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Unscheduled DNA Synthesis in Rat Lens Epithelial Cells Induced by Alkylating Agents[1] (40123)

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Alkylating agents have been used frequently as tools to produce cataracts in experimental animals (1). Alkylating agents are known to interfere with normal cell division in lens epithelial cells (2), but the mechanisms by which the cataracts are produced remain obscure. Such compounds are well known for producing damage to DNA of other cell types which results in DNA repair. Recently we have shown that DNA repair synthesis is active in the lens cells following damage by uv light (3). The present experiments were done to determine whether alkylating agents could also induce such unscheduled DNA synthesis (DNA repair synthesis) (4) in lens epithelial cells. The ability to induce repair synthesis would demonstrate that alkylating agents interacted with and presumably damaged the lens epithelial cell DNA.

Materials and methods. Whole lenses from male Osborne-Mendel rats (175–200 g) were incubated in 4 ml of medium (5) containing nitrogen mustard (HN_2, 10^{-4}, 4-nitroquinoline-1-oxide (4-NQO, 2×10^{-5} *M*), or no drug. The HN_2 was made up directly in the medium. 4-NQO was made up as a 10^{-3} *M* solution in 30% ethanol and an aliquot of 80λ was added to 4 ml of the medium. The control lens received 80λ of 30% ethanol. After one-half hour, the lenses were placed in fresh medium containing 5 μCi/ml of tritiated thymidine (^{3}HTdR, 40–60 Ci/m*M*, New England Nuclear) and incubation continued for 2 hr. After that time, the lenses were rinsed three times in phosphate buffer, and placed in Carnoy's fixative. Whole mounts were prepared from lens epithelium (6), and were dipped in Kodak NTB-2 emulsion. The slides were developed for 2 weeks under refrigeration, developed with Kodak D-19, fixed with Rapid Fix, and stained with hematoxylin and eosin.

Results. Figure 1a demonstrates the typical nuclear labeling pattern which was observed following incubation of a rat lens with NH_2 followed by ^{3}HTdR. This type of diffuse nuclear labeling is typical of DNA repair synthesis such as has previously been observed in HeLa (7) and lens cells (3). This type of labeling was not observed in control lenses (Fig. 1b) which were not subjected to the alkylating agent. The heavily labeled cells which are seen in both figures are typical of replicative DNA synthesis (8), which can be seen to persist in the NH_2 treated lenses.

A diffuse labeling pattern consistent with DNA repair synthesis was also induced by exposure of the lens to 2×10^{-5} M-NQO. This alkylating agent also did not interfere with replicative type labeling at the concentration used.

Discussion. Many alkylating agents, including HN_2 (1), have been shown to cause cataracts. The opacities are generally posterior subcapsular cataracts which do not appear for months or years after the initial exposure. Such similarities to ionizing radiation effects have caused alkylating agents to be referred to as "radiomimetic" (1). Evidence is strong that the primary site of action of X-rays in the lens is to the epithelial cells rather than to the cortical fibers already formed (9). Our results show that the lens cell DNA is also one target of alkylating agents since unscheduled DNA synthesis in the epithelial cells followed exposure to the drugs. In other cell systems, DNA damage which is unrepaired or "misrepaired" (10) has been proposed to cause cellular aging (11) and mutations (12). One might expect these agents to produce similar effects in the lens cells.

The results reported here support the suggestion that certain cataractogenic agents have their initial effect on the lens epithelial

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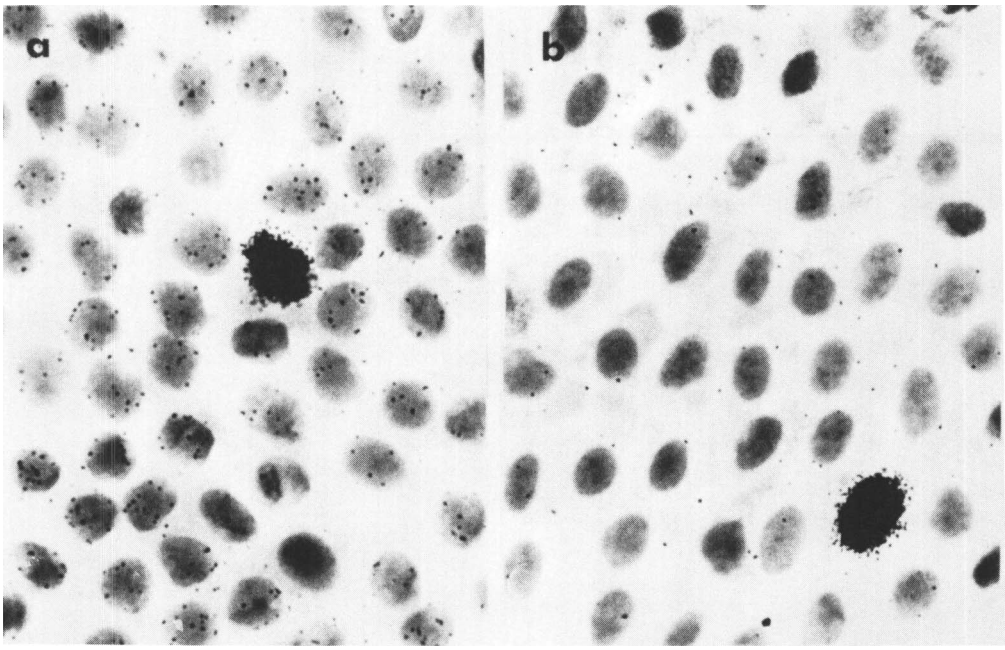

FIG. 1. Autoradiograms of epithelia prepared from whole rat lenses incubated *in vitro* with 3 HTdR (a) following one half hour's incubation with 10^{-4} *M* HN_2 (b) untreated control.

cell DNA. If this initial damage is not repaired, or is misrepaired, the cell may express itself abnormally in terms of cell division, protein synthesis, and differentiation into fibers with ultimate opacification. These events may occur with considerable time delay due to the normal differentiation scheme for lens epithelium.

The importance of the repair process for maintenance of normal lens clarity is suggested by split dose ionizing radiation studies. A single dose of X-ray which produces opacification fails to produce cataracts if the total dose is split and given in several small increments. In the case of neutron irradiation, splitting a cataractogenic dose does not decrease the severity of the cataract produced (13). In contrast to X-rays, which produce repairable single strand breaks, neutrons produce a high proportion of nonrepairable double strand breaks (14). Thus, splitting an X-ray dose allows time for the smaller amounts of damage to be repaired before additional damage is imposed by subsequent doses. No benefit results from splitting the neutron dose since little repair occurs in the interim.

Summary. Two alkylating agents, HN_2 and 4-NQO were found to induce repair synthesis of DNA in rat lens epithelial cells. HN_2 is known to produce cataracts similar to radiation cataracts. A mechanism by which radiomimetics may produce cataracts is proposed.

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