A COMPARISON OF THE CYTOLOGICAL EFFECTS PRODUCED BY
IONIZING RADIATIONS OF DIFFERENT LET.

Progress Report

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A COMPARISON OF THE CYTOLOGICAL EFFECTS PRODUCED BY
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Technical Progress Report, Contract AT(11-1)-1024
April, 1974

Radiation Research Laboratory
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Summary of Recent Project Activities

Our last progress report, C00-1024-48, was submitted in March 1973. Since then, we have done the following things:

(1) We have neutron irradiated about 300 mice at the Argonne National Laboratory Janus Reactor. We purchased female B6CF₁ mice from the ANL Animal Facilities to avoid bringing outside animals into the area. About 150 mice were given a single dose of 200 rad. Another 150 mice were given 4 doses of 50 rad each and spaced 1 week apart. Approximately 1 hour after irradiation, about one fourth of the mice in each group were anesthetized and the skin and hair were stripped off of the top of the head and both eyes were wounded. Then, beginning at 12 or 14 hours after wounding and stripping, animals were lightly anesthetized and 5 μ Ci of ³H-TdR were applied to each eye and the denuded skin on the head. Animals were sacrificed 2 hours later. The cell labeling and tissue sampling procedure was repeated 18 - 20 times, every 2 hours, until 48 - 50 hours after lens wounding and skin stripping. Autoradiographs of whole mounts of lens epithelia and autoradiographs of skin sections have been prepared. Evaluation of cell proliferation in the tissues responding to stress is now in progress. Samples of control, unirradiated tissues were also obtained. The "1 hour" testing procedure was repeated at 1 week, 2 weeks, or 4 weeks after irradiation. Mice have also been given a single X-ray dose of 1000 rads (head only) and tissues tested and sampled at 1 hour, 1 week,

2 weeks, and 4 weeks after irradiation. Tissues are now being processed. Another group of mice is being exposed to 4 doses of X-radiation, 250 rads each and spaced 1 week apart. Tissues will be tested and sampled as above. Data from the first experiments are now emerging; these preliminary observations will be discussed later in this report.

(2) Mr. Richard C. Miller has been investigating the wound response in the rat lens epithelium after X-irradiating only one half of the lens. His plan is to wound at 1 hour, 3 days, 1 week, 2 weeks, and 4 weeks after irradiation and then assay cell proliferation in both halves of the epithelium responding to the stimulus produced by wounding. The wound is usually, but not always, near the center of an epithelium. When it is off center, he sometimes has a chance to observe the effect of wounding in an irradiated half and sometimes the effect of wounding in the shielded half of a lens. Preliminary observations suggest that the stimulus is produced, but that the irradiated cells can not respond at times soon after irradiation. He is comparing the early effects and the first 30-day recovery in the half-irradiated/half-shielded lenses with the effects observed after the entire lens is irradiated.

(3) A paper, based on the Ph.D. research and thesis of Dr. Alice L. Lindgren, has been published in Radiation Research [Radiat. Res. 54:411-430, 1973, C00-1024-47]. This, of course, dealt with the effects of a single 1000 rad dose of X-radiation on the ability of the rat lens epithelium to respond to wounding at 1 hour, 3 days, 7 days, or 30 days after irradiation. Dr. Bruce Austin submitted an acceptable revision of his Ph.D. thesis. This study paralleled that of Dr. Lindgren to compare the effects of neutrons on the wound response with the effects of X-rays. Some of Dr. Austin's observations were incorporated into a paper presented during a symposium at Neuherberg, Germany, 22-26 October 1973, and to be published by the I.A.E.A. [C00-1024-50]. A few comments about

this paper will be made later in this report.

(4) A paper describing the use of liquid scintillation counting methods to assay the amount of tritiated thymidine taken up by the chick embryo after topical application onto the inner shell membrane had been published in Proc. Soc. Exp. Biol. and Med. 140:208-211, 1972 [C00-1024-46]. A second paper describing some effects of single and split doses of X-radiation on the ^3H -TdR uptake by chick embryos was submitted to the same journal. It was returned with some suggestions for revision and a suggestion that it be submitted to a journal specializing in radiation effects. This second paper is now being revised accordingly.

(5) Mr. R.C. Miller and E.F. Riley participated in a workshop on lens differentiation held at Oakland University in Rochester, Michigan, 21-23 March 1973. We had decided that it would not be feasible to utilize mice for our irradiation and wounding-of-the-lens experiments. Discussions with Dr. Nancy Rafferty at that workshop changed our minds and, when we agreed to use A.N.L. mice, expedited our neutron irradiations at the A.N.L. Janus Reactor.

(6) Mr. R.C. Miller presented a paper, "Delayed Utilization of ^3H -TdR After Pulse Labeling of the Rat Lens Epithelium", at the 21st Annual Meeting of the Radiation Research Society in St. Louis, 29 April - 3 May 1973. [Abstract printed in Radiat. Res. 55:542, 1973; or see C00-1024-49 and also see our March 1973 Technical Progress Report, C00-1024-48] He will present another paper at the 5th International Congress of Radiation Research, 14-20 July 1974. This will report some observations of the wound response in the one-half irradiated rat lens. At the same meeting, E.F. Riley will present a paper on the persistence of latent radiation damage in neutron and X-irradiated rat lens epithelia.

Projected Activities; Percent Effort

We will soon complete a series of fractionated X-ray doses to the mouse head. Then we will begin the month-long period of tissue testing and sampling. Then the period of autoradiograph preparation begins. Finally, the prepared tissues must be evaluated by study with our light microscope. We hope this aspect of our study can be completed by next Summer, 1975. We will not have many academic (teaching) commitments this summer (1974) and next spring (1975). The Principal Investigator may be able to devote more than 50% of his effort to the project during those periods. This should more than compensate for a reduced effort (perhaps only 35-40%) applied to the project during the fall semester with its greater teaching load. Mr. Miller has completed most of his course work and should be able to devote most of his time to our mouse experiments and his own Ph.D. research on the partially shielded rat lens. We hope Mr. Hite will also be able to complete his study of effects on the mouse skin given single or fractionated doses of fission neutrons or X-rays.

Recovery in the Rat Lens Epithelium

In our last Technical Progress Report (March 1973, C00-1024-48), we presented some of the cellular recovery patterns observed in the rat lens epithelium responding to a wound stimulus at various times from 1 hour to 1 month after a single dose of neutron or X-radiation. The same data were presented at the IAEA Symposium in Neurherberg, Germany, October, 1973. After submitting our last Technical Progress Report and before the IAEA Symposium, we prepared a bar graph to compare recovery in neutron and X-irradiated epithelia. The figures previously submitted in a Technical Progress Report are presented again with the new bar graph added.

Figure 1 depicts the amount of ^3H -TdR cell labeling observed at various times after wounding of a control (unirradiated) rat lens epithelium. Figure 1 also depicts the amount of cell labeling observed when epithelia were wounded (a) 1 hour, (b) 3 days, (c) 7 days, or 30 days after a single 1000 rad dose of X-radiation. At 1 hour post-irradiation, the cell labeling was delayed and suppressed. The peak of ^3H -TdR cell labeling gradually increased with increased time after irradiation. At 30 days after irradiation, the response to wounding occurred without delay, but the peak response was only about 50% of the peak response in control epithelia. Mitotic abnormalities were observed in the cells responding to wounding at 30 days post-irradiation.

A similar pattern was observed in neutron irradiated epithelia (Fig. 2) excepting that the peak of the response to wounding did not seem to increase during the first seven days after neutron irradiation. The responses were similar at 1 hour and at 30 days after neutron or X-irradiation. The persistence of latent radiation damage was again demonstrated by mitotic abnormalities in cells responding to wounding at 30 days after neutron irradiation.

The ability of neutron or X-irradiated epithelia to respond to wounding has been compared in Figure 3. The peaks of cell labeling depicted in Fig. 1 and Fig. 2 have been shown as percentages of the peak of cell labeling in control epithelia. An increase of the ability to respond to wounding is indicative of recovery. The X-irradiated epithelia recovered more than the neutron irradiated epithelia during the first 7 days after irradiation. We believe that fractionated doses given 1 week apart will confirm and exaggerate this difference in recovery during the first week after neutron or X-irradiation.

Preliminary Mouse Lens Data

Figures 4, 5, and 6 show the amount of 2-hour cell labeling observed at various times after wounding of the control, unirradiated mouse lens epithelium. The ^3H -TdR cell labeling, of course, indicates DNA synthesis in cells responding to the stimulus produced by wounding. To facilitate cell counting, we utilize a microscope eyepiece reticule that outlines an area that is 100 x 100 μm at a 500X magnification. The position of the whole mount is adjusted so that one edge of the reticule coincides with the inner edge of the distinctive, parallel rows of cells at the equator of the lens. This is called area 1 (position 1). The whole mount is then shifted to outline successive counting areas moving centripetally from area 1 toward the wound. Most of the cell proliferation normally occurs in the peripheral region of the lens epithelium (near the equatorial region of the epithelium). Areas 5-6 and 7-8 are located in the region of relatively high mitotic activity. [Even in this region of greatest mitotic activity, the mitotic activity is low and the cell population turnover time is long compared to other cell populations such as the cells in the crypts of the small intestine.] The amount of cell proliferation in the central zone (extending about half way from the anterior pole to the equator of the lens) is usually no more than one fifth or one tenth of the amount in the peripheral zone.

In the rat and the rabbit lens epithelium, the wave of DNA synthesis activity starts near the wound and spreads outward toward the peripheral zone. [C.V. Harding, et al., Internat'l Rev. of Cytol. Vol. 31, pp. 215-300, Academic Press, N.Y., 1971.] The cells in the peripheral zone are not very much affected by the stimulus produced by wounding. In contrast, the response to wounding seems to start 3-4 hours sooner in the peripheral zone than in the

central zone of the smaller mouse lens (compare Fig. 4 and Fig. 5 with Fig. 6). Our observations confirm those of Rafferty [Invest. Ophthalmol. 12:156-160, 1973]. There seemed to be three successive waves of DNA synthesis in areas 5-6; the three waves were less obvious in areas 7-8 and, again, more obvious in the central zone. [Incidentally, the error bars show standard deviations and not the smaller standard errors often depicted.]

The preliminary data in Figure 7 indicate that there was about a 6-hour delay and a 30% suppression of the wound response in areas 5-6 at 1 hour after a single, 200 rad dose of fission neutron radiation (compare Fig. 4 and Fig. 7). The data in Figure 8 shows no obvious delay and little, if any, suppression of the wound response in areas 5-6 at 1 hour after the last of four neutron doses of 50 rad each and spaced 1 week apart (compare Fig. 4 and Fig. 8). The data suggest a considerable amount of recovery between fractionated doses of neutron radiation. We had not expected as much recovery as was indicated by our preliminary data. There did seem to be some suppression of the wound response in areas 7-8 and in the central zone at 1 hour after the fourth 50 rad dose of neutrons (compare Figures 5 and 10 and Figures 6 and 12, respectively). We do not at this time have data to compare the effects of single and fractionated doses of neutron radiation at 1, 2, or 4 weeks after irradiation or to compare the effects of neutron radiation with the effects of X-radiation.

Several years ago, we split a cataractogenically effective dose into 9 equal fractional doses given 1 week apart over a period of 8 weeks. When mouse eyes were given 9 X-ray doses of about 122 rad each, the total dose of 1100 rad was no more cataractogenic than a single dose of 400-500 rad. The eyes recovered from a considerable amount of radiation damage during the

8 week period of irradiation. When mouse eyes were given 9 neutron doses of about 22 rad each, the 200 rad total dose was as cataractogenic as a single dose of 200 rad. There was no discernible recovery during the 8 week period of neutron irradiation. The effectiveness of the different irradiation patterns was evaluated about 6 months after irradiation. The effectiveness of irradiation was directly related to the amount of abnormal lens fiber differentiation and the clouding of the lens.

Based on the results summarized in the preceding paragraph, we had expected a single 200 rad dose of neutrons and 4 neutron doses of 50 rad each and spaced 1 week apart to have about the same impact on the response to wounding in the mouse lens epithelium. We were surprised to find that the fractionated neutron doses had much less impact than the single dose when the lens epithelium was stressed by wounding at 1 hour after irradiation. There may be less difference at 1 month post-irradiation. On the other hand, the ability of intermitotic cells to respond to a wound stimulus and initiate DNA synthesis may not be affected by neutron irradiation in the same way that lens fiber differentiation is affected. Different processes with different radiosensitivities and different potentials for recovery may be involved in the short-term wound response and in long-term cataract development. We hope the dose-effect relationships will not be as puzzling when all of our wound response data have been collected. We hope our study will then contribute to a better understanding of the ability of normal tissues to "tolerate" some doses of radiation. One objective of radiation therapy, of course, is to kill all but an inconsequential number of the tumor cells. The other side of the coin, and one of the factors limiting the possibilities of radiation therapy, is the radiation tolerance of normal tissues. [See reviews in Frontiers of

Radiation Therapy and Oncology (J.M. Vaeth, Editor), Vol. 6, "Radiation Effect and Tolerance, Normal Tissue", University Park Press, Baltimore, 1972.] It seems appropriate, therefore, that the radiation tolerance of a variety of normal tissues and normal tissues in a variety of species be investigated.

Fig. 1

Amount of ^3H -TdR cell labeling observed at various times after wounding of control epithelia and at various times after wounding of epithelia given 1000 rad of X-radiation (a) 1 hour, (b) 3 days, (c) 7 days, or (d) 30 days prior to wounding. [Means of average percentages and standard errors were plotted.]

Fig. 2

Amount of ^3H -TdR cell labeling observed at various times after wounding of epithelia given 200 rad of fast neutron radiation 1 hour (circle), 3 days (square), 7 days (star), or 30 days (diamond) prior to wounding. [For clarity, the standard errors have not been shown.]

Fig. 3

Cellular recovery observed in G_0 cells of the rat lens epithelium during the first 30 days after irradiation with fast neutrons (200 rads) or X-rays (1000 rads). Recovery was indicated by an increased ability to respond to wounding with a resultant increase in ^3H -TdR cell labeling.

Percentage of Cells Labeled with ^3H -TdR in Response to Wounding
of Control Epithelium (peripheral zone, areas 5 - 6)

Figure 4

Figure 5

Percentage of Cells Labeled with ^3H -TdR in Response to Wounding
of Control Epithelium (central zone)

Figure 6

Percentage of Cells Labeled with ^3H -TdR in Response to Wounding at 1 Hour
after Single, 200 rad Neutron Dose (peripheral zone, areas 5 - 6)

Figure 7

Percentage of Cells Labeled with ^3H -TdR in Response to Wounding at 1 Hour
after Fourth 50 rad Neutron Dose (peripheral zone, areas 5 - 6)

Figure 8

Percentage of Cells Labeled with ^3H -TdR in Response to Wounding
at 1 Hour after Single, 200 rad Neutron Dose (peripheral zone, areas 7 - 8)

Figure 9

Percentage of Cells Labeled with ^3H -TdR in Response to Wounding at 1 Hour
after Fourth 50 rad Neutron Dose (peripheral zone, areas 7 - 8)

Figure 10

Percentage of Cells Labeled with ^3H -TdR in Response to Wounding at
1 Hour after Single, 200 rad Neutron Dose (central zone)

Figure 11

Percentage of Cells Labeled with ^3H -TdR in Response to Wounding at 1 Hour
after Fourth 50 rad Neutron Dose (central zone)

Figure 12