The Effect of X-Irradiation on Hydrocarbon Metabolism and on Hydrocarbon-Induced Lethality and Transformation in Cells Derived from Mouse Prostate*

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Summary. In M 2 cells derived from C 3 H mouse prostate which are X-irradiated (250 r) 48 h before treatment the lethality induced by 3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene is decreased, that induced by the K-region epoxide of 3-methylcholanthrene is significantly increased, whereas that induced by N-methyl-N'-nitro-N-nitrosoguanidine is not altered. The effect of prior X-irradiation on the rate of malignant transformation in vitro is as follows: that caused by 3-methylcholanthrene is markedly decreased, that caused by the K-region epoxide of 3-methylcholanthrene is significantly increased, while that caused by dimethylbenz(a)anthracene or by N-methyl-N'-nitro-N-nitrosoguanidine is not influenced. The data suggest that the effect of X-rays on methylcholanthrene-induced transformation in vitro is due to altered methylcholanthrene metabolism.

Introduction

Recently, Di Paolo et al. were able to demonstrate enhancement of hydrocarbon-induced transformation in hamster embryo cells by prior X-irradiation (Di Paolo et al., 1971 a). Since X-radiation (Brown et al., 1971; Errera and Forssberg, 1961; Ichii et al., 1969; Varagic et al., 1964; Winne, 1962; Yam and Du

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1 The abbreviations used are: MCA, 3-methylcholanthrene; DMBA, 7,12-dimethylbenz(a)-anthracene; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine.
Bois, 1967) and radiomimetic drugs (Tarditt and Du Bois, 1969) are known to inhibit the activity of microsomal mixed-function oxidases and since at least some carcinogenic polycyclic hydrocarbons are activated in vitro by the same oxidases (Grover et al., 1971; Huberman et al., 1972; Marquardt and Heidelberger, 1972a; Marquardt et al., 1972b), the findings described by Di Paolo et al. may have been due to an effect of X-rays on microsomal metabolizing enzymes. This possibility is studied in the present work using cells derived from C 3 H mouse prostate in which hydrocarbon metabolism is very low (Huberman et al., 1971; Marquardt and Heidelberger, 1972a), and where activation of carcinogenic hydrocarbons to an ultimate carcinogen may be the rate-limited step. Studies of the hydrocarbon metabolism in hamster embryo cells were also undertaken in order to get additional information on the biological effects of X-rays on these cells.

**Materials and Methods**

*Chemicals.* Acetone (reagent grade) was purchased from Merck and Co., Rahway, N. J.. Polycyclic aromatic hydrocarbons used and MNNG were obtained from Aldrich Chem. Co. Inc., Milwaukee, Wis. The K-region epoxide of MCA was kindly provided by Drs. P. L. Grover and P. Sims, Chester Beatty Research Institute, London, U. K. (Sims, 1966).

*Culture Media.* Eagle’s BME supplemented with antibiotics and 10% Fetal Calf Serum were used and both were obtained from Grand Island Biological Laboratories, Grand Island, N. Y..

*Transformation Assay.* Cells were obtained from mouse prostate (C 3 H/HeJ mice purchased from Jackson Memorial Laboratory, Bar Harbor, Maine) and established as a line using the procedures described by Chen and Heidelberger (Chen and Heidelberger, 1969). From the original mass culture a pure clone was obtained by thrice cloning and designated M 2.

The cells were irradiated as subconfluent monolayers by means of a General Electric Maxitron 300 (300 KVP, 20 mA, 2 mm Cu HVL, distance 50 cm, 120 r/rain); the doses delivered were calculated in air. Immediately after irradiation, 10^6 cells were plated in 60 mm dishes for assay of transformation. For estimation of plating efficiency 200 cells were introduced into a 60 mm dish. At 48 h after plating test compounds dissolved in acetone were added (final media concentration of acetone of 0.5%). 24 h later the test compounds were removed by change of media. Thereafter, the media were changed twice weekly. After 7—14 days the dishes plated with 200 cells were fixed in methanol, stained with Giemsa, and colonies were counted to determine plating efficiency. After 50 days the dishes plated with 10^6 cells were fixed and stained and scored for transformed foci (Chen and Heidelberger, 1969).

*Determination of Hydrocarbon Metabolism in vitro.* This method, which utilizes the formation of water soluble products from hydrocarbons to measure their metabolism, was performed as previously described (Huberman et al., 1971). It should be noted that as described in this reference (Huberman et al., 1971) autooxidation of the hydrocarbons was measured and it was found that the formation of water soluble products by autooxidation was less than 0.3% of that by cells. In all experiments, 290 pmoles of tritiated MCA (sp. act., 500 mCi/mmole), or tritiated DMBA (sp. act., 5 Ci/mmole) obtained from Amersham-Scarle Corp. (Arlington Heights, Ill.) were added to cells. In these experiments M 2 fibroblasts derived from mouse prostate as well as Syrian hamster embryo fibroblasts (secondary culture) were used.

*Precursor Incorporation into DNA.* Tritiated thymidine (spec. act., 15 Ci/mmole) was purchased from Amersham-Scarle (Arlington Heights, Ill.), and was added to cells at a concentration of 0.25 μCi/ml for 30 min before harvesting. Cells were harvested in 0.02% EDTA-solution, lysed with 1 N NaOH, and DNA was extracted with hot trichloroacetic acid. The radioactivity of DNA was determined by liquid scintillation counting of the extract, and the specific activity of the DNA is reported in dpm/10^6 cells.