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Annual Technical Progress Report

on

Correlation between radiation and chemical
induced molecular damages and their
biological expression in mammalian cells.*

INVESTIGATOR

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During the months from June, 1968 to the time of this report, several aspects of this contract have been completed and several belated projects were undertaken. The following report summarizes the results to date on our attempts (1) to delineate the molecular mechanisms of ultraviolet light (u.v.)-induced damage and their associated repair processes in higher organisms (plant and animal); (2) to develop techniques to determine the molecular organization of the higher organism-chromosome; (3) and to use these techniques in measuring the molecular basis of radiation or chemical-induced chromosomal damage in mammalian cells.

During this year, I have been able to broaden my research efforts by collaborating with several other investigators (Dr. Barnett Rosenburg - Biophysics Department, Dr. B. Zimmerman - University of Santa Cruz, Dr. J. E. Cleaver - Laboratory of Radiobiology, University of California, and Dr. P. Markikis, Food Science Department), who have been able to use the techniques that I have developed. All of the work has been done in the context of my involvement in under graduate teaching (3 terms/year; 11 contact hours/term). All the work reported here was made possible only because of the experience and dedication of my technician, Mrs. Virginia H. Mansour.

I. U.V. effects on plant tissue culture cells.

A.] Enclosed with this report is a manuscript which gives the details of our results. Last year, we found that some higher plants, Nicotiana tabacum L. var. Xanthi, could ameliorate some of its u.v.-induced molecular damages (pyrimidine dimers) after photoreactivating light posttreatment (1). We have subsequently found that a haploid line of Ginkgo biloba L.also can be photoreactivated to remove u.v.-induced pyrimidine dimers (2). This is of particular interest because if u.v.-induced mutations are caused by pyrimidine dimers (3), then this in vitro plant system might be used in biochemical-mutation studies.

Since any u.v.-induced recessive mutation could be detected in these haploid cells, one needs only to work out a synthetic medium assay system to study some aspects of mutations in higher plants.

B.] In our attempts to determine if the u.v.-induced pyrimidine dimer plays the same role as it seems to in bacteria, (i.e., block DNA synthesis), we have developed a technique to measure DNA synthesis, (i.e., incorporation of ^{3}H-thymidine into TCA-insoluble material) in tobacco tissue culture cells. Basically, by modifying the technique of Bollum (4), we irradiate the tissue culture cells, posttreat, then add ^{3}H-thymidine to the cells. At various times after the u.v. posttreatment, equal aliquots of cells are withdrawn, sonicated and aliquots precipitated on paper disks. Results are shown in Figure 1, These indicate that posttreatment of u.v.-irradiated cells with 3600 A° light increases the incorporation of ^{3}H-thymidine into DNA as well as the increased growth potential of the tobacco cells. Hence one could tentatively conclude that, although u.v.-induced biological damage may be due to many lesions, the pyrimidine dimers might be partially responsible for u.v.-inhibition of growth by interfering with DNA synthesis. This can only be said of tobacco cells at present, since we have not measured the effect of dimers on DNA synthesis in Haplopappus cells, which do not excise or photoreactivate u.v.-induced dimers (yet they are more resistant to u.v. radiation than the tobacco cells which also do not excise dimers but do photoreactivate them).

II. Radiation-induced single strand DNA chain breaks in mammalian cells.

We feel we have made a possible major contribution in the study of agents which affect mammalian cells via damage to DNA. Since the development of the technique to measure and detect single strand breaks on an alkaline-sucrose gradient (5), I have tried to modify the technique for the study of mammalian DNA. Up until now, no technique has been available to measure the in situ

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molecular weight of mammalian DNA because the techniques used to isolate the DNA have sheared the DNA into small pieces. The direct application of the McGrath-Williams technique can not work because of the large amount of interfering cytoplasmic material which causes aggregation-type problems during centrifugation.

We have developed a technique (see enclosed abstract) which enables us to measure huge DNA from Chinese hamster or human cells. We isolate ^{3}H-thymidine-labeled nuclei from control or treated cells (radiation or chemical). These nuclei are then treated with RNase (de-DNased) for a couple of hours, then placed on a 5-20%, pH 12.5 alkaline-sucrose gradient which has a small layer of pronase-duponal mixture. This enables us to transfer the DNA in the intact nuclei (membrane prevents mechanical-shear breaks) to the gradient where the duponal dissolves the membrane, releasing the DNA so that the pronase can digest any interfering proteins. The DNA is then spun at 30,000 RPM for 1-1 1/2 hours at 20°C in a SW-39 swinging bucket centrifuge. Drops from the tube are collected and radioactivity per fraction is determined by liquid scintillation counting.

Results of a typical experiment are shown in Figure 2. To test the hypothesis that the "BUdR-sensitization" of u.v. induced biological damage (killing, chromosome breaks) is due to u.v.-induced BUdR photoproducts which break the DNA, we irradiated Chinese hamster cells which had BUdR in their DNA molecules. We irradiated some of these cells in the presence of cysteamine which has been shown to ameliorate some of the BUdR-sensitization of biological damage (6, 7). We isolated the nuclei and performed our assay. The results indicate (1) that control Chinese hamster cells' DNA is at least 800 x 10^6 M.Wt. units compared to our marker T$_4$-DNA (130 x 10^6 M.Wt.); (2) that u.v. induces an "alkaline lability" or single chain break in the BUdR-substituted

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DNA (we did not find this "alkaline-lability" in control DNA); and (3) that
at this dose, cysteamine did not ameoliorate this "alkaline lability."

Because of the large amount of time to do these experiments and because
of the limited access to two pieces of major equipment needed for these studies
(Model L-centrifuge and liquid-scintillation spectrometer), these studies will
be prolonged before they will be completed and reported.

III. <u>Effect of Platinum compounds on cell division and DNA synthesis inhibition.</u>

With the cooperation and collaboration of Dr. Barnett Rosenberg of MSU's
Biophysics Department, I have analyzed a platinum compound's molecular mode
of action on human cells in culture. Dr. Rosenberg found a platinum compound
$[Cis-Pt (IV) (NH_3)_2 Cl_4]$ which inhibited cell division in bacteria (8) and
which inhibits growth of sarcoma 180 and leukemia L1210 tumors in mice. We
tested this compound on human cells (AV-3) in culture and found that it severely
represses DNA synthesis in human cells (unlike its effect in bacteria) and that
the platinum compound also caused some membrane effects (see accompanying manu-
script). The exact mechanism of inhibition will have to be worked out.

IV. <u>Caffeine-sensitization of u.v.-induced damage.</u>

It has been reported that caffeine enhances the u.v. sensitivity of
bacterial (9) and mammalian (10) cells. Recently it has been shown that DNA
polymerase activity is inhibited by caffeine (11).

Also, it has been shown that caffeine seems to inhibit excision of u.v.-
induced pyrimidine dimers in bacteria(12). However, it does not inhibit an
excision-like process in Hela cells (13). Dr. Burke Zimmerman (formerly of
MSU's Biochemistry Department and now at the University of Santa Cruz) and I
have collaborated on the possible mode of caffeine sensitization on human cells.
Dr. Zimmerman has performed <u>in vitro</u> DNA polymerase assays on extracts of human

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cells, treated with u.v. light with or without caffeine. He will be reporting
the data soon (see accompanying abstract). On my part, I have demonstrated
that caffeine <u>stimulates</u> incorporation of ^{3}H-thymidine into DNA of human cells
(see Figure 3). These cells do <u>not</u> excise pyrimidine dimers and yet are sensi-
tized by the caffeine after u.v. irradiation. ·This leads us to believe that
caffeine sensitizes the u.v. irradiated cells by lending itself to bridging
the dimers in the DNA template during DNA snythesis. Further studies will be
forthcoming.

V. <u>Effect of x-rayed sucrose on DNA synthesis of human cells grown in culture.</u>

Collaborating with Miss Neomi Diaz (graduate student under Dr. P. Markakis
of Food Science Department at MSU), I have demonstrated that x-rayed sucrose
(4% solution given 2 megarads) significantly repressed the incorporation of
^{3}H-thymidine into the DNA of human cells (see Figure 4). This effect was dose-
dependent. We have not had an opportunity to check if this is a general or
specific effect.

VI. <u>Ultraviolet irradiated Chinese hamster cells: Comparison between two strains</u>
    <u>having different u.v. sensitivities.</u>

This work was done in collaboration with Dr. James E. Cleaver of Laboratory
of Radiobiology, University of California Medical Center, San Francisco, Cali-
fornia. He has found a line of Chinese hamster cells (V79-79) which exhibits
higher u.v. sensitivity than its parent line (V79). I have examined both lines
for their ability to form pyrimidine dimers with the same dose of u.v. light
and for their ability to excise the dimers. The data indicate (Table I) that
neither cell line excised the dimers in TCA-soluble pieces, yet each had the
same number of dimers formed. Hence one must conclude at this time that either
u.v. sensitization of the one line has nothing to do with dimers or that one
cell copes with the dimer (albeit not via excising them) in a manner which the

other can not. He will be reporting these results soon.

VII. <u>Molecular basis of the u.v.-induced skin disease of Xeroderma pigmentosum.</u>

Dr. James Cleaver has shown that the u.v.-induced stimulation of
^{3}H-thymidine incorporation into mammalian DNA does not occur in cells of
patients having the disease, Xeroderma pigmentosum (14). We are now in the
process of determining if normal skin can excise dimers (i.e. as Hela cells)
and if Xeroderma cells can not.

VIII. <u>U.v.-induced pyrimidine dimers' role in human cells.</u>

Dr. James Regan of Oak Ridge National Laboratory and I had shown that
Hela cells could remove up to 50% of the u.v.-induced pyrimidine dimers (13).
However, we have been unable to show that this "excision" has anything to do
with biological repair. I have also shown that another human cell line (AV-3)
does not excise or photoreactivate u.v.-induced dimers (Table 2). This leaves
us with a dilemma...do only certain human cells excise some of their dimers
and if so why? Just what role, if any do these dimers play in the u.v.-induced
biological damage manifested by the cells. At any rate, addition studies have
been designed to test some of my ideas and will be performed during the next
year (see proposed research in next section).

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