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Annual Technical Progress Report

on

Correlation Between Radiation and Chemical
Induced Molecular Damages and Their
Biological Expression in Mammalian Cells*

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In this annual progress report (3rd year of my contract), I will outline the type of research engaged in this year, results obtained and new programs to be pursued.

Basically, the report summarizes the results of my attempts (1) to understand the nature of the repair mechanisms associated with radiation and chemical induced molecular damage to higher organisms and (2) to study how exogenous environmental agents, such as radiations and drugs, might cause biological damage to human cells.

During this year, I was able to continue my collaborative research efforts with several investigators (Dr. J.E. Cleaver, Laboratory of Radiobiology; Dr. Barnett Rosenberg and Mr. Harold Harder, Biophysics Department, Michigan State University; Dr. Pericles Markakis and Miss Noemi Diaz, Food Science Department, Michigan State University; Mr. W.L. Carrier, Oak Ridge National Laboratory; and Dr. B.K. Zimmerman, Department of Biophysics, Johns Hopkins University).

Also, at the time of writing this report, I will have changed my academic position within the university (April 1, 1970), in that I will be transferred out of the Natural Science Department, University College, into a joint appointment with the Department of Human Development, College of Medicine, and Justin Morrill College [an undergraduate college within the University]. I will spend two terms teaching in Justin Morrill College/year (although I'll have new courses to teach, my ultimate teaching load will be reduced... 120 students/term, 11 contact hours to approximately 30 students/term, 3 contact hours). My teaching duties in the Human Development Department will be unstructured and will coincide with my training and interests. My home department will be the Human Development Department and as soon as the new Life Science I building is completed, I will have a large, well-equipped laboratory. The medical school at Michigan State has been authorized to be a 4-year school.

I also wish to note that the work performed this year was performed primarily by my new technician, Mrs. Miriam Isoun. As of this moment, I still am working without either undergraduate or graduate students.

I. Lack of dark repair in Xeroderma pigmentosum cells.

In collaboration with Dr. James Cleaver, Laboratory of Radiobiology, San Francisco, I have examined cells of xeroderma pigmentosum patients with regard to their ability to excise ultraviolet light (UV)-induced pyrimidine dimers. Cleaver had earlier observed that cells of xeroderma pigmentosum could not "repair-replicate" (1-2). Direct analysis of dimers in UV-irradiated and in UV-irradiated + dark incubated xeroderma cells indicated that these cells are deficient for the dark repair enzyme activity [Figure 1].

II. Saturation of dark repair in human cells in vitro.

Although it had been demonstrated that human cells could excise some of their UV-induced pyrimidine dimers (2), I found by accident that, at relatively low UV doses (300 ergs/mm^2 , 2537°A), the repair of pyrimidine dimers by excision reaches saturation level (Table II). Concomitantly, at these doses, the survival of cells is very low. The mechanism of saturation of repair is at this time unknown. This might be a fertile area to explore in the future. ✓

III. Synergistic effects of ultraviolet light and caffeine in human cells.

It has been demonstrated that caffeine can sensitize UV-irradiated mammalian cells (4-8). We demonstrated last year that high concentrations of caffeine ($9 \times 10^{-3} \text{M}$) can stimulate DNA synthesis in UV irradiated cells. This year we have extended the work and have observed that (1) caffeine does not inhibit excision of pyrimidine dimers in human cells; (2) that it has no measureable effect on the activity of DNA polymerase; (3) that it is not incorporated into the DNA of mammalian cells; (4) that it probably inhibits the rejoining of single strand breaks in the DNA as has been postulated (9) [see enclosed manuscript]. These studies will be the basis for some extended studies on UV-induced carcinogenesis studies in mice [see Renewal proposal].

IV. Effects of natural sunlight on the production of pyrimidine dimers in human cells.

Since it is known that cancers can be induced by sunlight (see 10), and that xeroderma patients, which can not repair UV-induced pyrimidine dimers, have cancers, I reasoned that if sunlight induces enough dimers in human skin, the non-repair or misrepair of the DNA might ultimately lead to skin cancer.

Hence, last summer, I devised a technique in which monolayers of ^3H -thymidine labeled human cells were covered with a monolayer of non-labeled cells. These cells were then exposed to natural sunlight for various lengths of time. Cells were analyzed for dimers. In Table II, it is noted that a 5-hour exposure, which can cause severe sunburn, can also induce a significant amount of pyrimidine dimers. This sunlight exposure is equivalent to 500 ergs/mm^2 of $2650 \text{ \AA}$ UV light. This supports recent findings of Harm (11).

V. Lack of evidence for photoreactivation in human cells.

Although it has been demonstrated via different experimental approaches that many mammalian cells, including those of man, do not have the phenomenon of photoreactivation (12-14), Roth (15) has interpreted his findings as indicating direct photoreactivation in human cells. To test this hypothesis out, direct monomerization of UV-induced pyrimidine dimers in human cells was looked for, as well as photoreactivation of DNA synthesis in these cells. Table III and IV indicate that direct photoreactivation, as manifested by monomerization of UV-induced pyrimidine dimers does not exist in the strain of human cell lines we examined (AV₃ or Hela). Figure 2 illustrates that DNA synthesis is also not photoreactivated.

VI. Effect of platinum compounds on human cells.

Recently, a class of potential antitumor drugs has been shown to inhibit

several tumors in mice (16). Mr. Harold Harder, graduate student of Dr. Barnett Rosenberg of MSU's Biophysics Department, has been using my laboratory and several of the techniques developed in our laboratory to examine the possible mechanism of tumor inhibition by platinum compounds. Some of his findings are illustrated in Figure 3 and 4. Figure 3 shows that three more or less effective S-180 tumor inhibiting platinum compounds inhibit DNA synthesis in AV_3 cells by 90% after 10 hours at 25 μ M, whereas two ineffective tumor inhibiting platinum compounds inhibit only to a small extent. Figure 4 shows that, whereas DNA synthesis is still effectively inhibited at 5 μ M Cis Pt II $(NH_3)_2$, there is only a small effect on RNA and protein synthesis. The same compound has also been shown to inhibit excision of UV induced-pyrimidine dimers and DNA polymerase.

VII. Effect of irradiated sugars on human cells grown in vitro.

One of the major components of the solution to the world food problem is food preservation. The technology of irradiated foods has been suggested as a means to help preserve foods. Miss Noemi Diaz, graduate student of Dr. P. Markakis of the Food Science Department of MSU, has been examining the effect of irradiated sucrose, fructose and glucose on human cells in culture. Figure 5 illustrates some of her findings to date. These findings dramatize the potential dangerous effects of irradiated foods.

VIII. Effect of trisoralen on human cells.

It has been demonstrated that many fucocoumarin compounds can photosensitize human cells (17, 18). Most of these compounds form photoproducts with DNA of cells at long wave length ultraviolet energies. The purpose of my experiments was to examine the effect of these photoproducts on DNA synthesis in human cells. Figures 6 and 7 and Table 5 illustrate the effect of concentration and radiation energies on the photosensitizing effect of trisoralen on human

cells. This work may have some clinical significance, since with these findings, one may be suspicious of the value of trisoralen for repigmentation of skin. It is known to be carcinogenetic with ultraviolet light. Farther work on this drug needs to be done.

IX. DNA-degradation products from mammalian cells irradiated with ultraviolet light.

Chinese hamster cells begin to die and degrade their DNA within 6 to 8 hours after 140 erg/mm^2 and 700 erg/mm^2 of UV light. Cell lysis releases macromolecular double-strand DNA and DNA degradative enzymes into the medium. Extracellular DNA degradation leads to an accumulation of thymidine and thymine in the medium. Intracellular degradation also occurs, and the acid-soluble fraction of the cells contains polynucleotides, phosphorylated derivatives of thymidine and thymine. Specific excision of thymine dimers in an acid-soluble form does not occur, and the contribution of DNA repair mechanisms to the observed degradation products is probably insignificant. No detectable amounts of DNA-degradation products (i.e. less than 0.05 per cent of total cellular DNA) were used for post-irradiation synthesis [See enclosed reprint].

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TABLE LEGENDS

1. Effect of various doses of ultraviolet light on the removal of pyrimidine dimers from human AV₃ and Hela cells.
2. Effects of sunlight on the induction of thymine-containing dimers in human cells grown in vitro.
3. Lack of an effect of photoreactivating light on monomerization of thymine-containing dimers in ultraviolet irradiated human cells.
4. Lack of an effect of sunlight on the monomerization of ultraviolet light-induced pyrimidine dimers.
5. Lack of a modification in thymine-containing dimer production by trisoralen photoproduct in DNA of human cells.

TABLE I

TREATMENT	HARVEST TIME (post UV)	PYRIMIDINE DIMERS Thymine	%XT* T
<u>HELA</u>			
Control (noUV)		26/288,000	0.007
100 ergs/mm ²	0 hr.	79/171,400	0.046
	24 hr.	35/134,500	0.026
200 ergs/mm ²	0 hr.	127/164,800	0.078
	24 hr.	34/71,900	0.047
400 ergs/mm ²	0 hr.	172/139,000	0.124
	24 hr.	203/180,200	0.112
600 ergs/mm ²	0 hr.	258/131,200	0.195
	24 hr.	251/155,000	0.162
<u>AV₃</u>			
Control (no UV)		52/471,000	0.011
100 ergs/mm ²	0 hr.	176/221,300	0.080
	24 hr.	116/199,700	0.058
200 ergs/mm ²	0 hr.	243/203,000	0.120
	24 hr.	118/207,000	0.057
400 ergs/mm ²	0 hr.	343/193,400	0.178
	24 hr.	306/195,000	0.156
600 ergs/mm ²	0 hr.	454/191,000	0.238
	24 hr.	374/193,000	0.185

* XT refers to both TT and UT dimers, since chromatography procedures used here do not separate them from each other.

TABLE II

TREATMENT	XT/T (Counts/min.)	% OF THYMINE AS DIMER
Experiment 1, July 26, 1968*		
<u>2 cell layers</u>		
Sunlight 2 hrs.	89/121,700	0.07
Sunlight 3 hrs.	123/98,200	0.12
Sunlight 5 hrs.	318/137,000	0.23
Sunlight through 1 cm. plate glass	0/216,500	0.00
Dark control	0/167,800	0.00
Experiment 2, August 11, 1968#		
<u>1 cell layer</u>		
Sunlight 2 hrs.	822/582,300	0.11
Sunlight 3 hrs.	1053/607,000	0.17
Sunlight 5 hrs.	1040/283,500	0.36
<u>2 cell layers</u>		
Sunlight 2 hrs.	441/583,700	0.07
Sunlight 3 hrs.	1029/682,100	0.15
Sunlight 5 hrs.	1225/463,500	0.26

*Average dosimetry readings of sunlight, taken at 5 different times from 10:30-3:30 p.m., was 6.5×10^5 erg/mm²/sec. under 1 cm. of plate glass and 7.8×10^5 erg/cm/sec. without glass.

#Average dosimetry readings of sunlight, taken at 5 different times from 10:30 a.m., was 6.8×10^5 erg/mm²/sec. under 1 cm of plate glass and 8.4×10^5 erg/mm²/sec. without glass.

TABLE III

CELL TYPE	TREATMENT	XT/T (Counts/min.)	%THYMINE AS DIMER
<u>HELA</u>	UV-Alone*	540/289,200	0.18
	UV + PR ₁ ⁺	520/256,500	0.20
	UV + PR ₂ ⁺	459/234,700	0.19
AV ₃	UV Alone	292/187,800	0.15
	UV + PR ₁	350/220,000	0.15
	UV + PR ₂	312/213,700	0.14

*Cells were irradiated with 300 erg/mm² of ultraviolet light at 2537 Å: Dose rate was 20 erg/mm².

+PR₁ refers to 2.8 x 10⁵ erg/mm² of photoreactivating light at 4800 erg/mm²/min.; whereas PR₂ refers to 5.6 x 10⁵ erg/mm² at 4800 erg/mm²/min.

TABLE IV

TREATMENT	XT/T (Counts/min.)	%THYMINE AS DIMER
UV alone ⁺	1376/920,000	0.14
UV + 2 hr. sunlight (Cells in MEM medium)	737/536,400	0.13
UV + 4 hr. sunlight (Cells in MEM medium)	898/629,300	0.14
UV + 4 hr. dark incubation (Cells in MEM medium)	978/858,837	0.11
UV + 2 hr. sunlight (Cells in Hank's Buffer)	1180/800,300	0.14
UV + 4 hr. sunlight (Cells in Hank's Buffer)	588/370,300	0.15

*Average dose rate of sunlight taken from 5 readings between 10:30 a.m. to 3:30 p.m. was 5.7×10^5 erg/cm²/sec. (determined through 1 cm. of plate glass with a YSI-Model 65 Radiometer).

⁺Cells were irradiated with 300 erg/mm² of ultraviolet light at 2537 Å. Dose rate was 30 erg/mm²/sec.

TABLE V

TREATMENT	XT/T (count/min.)	% THYMINE AS DIMER
Control medium + no irradiation	0/334,800	0.00
Control medium + 2537 Å irradiation ⁺	486/570,200	0.08
Control medium + 3600 Å irradiation ⁺	0/578,500	0.00
Trisoralen medium + no irradiation	0/577,300	0.00
Trisoralen medium + 2537 Å irradiation	703/731,600	0.09
Trisoralen medium + 3600 Å irradiation	0/666,400	0.00
Trisoralen medium + 3600 Å irradiation + 2537 Å irradiation	510/566,700	0.09

⁺Total incidence dose was 200 erg/mm² at a rate of 20 erg/mm²/sec.

*Total incidence dose was 10.2×10^4 erg/mm² at a rate of 4100 erg/mm²/min.

Figure Legends

- Figure 1. Percentage of ^3H activity in pyrimidine dimers (XT/T) as a function of dose. 0 value immediately after irradiation, 0 value 24 hrs. after irradiation. Top-normal fibroblasts, bottom - homozygous xeroderma pigmentosum (de Sanctis Cacchione) fibroblasts. Bars represent standard deviations of 10 percent for single estimates of (XT/T) or standard errors.
- Figure 2. Effect of photoreactivating light on DNA synthesis of non- and UV-irradiated human cells.
- Figure 3. Relative inhibition of DNA, RNA and protein synthesis by 5.0 μM of $\text{Pt II (NH}_3)_2\text{Cl}_2$.
- Figure 4. Inhibition of DNA synthesis by 25 μM CisPt II, CisPtIV, PtIIE $\bar{\text{D}}$, Trans PtIV, PtII $^{++}$.
- Figure 5. The effect of 3 Mrad gamma irradiated sucrose, glucose and fructose 0.02 M solutions on DNA synthesis of human cells in culture under controlled pH.
- Figure 6. The effect of 2537 and 3600 $\text{\AA}$ irradiation on DNA synthesis in control and trisoralen treated human cells.
- Figure 7. Effect of trisoralen concentration on DNA synthesis in 3600 $\text{\AA}$ -irradiated human cells.

PERCENTAGE ^3H ACTIVITY IN PYRIMIDINE DIMERS ($\hat{x}/T\%$)

ULTRAVIOLET DOSE (Erg/mm²)

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

HOURS IN H^3 -THYMIDINE

FIGURE 7