PHOTOENZYMATIC REPAIR IN MAMMALIAN CELLS REVEALED BY AN ACRIDINE MARKER.

Daniel Roth

Department of Pathology, University Hospital

560 First Ave., New York, N.Y. 10016

Received October 15, 1968

SUMMARY:

The UV-dimerization of thymine and its photoenzymatic repair were demonstrated in actively growing mammalian cells as well as in inactive desquamated human buccal cells by an acridine marker specific for thymine sequences of intracellular DNA. The stoichiometry of the reaction enabled quantitative measurements of the dye (acriflavine) taken up by the tested cells. The response followed the known kinetics of photoreactivating enzyme derived from bakers' yeast. Active synthesis of DNA does not appear to be necessary for this form of photo repair.

INTRODUCTION:

Photoenzymatic repair has not been observed in mammalian cells.(1) By using an acridine marker for thymine photodimer we have found evidence of the phenomenon in a murine and two human cell lines, as well as in inactive squamous cells shed from human buccal mucosa.

The procedure uses limiting amounts of acriflavine in a standardized experimental model, under conditions allowing the selective and near-stoichiometric association of the dye with intracellular DNA. (2) We have proposed that the dye localizes between adjacent thymines on a single strand of DNA, specificity for this interaction being conferred by the 5-methyl groups of the adjacent thymine molecules. (3)

Inasmuch as the major photobiological effect of UV irradiation is widely held to be localized, similarly, at adjacent thymines, causing their dimerization (4,5,6), we explored the potential of the dye-binding procedure as a marker for this reaction. We found clearcut, replicable changes in dye-binding which paralleled treatment calculated to induce both photodimerization and photoenzymatic repair in the cells we studied.

MATERIALS AND METHODS:

Cultured cell lines consisted of 3T3 mouse fibroblasts obtained from Dr. Howard Green, Department of Cell Biology, New York University School of Medicine, HeLa S3 human polyploid cells, and WI-38 human diploid cells. The 3T3 and HeLa cells were maintained in Eagle's minimum essential medium supplemented with 10% calf serum; the WI-38 cells were maintained in Eagle's basal medium supplemented with 10% calf serum. At confluency, passage was performed and portions of the cell harvest were taken for dye-binding determinations by suspending in 0.05M phosphate-buffered saline solution pH 6.0-6.2. The buccal cells were obtained by gentle abrasion of the buccal mucosa of non-smokers with cotton-tipped applicators after a preliminary cleansing rinse with saline solution. The harvested cells were transferred by swirling into buffered saline solution.

Aliquots of 50,000 cells were obtained by hemacytometer count. They were then washed twice, centrifuged, drained to dryness, and stained under conditions previously described in detail. (2) Briefly, this involved the exposure of the unfixed cells to 0.055 μg of freshly diluted acriflavine in buffered saline solution. Staining was carried out in the dark at 35°C for 10 min. The cells were then centrifuged and the unreacted supernatant dye was determined fluorometrically. Inasmuch as the reaction had been found to be near-stoichiometric and specific, the amount of dye bound per average cell could be determined by measuring the loss of dye from the supernate. Results were recorded in picograms of dye per cell. A reproducibility of $\pm$ 5% was obtained with this procedure.

UV irradiation was carried out in a light-proof chamber with an adequately "aged" 4 watt GE G4T4/1 germicidal lamp delivering an estimated radiation dose of 7.5×10^5 ergs/mm^2. During irradiation the cells were kept suspended by frequent, gentle agitation in 1 ml of buffered saline solution, within quartz cuvets having an inside diameter of 1 cm.

Photoreactivation was carried out with a GE Photoflood lamp set 30 cm above the cells, its light passing through plate glass 1 cm thick. Concurrently, photoreactivating enzyme, cytochrome b2 (Sigma), was added to the cell suspension at a standardized concentration of 3/4 units. Aside from the studies of enzyme kinetics, treatment was carried out routinely at 35° C for periods of 10, 20, and 30 min. The 3/4 unit dose had been established earlier to provide sufficient enzyme to cover the maximal requirements of the cells under investigation. After treatment, the cells were washed three times with buffered saline solution and were then stained in the usual manner.

TABLE I

EFFECTS OF UV LIGHT AND PHOTOENZYMATIC REPAIR ON SEVERAL CELL TYPES.

	DYE UPTAKE (picogram per cell)				
CELL TYPE	BASAL	UV-IRRADIATED	PHOTOREACTIVATION AFTER UV		
			10 min	20 min	30 min
WI-38	0.18	0.12	0.15	0.18	0.18
3T3	0.19	0.12	0.18	0.18	0.19
BUCCAL-a	0.22	0.15	0.20	0.22	0.22
BUCCAL-b	0.24	0.15	0.22	0.24	0.24
HeLa S3	0.35	0.22	0.23	0.23	0.25

TABLE II

COMBINATIONS OF UV LIGHT, VISIBLE LIGHT, AND ENZYME IN 2 BUCCAL CELL POPULATIONS.

DYE UPTAKE (picogram per cell)				
BASAL	UV-IRRADIATED	UV plus VISIBLE LIGHT (no enzyme)	ENZYME plus VISIBLE Lt. (no UV Lt)	UV plus VISIBLE Lt & ENZYME
0.24	0.19	0.19	0.23	0.24
0.22	0.15	0.15	0.22	0.22

RESULTS:

Table I lists the dye-binding values in picograms per cell for the treated cells in their basal state, after UV irradiation, and after treatment with visible light and enzyme over the three time intervals. Controls for 2 populations of buccal cells are listed in Table II.

That the reaction is enzyme-dependent is demonstrated in figs. 1 and 2. These represent the binding of dye by buccal cells in relation to temperature

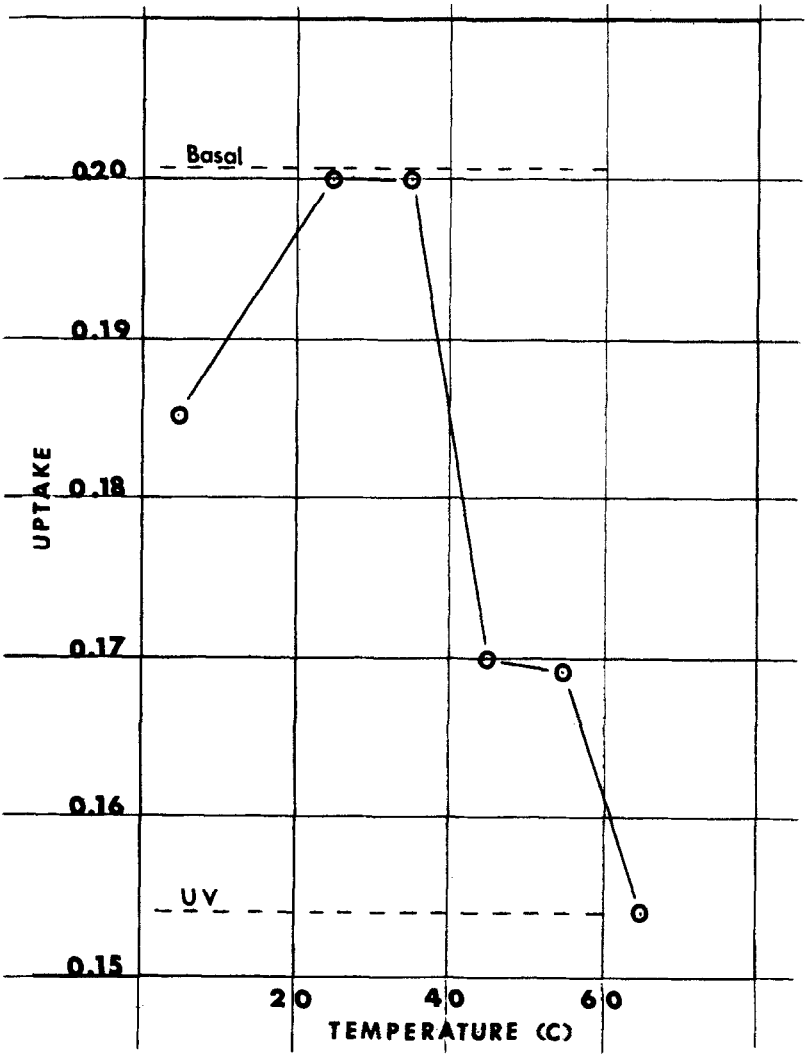

Fig. 1): Plot of dye uptake (in picogram per average cell) against photoreactivating temperature. Considerable enzyme activity at 5°C; peak activity at 25°-35°; no activity recorded at 65°. Treatment with 3/4 Sigma units of yeast-derived enzyme for 30 min, enzyme pre-heated to reaction temperature for 5 min.

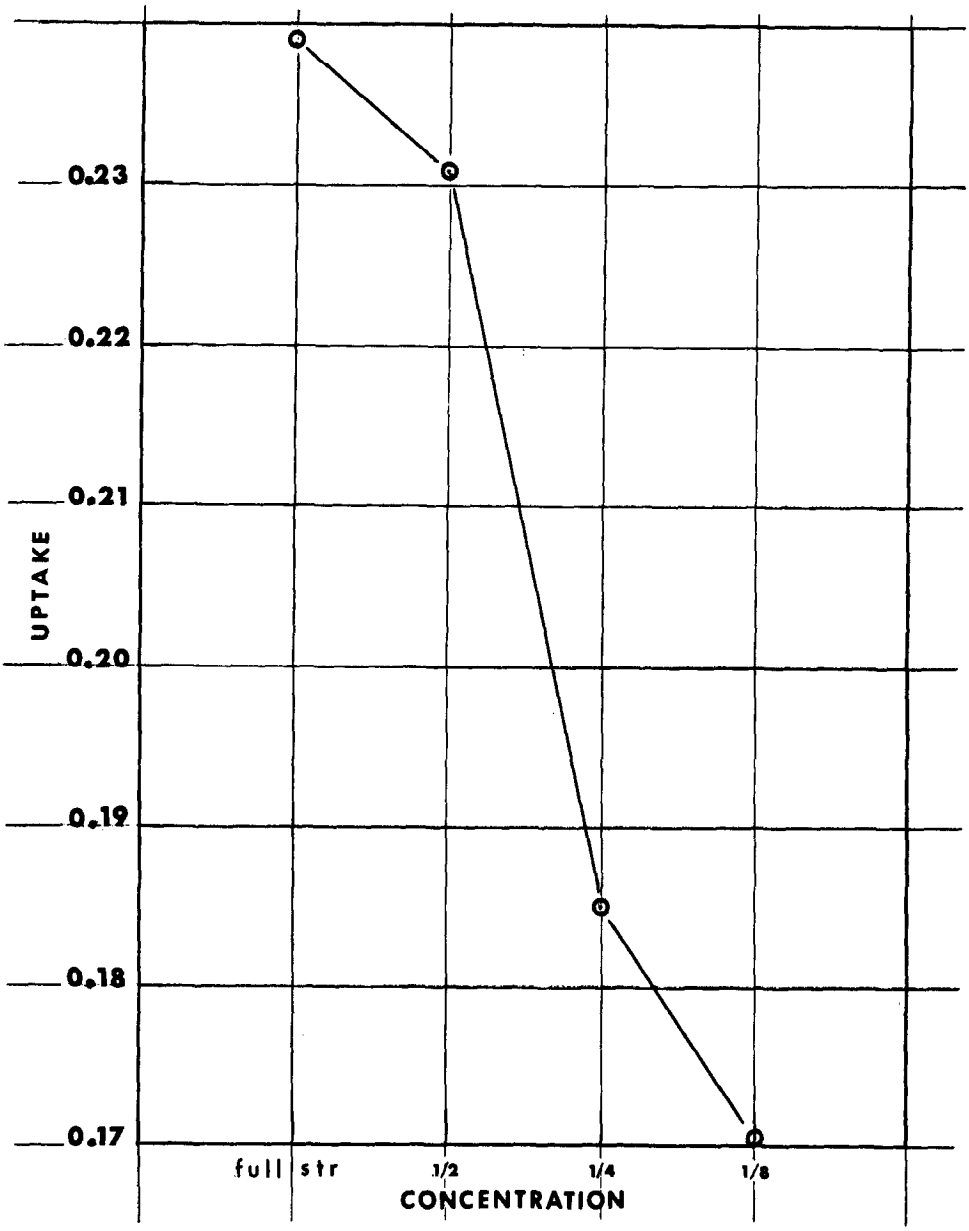

Fig. 2): Dye uptake with dilution of enzyme, starting at full strength, 3/4 Sigma units.

and to enzyme concentration, and show the characteristics of the yeast-derived enzyme for these functions. (7)

DISCUSSION:

All tested cells except Hela exhibited complete recovery of

their basal dye-binding levels after 20 minutes of treatment with enzyme and visible light. Doubling the enzyme concentration in the case of the HeLa cells did not alter their recovery rate significantly. Prolonging the photoreactivation time to 60 minutes, however, did result in nearly complete recovery, indicating a reduced time-rate response for this cell line.

If the greater part of the adsorbed dye is indeed situated between adjacent thymine molecules of cellular DNA, these quantitative alterations in dye-binding after UV-irradiation and photoreactivation appear to serve as a marker for thymine dimer formation and photoenzymatic "repair". Inasmuch as inactive, desquamated buccal cells exhibited the same response as exponentially growing cells, it is suggested that this repair mechanism is independent of DNA replication, thus supporting the concept of Hanawalt and Haynes. (8) In company with other, recent reports of repair of UV injury in a number of mammalian cell lines, including human, by such mechanisms as dark repair and repair replication of DNA, it appears that normal mammalian cells are well equipped to repair genetic injury caused by ultraviolet light. (9,10,11,12,13)

ACKNOWLEDGMENT:

Supported by the Division of Cancer Control and Research of the New York City Department of Health.

REFERENCES:

1) Cleaver, J.E., Biochem. Biophys. Res. Commun., 24, 569, (1966).
2) Roth, D., Manjon, M.L., and London, M., Exptl. Cell Res., in press.
3) Roth, D., and Manjon, M.L., submitted, (1968).
4) Beukers, R., and Berends, W., Biochim. Biophys. Acta, 41, 550, (1960).
5) Setlow, R.B., and Setlow, J.K., Nat. Acad. Sci. (U.S.) Proc., 48, 1250, (1962).
6) Setlow, R.B., Swenson, P.A., and Carrier, W.L., Science, 142, 1464, (1963).
7) Rupert, C.S., J. Gen. Physiol., 45, 703, (1962).
8) Hanawalt, P.C., and Haynes, R.H., Biochem. Biophys. Res. Commun., 19, 462,(1965).
9) Rasmussen, R.E., and Painter, R.B., J. Cell Biol., 29, 11, (1966).
10) Rauth, A.M., Radiat. Res., 31, 121, (1967).
11) Regan, J.D., and Trosko, J.E., Radiat. Res. 31, 548, (1967).
12) Horikawa, M., Nikaido, O., and Sugahara, T., Nature, 218, 489, (1968).
13) Cleaver, J.E., Nature, 218, 652, (1968).