activation of synaptic vesicle PLA_2 may be one of the mechanisms involved in Ca^{2+}-mediated stimulus-secretion coupling in axon terminals. The fact that this enzyme can be modulated by a variety of compounds present in the brain and known to modify neuronal activity suggests that it may have an important role in presynaptic neuronal events.

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24. When light-scattering experiments were performed at *p*H 9.0, the results were similar to those in Table 2. However, at *p*H 9.00 parabromophenacylbromide induced a 24.3 percent (±8.4) greater inhibition of light scattering than at *p*H 6.5. This was in line with the observation that this compound exerts its maximum inhibitory effect on PLA_2 at basic *p*H values (*23*).
25. The physiological importance of synaptic vesicle PLA_2 in function is underlined by the effects of these compounds on vesicle-vesicle interaction at physiological cellular *p*H (6.5). Although maximum PLA_2 activity as determined by the enzymatic assay was achieved at *p*H 9.0, we emphasize that this assay measured the amount of exogenously added phosphatidylcholine capable of being hydrolyzed by endogenous synaptic vesicle PLA_2. This high *p*H may be necessary for the maximum exposure of synaptic membrane PLA_2 to come into contact with and subsequently hydrolyze exogenous substrate. The nephelometer experiments, however, represent a functional manifestation of synaptic vesicle PLA_2 hydrolysis of endogenous synaptic vesicle phosphatidylcholine. At *p*H 6.5 there probably is adequate interaction between the substrate and enzyme.
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X-ray Induction of Persistent Hypersensitivity to Mutation

Abstract. *The progeny of x-irradiated V79 cells are hypersensitive to PUVA-(8-methoxypsoralen plus longwave ultraviolet light) induced mutation at the locus for hypoxanthine-guanine phosphoribosyl transferase. This hypersensitivity is most evident at low doses of PUVA that do not induce mutation in non–x-irradiated cells. The hypersensitivity is evoked by x-irradiation delivered as a single dose or as multiple fractions over a long period and persists for at least 108 days of exponential growth. This radiation-induced hypersensitivity to subsequent mutation is a new phenomenon that may be relevant to multistage carcinogenesis.*

The reaction of a human population to various agents may depend in part on the population's previous exposure to radiation and chemicals. For example, psoriatic patients show an increased risk for cutaneous carcinoma after PUVA therapy (8-methoxypsoralen plus longwave ultraviolet light) if they have previously been treated with ionizing radiation (*1*). The incidence of cutaneous carcinoma in psoriatic patients is 15 times higher than expected for an age, sex, and geographically matched population (*2*). The present investigation was designed to determine whether the progeny of cells treated in vitro with x-radiation display a subsequent hypersensitivity to the mutagenic effects of PUVA.

Preliminary studies indicated that progeny of V79 cells irradiated with a single, high dose of x-radiation (600 to 900 rads) were sensitive to mutation induced by low doses of PUVA. These studies also indicated that the dose-response pattern of the induced hypersensitive state could best be studied by selecting from the progeny of x-irradiated cells a number of cells (10^4) that would make the probability of including x-ray–induced mutants in the population rather small. The maximum number of mutants observed after x-irradiation in these cells was 25×10^{-6}. From each of ten populations of x-irradiated cells we cultured 10^4 survivors; the progeny of these survivors exhibited background levels of mutation that were similar to those of non–x-irradiated cells ($13.4 \pm 1.0 \times 10^{-6}$ mutants per survivor). All ten populations, however, exhibited increased sensitivity to PUVA-induced mutation at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus when compared to non–x-irradiated cells. Although increased sensitivity to PUVA-induced mutation was always observed when larger numbers of cells were selected from the x-irradiated populations, the increased number of x-ray–induced mutants partially obscured the pattern of increased mutation induced by low doses of PUVA.

From four populations of x-irradiated V79 cells we cultured 10^4 survivors. Twelve days later we exposed the progeny of these cells, and of control (non–x-irradiated) cells, to PUVA, and examined their cytotoxic and mutational responses. The survival pattern, which was characteristic of cells exposed to PUVA (*3*, *4*), was essentially the same for the x-irradiated and control populations (Fig. 1A). In contrast, the rate of mutation at the HGPRT locus (indicated by 6-thioguanine resistance) was significantly greater in the x-irradiated cells than the control populations. The frequency of PUVA-induced mutation in the progeny of x-irradiated cells is the difference between the frequency after PUVA treatment and the background frequency in replicate cultures of x-irradiated cells not exposed to PUVA. Similarly, the frequency of PUVA-induced mutations in non–x-irradiated control cells is the difference between the frequency in non–x-irradiated cells treated with PUVA and the background frequency in replicate cultures exposed neither to x-rays nor PUVA. Historical or laboratory control levels are not used for any calculation; each data point in Fig. 1B has its individual non–PUVA-treated control.

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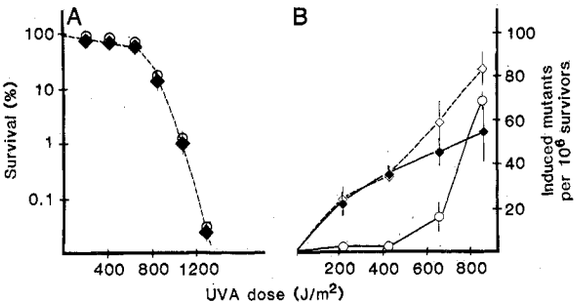

Fig. 1. Survival and mutation plotted as a function of ultraviolet A (*UVA*) dose. The progeny of x-irradiated cells along with controls were exposed to a $10^{-5}M$ solution of 8-methoxypsoralen for 20 minutes at 37°C, then irradiated by longwave ultraviolet light (320 to 400 nm) by Sylvania PUVA tubes. These cells were then examined for cytotoxic and mutagenic responses. For cytotoxicity testing the cells were incubated in complete medium supplemented with 15 percent fetal calf serum for 1 week, then fixed, stained, and counted. For mutagenicity testing, respreading techniques (*8*) were used for a 7-day expression period. Cells were then replated in dishes with normal medium containing 6-thioguanine (3 μg/ml). Colonies were counted 1 week after exposure to 6-thioguanine. Symbols: ○, non–x-irradiated cells; ◆, x-irradiated with 900 rads 12 days before exposure to PUVA; ◇, x-irradiated with 15 rads per day for 60 days prior to exposure to PUVA. "Induced mutants" means that background has been subtracted. Data points represent means ± standard error. No error bar indicates that 1 standard error is less than the symbol size.

The progeny of x-irradiated cells displayed an increased sensitivity to mutation at its HGPRT locus when low doses of PUVA were used (Fig. 1B). In further experiments the progenitor populations were exposed to 900 rads of x-irradiation either as a single dose or as 60 consecutive doses of 15 rads per day. The progeny of each x-irradiated population and a nonirradiated control population were exposed to PUVA, and after 7 days were examined for colony-forming ability in the presence of 6-thioguanine. When compared to controls, the progeny of cells irradiated according to either schedule were hypersensitive to PUVA-induced mutation up to doses of 648 J per square meter of ultraviolet light ($10^{-5}M$ 8-methoxypsoralen). Above this dose of ultraviolet light, variation in mutation frequency for all groups was high, with no statistically significant differences ($P > .05$). The data indicate that cells irradiated over a long period are sensitized to an equal or greater extent than cells exposed to a single dose of x-rays. This observation suggests that even small, protracted doses of x-radiation can lead to a long-lasting hypersensitivity to the mutagenic effects of PUVA.

The time required for the appearance of hypersensitivity was investigated by varying the interval between x-irradiation and PUVA treatment (Fig. 2). The frequency of induced mutation in the progeny of x-irradiated cells was approximately 25 per 10^6 survivors (25 ± 2, mean ± 1 standard error) for all points examined. The mutation frequency in non–x-irradiated controls exposed to the same dose of PUVA was not significantly different from background levels. The data indicate that maximum hypersensitivity is observed as early as 1 day after x-irradiation, and that it persists for at least 108 days (~ 270 mean population doublings). Since there is usually a delay of 4 to 9 days before most specific locus mutations are expressed phenotypically (*5*), the early appearance of the hypersensitive state in x-irradiated V79 cells suggests either a nonmutational mechanism or a mutation that is expressed more rapidly than mutations at other loci studied previously. An example of a heritable alteration capable of modifying gene expression without changing nucleotide sequence has been documented (*6*). This heritable change involved an induced alteration in DNA methylation patterns. Although no specific example has been described, it is possible to conceive of a mutation at a regulatory site that would not require gene product dilution for expression, and therefore could be expressed rapidly. Whether the change is mutational or not, Fig. 2 clearly demonstrates that the induced hypersensitive state is stable during extensive cellular proliferation.

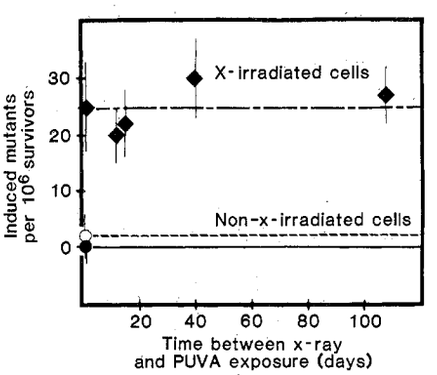

Fig. 2. Mutation plotted as a function of the time between x-irradiation (900 rads at 100 kilovolt peaks) and PUVA exposure ($10^{-5}M$ 8-methoxypsoralen for 20 minutes followed by ultraviolet light, 216 J/m²). Methods for mutation assay were as described in Fig. 1. Symbols: (◆) x-irradiation with 900 rads 1 to 108 days before PUVA; (○) PUVA-induced mutation frequency in non–x-irradiated cells; (●) background mutation frequency (in cells exposed neither to x-irradiation nor PUVA). Induced mutations have all been corrected for specific background levels in replicate cultures not exposed to PUVA. Data points indicate means ± standard error.

This study demonstrates that cells can retain the effects of x-irradiation as expressed by sensitivity to PUVA-induced mutation. The observed hypersensitivity of the progeny of x-irradiated V79 cells is consistent with the observation made by Stern *et al.* (*1*) that psoriatic patients previously treated with x-rays are more sensitive to the carcinogenic effects of PUVA therapy than are patients without histories of ionizing radiation.

The phenomenon of hypersensitivity to subsequent mutagen exposure may also be pertinent as a general model for carcinogenesis. Cancer appears to be a multistage process with the relative effectiveness of so-called initiators and promotors dependent on exposure sequence. Empirically, mutagens have been associated with the early steps in the cancer process, although recent investigators have questioned whether the initiating event is a mutation per se (*6, 7*). The phenomenon described here has characteristics that should be considered in multistage carcinogenesis: it is induced by a known mutagen; it persists, perhaps indefinitely, in dividing cells; and it predisposes cells to the action of subsequent exposure to a second agent. The actual role that induced hypersensitivity may play in pathological processes can only be defined through further experimentation.

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