected to ultraviolet irradiation (eight subjects) are compared with mean values for sparsely labeled nuclei in skin of XP patients subjected to ultraviolet irradiation (three patients). The difference between the counts in each cell type of the normal skin and skin of patients with XP revealed a $P < .01$ with the t-test for comparison of means and standard deviations. The subjects were injected with [^{3}H] TdR 15 minutes after ultraviolet irradiation.

Table 1. Sparse labeling of cutaneous nuclei. The results are expressed as the number of nuclei containing 3 to 15 silver grains per 1000 cells of each type. Ultraviolet irradiation, UV.

Cell type	Normal skin*		Skin from XP patients					
			Patient A		Patient B		Patient C	
	No UV	UV	No UV	UV	No UV	UV	No UV	UV
Basal cell	5.4	466	9	6	8	4	10	111
Malpighian cell	9	622	2	0	0	2	8	77
Granular cell	1.2	548	0	0	0	0	6	82
Fibrocyte	4.4	449	0	0	4	6	8	20

* Mean values for the tabulations in the eight normal control subjects.

21 days at 4°C, developed with Brussels Amidol Developer, and fixed with Edwal Quick Fixer; the tissues were then stained with hematoxylin and eosin.

A dense labeling pattern of generally greater than 20 silver grains was present in about 5 percent of basal cell nuclei of the epidermis and in about 1 percent of the dermal fibrocytes in the nonirradiated skin (Fig. 1a). The number of cells with this dense labeling did not change significantly by 15 minutes after irradiation. However, approximately half of the epidermal and upper dermal cell nuclei of the irradiated skin in the control subjects showed a sparse labeling pattern of 3 to 15 silver grains which represented the unscheduled type of DNA synthesis (Fig. 1b). In contrast, essentially no sparse labeling occurred after irradiation in the epidermis or upper dermis of the two patients with the de Santis-Cacchione syndrome (patients A and B) (Fig. 1c). A minimum amount of sparse labeling, ranging from 4 percent of normal in the upper dermal fibrocytes to 20 percent of normal in the basal cell layer, was noted in the XP patient with only skin changes (patient C) (Fig. 2 and Table 1).

The biochemical mechanisms involved in the stimulation of unscheduled DNA synthesis induced by ultraviolet irradiation in normal skin in vivo have not been elucidated. However, it has been demonstrated by density gradient techniques that this type of DNA synthesis, observed autoradiographically in cultured mammalian cells, represents the insertion of small amounts of [^{3}H]TdR into the injured DNA molecule, presumably as a result of the dark-repair process (*1–3*). We examined incorporation of [^{3}H]TdR in vivo in the skin of three patients with XP whose fibroblasts in tissue culture showed little or no evidence of DNA repair, as demonstrated by autoradiographic and density gradient methods (*2*). Unscheduled synthesis was not observed after irradiation in the epidermis or upper dermis in the two patients with the de Santis-Cacchione syndrome and was present in a small number of cells in the patient with only cutaneous lesions. These results are in close agreement with those of Cleaver, who studied cells from the same three patients. Thus, our findings support the concept that the defect in DNA repair noted in vitro occurs in vivo as well. Since defects in repair result in an increased mutation rate induced by ultraviolet irradiation in bacteria (*5*), a similar mutation increase in the skin of patients with XP after exposure to the sun might explain the cutaneous carcinogenic potential in this disease. However, a direct relation between a lack of DNA repair and cancer formation needs to be determined.

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Immune Virolysis: Effect of Antibody and Complement on C-Type RNA Virus

Abstract. *In the presence of envelope antibody and complement, the AKR strain of mouse leukemia virus was lysed, with the result that (i) the viral nucleic acid became susceptible to ribonuclease digestion and (ii) the internal group-specific antigen of the virus was released. The internal localization of the group-specific antigen is confirmed, the evidence being based on the failure of group-specific antibody to lyse virus in the presence of complement.*

Complement (C′) can enhance the virus-neutralizing ability of specific immune serum (*1*, *2*), although its precise role is still not known with certainty. The most general assumption is that C′ strengthens virus-antibody complexes by contributing additional large protein molecules to these aggregates (*2*, *3*). However, enveloped viruses having a lipoprotein component are exceptionally sensitive to the action of antibody and C′. Berry and Almeida (*4*) in a study of infectious bronchitis virus observed that the virus particles may actually have been lysed by an unheated heterotypic antiserum. These and similar observations with rubella and influenza virus were described later under the term virolysis (*5*); however, no evidence of loss of internal components, which is obtained in cellular lytic systems, was presented (*6*).

The oncogenic C-type RNA viruses also mature and "bud" from the cell surface and acquire a lipoprotein envelope in the process. In attempting to develop a rapid, sensitive assay for envelope antibodies and antigens we have obtained evidence that the combined action of antibody and complement releases or exposes viral RNA and also releases the group-specific internal virion antigen in soluble form.

Our studies were carried out with the AKR leukemia virus obtained from an in vitro cell line established from a virus-induced lymphosarcoma in the rat (*7*); however, the basic observations hold true for other C-type viruses as well (*8*). The rat cell line was grown in 32-ounce (about 1 liter) bottles in Eagle's basal medium supplemented with fetal bovine serum (10 percent) and containing glutamine (2 percent),