Growth of Ultraviolet-Damaged Herpesvirus in Xeroderma Pigmentosum Cells (34312)

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When bacterial cells are infected with ultraviolet irradiated phage, a number of repair mechanisms may make it possible for the damaged phage to replicate. Mutants of *E. coli* have been isolated which are not only more sensitive to UV themselves, but which are also unable to repair UV-damaged phage. These mutants have been found to be deficient in certain enzymes involved in repair of damaged DNA strands (1). In mammalian cell culture systems, it was shown recently that while normal skin fibroblasts can apparently repair UV damage to their DNA, the cells of patients with Xeroderma pigmentosum seem to be deficient in these repair mechanisms (2). If the xeroderma pigmentosum skin fibroblasts are indeed analogous to the UV-sensitive *E. coli* mutants, they should be less able to repair UV treated animal DNA viruses. We have therefore studied the growth of UV-damaged herpes simplex virus (HSV) in human cell cultures derived from skin of a patient with xeroderma pigmentosum and compared this with growth of virus in cells of humans without this disease.

Materials and Methods. Virus. Herpes simplex virus strain 11124 was originally obtained from Dr. W. Ashe of NIDR, NIH and grown in our laboratory in HEp-2 cells. It was titrated in monolayer cultures of a line of LBN rat kidney cells (3) in 25-cm^2 Falcon flasks with a methylcellulose overlay similar to that used by Plummer and Benyesh-Melnick for cytomegaloviruses (4). Flasks were fixed, stained, and plaques were counted 3 days after inoculation.

Cells. HEp-2, HeLa, and WI-38 cells were obtained from the NIH Media Unit. A strain of fibroblasts (XP) was derived from a skin biopsy from a 23-year-old woman with xeroderma pigmentosum and multiple cutaneous carcinomas and melanomas. The cells were grown in 25-cm^2 Falcon flasks in a medium composed of 20% fetal bovine serum and 80% RPMI no. 1640. The fibroblasts have grown slowly in culture since November 1967 and have been carried through 24 subcultures. Two other strains of human skin fibroblasts from people with no evidence of xeroderma pigmentosum have been used in the present studies. One (E. W.) was derived from a normal 35-year-old man and the other (C. W.) was derived from a 10-year-old boy with decreased levels of serum alkaline phosphatase.

UV inactivation of virus. Two ml of undiluted virus pool ($10^{6.8}$ pfu/ml) were placed in a 60-mm plastic petri dish. The UV source was a shortwave mineral light whose output was calibrated with a Laterjet dosimeter. The petri dish was placed at a distance from the UV source at which 1500 ergs/cm^2/sec were emitted. The solution was mixed by gentle agitation during the exposure.

Experiments and Results. The HSV pool which contained $10^{6.8}$ pfu/ml when titered in LBN rat kidney cells was UV irradiated for 15 and 30 sec. After 15 sec it contained $10^{5.3}$ pfu/ml and after 30 sec it contained $10^{4.2}$ pfu/ml. The surviving fractions are depicted graphically in Fig. 1.

In phage experiments with UV-sensitive mutants of *E. coli*, the irradiated phage was plaque assayed on the mutant cells and the surviving fractions were determined. Since our xeroderma pigmentosum cells grew slowly, it was difficult to obtain the numbers of flasks that would be needed to do the plaque assays of irradiated virus on these cells. We therefore decided to study viral growth in flasks of xeroderma pigmentosum cells but to titrate the amount of virus produced after 24

hr by plaque assay in the LBN rat kidney cells. In typical experiments, 2.0 ml of undiluted virus was placed in the petri dish. Prior to irradiation, 0.6 ml was removed and 0.2 ml was inoculated into each of three 25-cm^2 Falcon flasks containing xeroderma pigmentosum cells or cells of one of the other lines from nonxeroderma pigmentosum patients

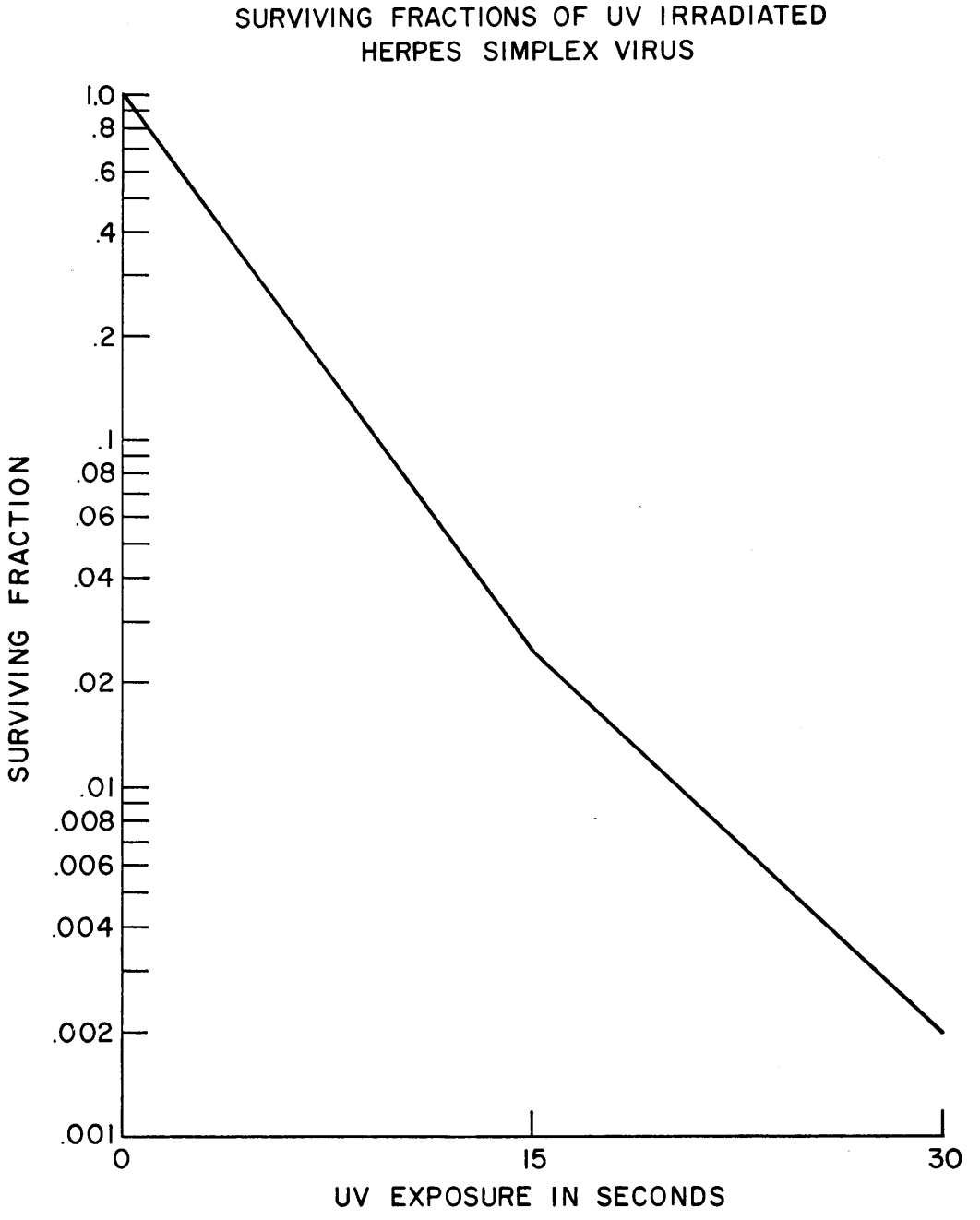

FIG. 1. Surviving fractions of herpes simplex virus after ultraviolet irradiation. A pool containing $10^{6.8}$ pfu/ml was irradiated for 15 and 30 sec (1500 ergs/cm^2/second) and the residual infectious virus was titrated by plaque assay on rat kidney cells.

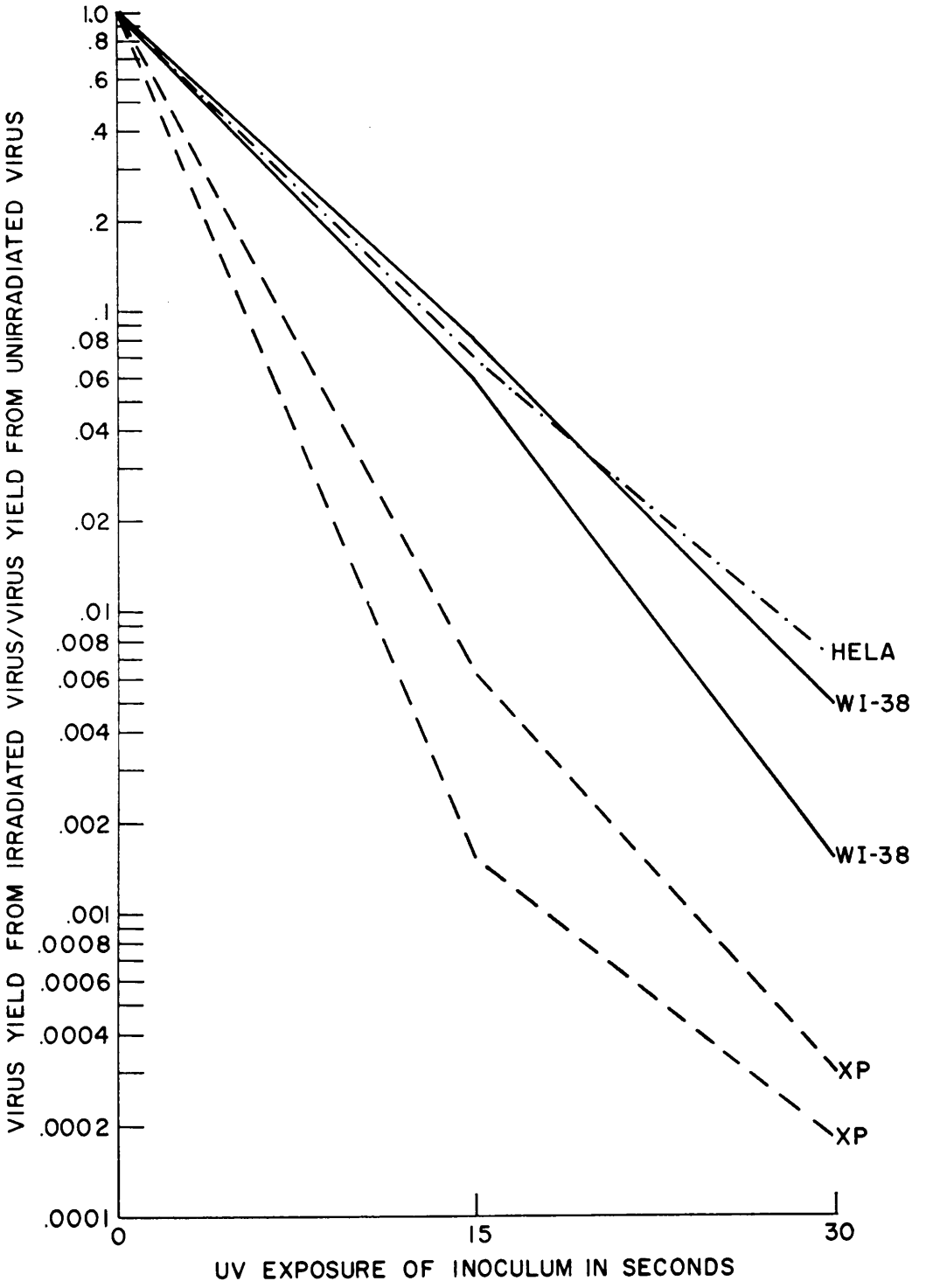

FIG. 2. Virus yield from ultraviolet-irradiated herpes simplex virus divided by the virus yield from a similar inoculum with no irradiation in HeLa cells, WI-38 cells and skin fibroblasts from patient

with xeroderma pigmentosum (XP). Unirradiated and irradiated inocula were grown for 24 hr in monolayer cultures of the various cells and total virus content (fluid and cells) of each culture was determined by plaque assay in rat kidney cells.

(skin fibroblasts from E. W. and C. W., WI-38 and HeLa). Each flask contained 10^5–16^6 cells. After 15 sec of UV treatment, another 0.6 ml was removed from the dish and 0.2 ml was inoculated into 3 flasks similar to those inoculated with unirradiated virus. The procedure was then repeated after 30 sec of irradiation. The inoculated flasks were then fed with 5 ml of medium, incubated at 35° for 24 hr, frozen and thawed 2 times, fluid and cell material was mixed well and titrated by plaque assay in the LBN cells. The results were then expressed as fraction of virus produced in cultures inoculated with 15 and 30 sec irradiated virus compared to amount of virus produced by the unirradiated inoculum.

As shown in Table I, in our initial experiment we compared the growth of unirradiated and UV irradiated virus in 2 groups of XP (xeroderma pigmentosum) cells and one group of WI-38 cells. Although the yield from unirradiated virus was similar in all 3 groups ($10^{5.76}$ to $10^{6.16}$ pfu/ml), the growth of the UV-irradiated virus was much less efficient in the XP cells than in WI-38. After 15 sec of UV irradiation, the inoculum produced $10^{4.84}$ pfu/ml in WI-38 cells while it produced $10^{3.87}$ and $10^{2.87}$ pfu/ml in the cells from the patient with xeroderma pigmentosum. When the ratio of virus yield with UV irradiated virus to virus yield with nonirradiated virus is calculated, it is considerably greater in WI-38 cells than in XP cells. The experiment was repeated with other cultures of the XP cells as well as with HeLa cells and WI-38 cells and the results were always similar (Fig. 2). Since it was possible that the differences reflected the fact that XP cells were skin fibroblasts while WI-38 cells were derived from embryo lung, similar experiments were carried out with skin fibroblasts from people with no evidence of xeroderma pigmentosum (Fig. 3). Again

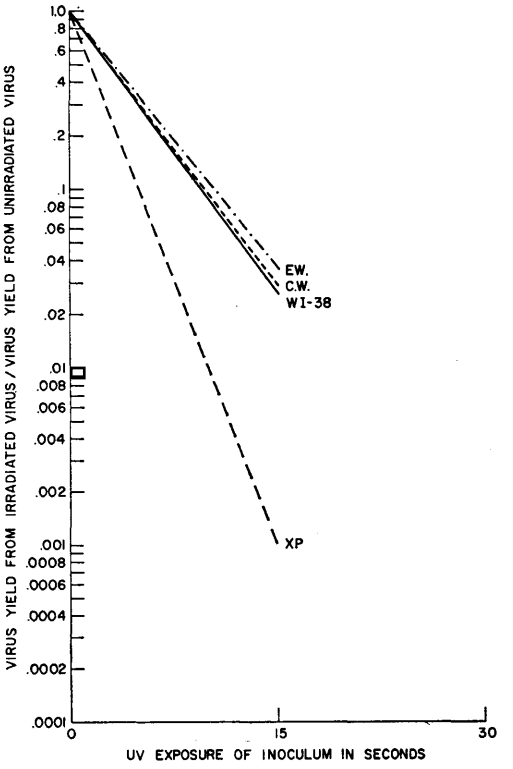

FIG. 3. Similar to Fig. 2 with growth of virus in skin fibroblasts from people with no evidence of xeroderma pigmentosum (E. W. and C. W.), WI-38 cells, and skin fibroblasts from patient with xeroderma pigmentosum (XP).

TABLE I. Growth of Unirradiated and UV-Irradiated Herpes Simplex Virus.

	XP cells		XP cells		WI-38 cells	
UV (sec)	Virus yield (pfu/ml)	Yield UV virus/ yield control virus	Virus yield (pfu/ml)	Yield UV virus/ yield control virus	Virus yield (pfu/ml)	Yield UV virus/ yield control virus
0 (control)	$10^{6.08}$	—	$10^{5.76}$	—	$10^{6.16}$	—
15	$10^{3.87}$	.0062	$10^{2.87}$	.0013	$10^{4.84}$	.048
30	$10^{2.60}$	.00033	$10^{2.00}$	.00018	$10^{3.28}$	.0013

there was a marked decrease in the growth of the irradiated virus in the XP cells when compared to the other types of fibroblasts.

Discussion. If the decreased growth of UV-irradiated herpes simplex virus in the xeroderma pigmentosum cells is related to a deficiency in repair mechanisms for UV-damaged viral DNA, our findings are consistent with those of Cleaver indicating defective repair of UV-damaged cellular DNA in xeroderma pigmentosum skin fibroblasts (2). They are also consistent with the recent observations of Burk and his associated that leukocytes from patients with xeroderma pigmentosum incorporated less tritiated thymidine after UV-irradiation than did leukocytes from normal subjects (5).

On the other hand, the differences that we have observed in growth of UV-irradiated virus may be unrelated to DNA repair. It is possible that the xeroderma pigmentosum cells have decreased absorption or decreased replication of the residual active virus in the irradiated preparations and that no repair takes place in the WI-38 cells or other cell lines. One method of examining this possibility which has been used in bacteriophage studies would be to study growth of UV-irradiated virus in UV-irradiated cells (6). If cellular repair mechanisms are involved, then UV-damaged WI-38 cells should be less able to repair the irradiated viral DNA than normal WI-38 cells. Another approach to the problem would be to study growth of single-stranded RNA viruses where the DNA repair mechanisms would not be operative. Such experiments are now in progress.

If it can be shown that our findings are indeed due to a deficiency of DNA repair mechanisms in the xeroderma pigmentosum cells, it might be of interest to study the cells from patients with a variety of diseases associated with increased sensitivity to ultraviolet light and with skin cancers with regard to their ability to repair UV-damaged viral DNA.

Summary. The growth of ultraviolet-irradiated herpes simplex virus was studied in cultures of skin fibroblasts from a patient with xeroderma pigmentosum and compared with growth in WI-38 cells, skin fibroblasts from people with no evidence of xeroderma pigmentosum and in HeLa cells. The irradiated virus grows less efficiently in the xeroderma pigmentosum cells than it does in the other cell lines examined, suggesting that mechanisms for repair of the UV-damaged viral DNA may be deficient in the xeroderma pigmentosum cells. This is consistent with previous observations of others that repair mechanisms for UV-damaged cellular DNA may be defective in skin fibroblasts and leukocytes from patients with xeroderma pigmentosum.

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