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Effect of X-rays on the Migration of Cells from Adult Tissue Explants.*

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A considerable literature exists on the effects of irradiating tissue cultures *in vitro* covering studies which have been made on the effect of radiation on mitosis, cell migration, cell morphology and viability.[1,2] In these investigations, generally, active growing cultures of various types of tissues have been used, but no work as far as we know has been done on the effects of radiation on freshly explanted normal adult tissues. When a piece of such normal adult tissue is removed from an animal and cultivated *in vitro*, a period of time called the latent period elapses before any cells appear at the margin of the explant.[3] Our present studies were undertaken to determine the effects of various doses of X-ray on the duration of the latent period.

Experimental method. Adult chicken cardiac muscle was cut into small fragments (approximately 2 mm^3). Groups of from 4 to 9 of these fragments were placed in Carrel flasks containing 0.5 cc of chicken plasma, 1 cc of Tyrode's solution and one drop of embryonic extract to facilitate formation of a coagulum. These flasks were then placed in an incubator at 38°C for a period of 20 minutes, exposed for 5 minutes to 5°C (the short 5°C exposures occurred during transport of cultures), after which they were again kept at 38°C for 20 minutes and then irradiated. During the entire period of irradiation (about 10 minutes) experimental as well as the control flasks were kept at 25°C. Following irradiation, the flasks were again subjected to 5°C for 5 minutes, after which they were kept in the incubator at 38°C. All flasks were removed from the incubator for 15 minutes every 6 hours in order to observe the tissue fragments under the microscope and note the appearance of the first cells. Each curve in Fig. 1 represents the collected observations on 30 to 40 pieces of tissue.

The source of the X-rays was a 180 Kv tungsten target experimental X-ray machine, running at 25 milliamperes and using a filtration of 0.5 mm Cu + 1.65 mm Al; the half value layer of transmitted X-rays was 0.8 mm

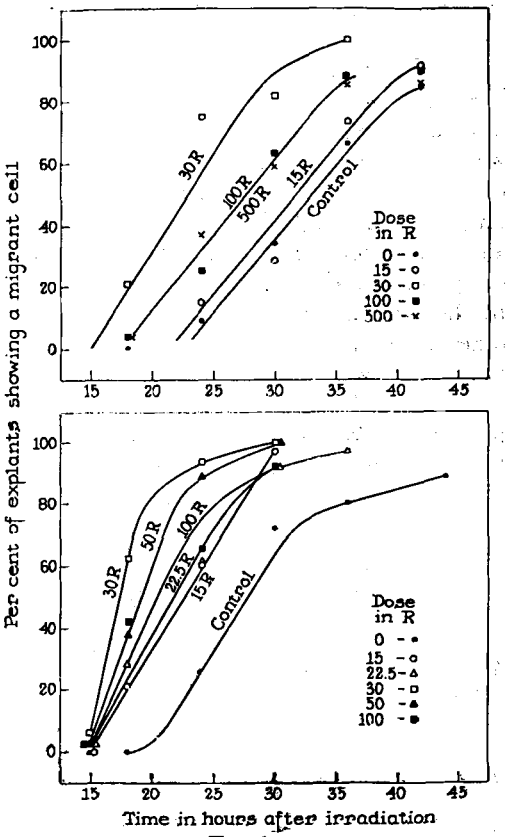

FIG. 1.

Curves showing how migration of cells from explants of adult chicken heart depends on the time after irradiation for various doses of X-rays.

* This work was supported by a grant furnished by the Committee on Growth of the National Research Council.

1 Spear, F. G., *Brit. J. Radiol.*, 1935, **8**, 68, 280.

2 Lea, D. E., Action of Radiations on Living Cells, Pub. Cambridge-Macmillan, 1947.

3 Hoffman, R. S., Goldschmidt, J., and Doljanski, L., *Growth*, 1937, **1**, 228.

Cu. The tissues were at a distance of 50 cm from the target. The studies reported here in detail involved doses of 500 r or less at a dose rate of about 25 r per minute. Some studies were also made using doses up to 10,000 r of unfiltered radiation at a higher dose rate.

Results. Explants given small doses of X-rays had a shorter latent period than unirradiated controls. This seems to be true for doses of X-rays at least as high as 500 r.

Data for two representative experiments are shown in the graphs, Fig. 1, which indicate the percentage of the explants which show one or more migrating cells as a function of the time after irradiation for various doses of X-rays. Typical sigmoid curves are obtained.

In order to compare the effects of different doses of radiation more easily, we have plotted in Fig. 2 data obtained from graphs like those in Fig. 1, giving the time after irradiation at which 50% of the explants show one or more migrating cells as a function of the dose of X-rays for the above experiments. Different heart preparations have different latent periods for both controls and irradiated explants. A fact which is consistent among all preparations, however, is that the minimum latent period occurs at a dose of about 30 r. At higher doses of irradiation the latent period increases slowly with dose and it is not until explants are irradiated with 1,000 r that the latent period is as long as in the controls. With doses of 10,000 r the latent period is approximately 50% longer than in the controls, but the result may not be strictly comparable since the dose rate was used for the 10,000 r is considerably higher than that with smaller doses.

One experiment using 11-day old embryonic chick heart fragments gave results similar to those obtained with adult heart tissue, although as is characteristic of embryonic tissue the latent periods were very short. The latent period was less than 2 hours for 30 r, approximately 3 to 4 hours for controls and 5 hours for 5,000 r.

Our data in Fig. 2 indicate that irradiation has two apparent effects on the duration of the latent period. At doses below 30 r the

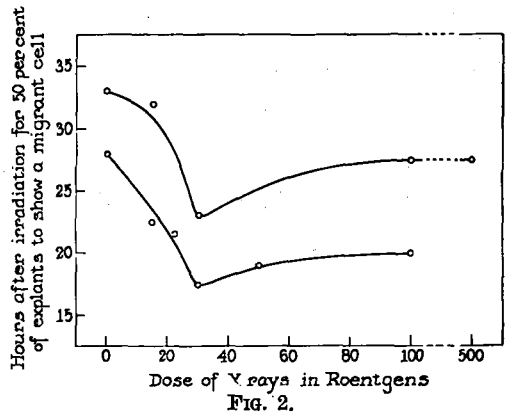

FIG. 2.

Curves showing how the time for 50% of explants to show one or more migrating cells depends on the dose of X-rays expressed in roentgens.

most obvious effect is a stimulating effect on the migration of cells from a fresh explant. With doses of X-rays higher than 30 r the gradually increasing latent period suggests a superimposed inhibition which increases with the dose of radiation. This inhibition of migration with larger doses of X-ray is not surprising since practically all the older literature shows that irradiation of cell cultures causes an inhibition which becomes evident only after a delay of approximately 24 hours. The stimulating effect of small doses of irradiation is somewhat more surprising. Ludford[4] observed in a few cases a stimulating effect of radiation on actively growing cultures but made no systematic and controlled study. He also observed that in growing cultures macrophages seem to inhibit the growth of fibroblasts. In these cultures the growth stimulating effect of irradiation was attributed to the inhibition of the macrophage activity by X-rays. In our experiments on fresh explants, however, the mechanism responsible for the stimulation or inhibition may be different.

Another explanation which has been offered[5] is that radiation damages some cells, causing a release of nutrients which can stimulate other cells into activity. This occurs with considerably larger doses of radiation than those used by us.

Our results may be of interest in connec-

[4] Ludford, R. S., *Proc. Roy. Soc.*, 1934, **115**, 278.

[5] Giese, A. C., *Quart. Rev. Biol.*, 1947, **22**, 253.

tion with the observation of Kaplan[6] who found that irradiation of a lymphosarcoma *in vivo* with 400 r significantly increased the number of metastases over the number occurring in unirradiated controls.

Summary. Evidence is presented that irradiation with small doses of X-rays decreases the time—latent period—required for a migrating cell to appear at the margin of a fresh explant of normal adult chicken heart. The maximum shortening of the latent period occurs with a dose of about 30 r. With larger doses of X-rays the latent period increases again, although a dose of the order of 1,000 r is necessary before the latent period is as long as that of the controls.

[6] Personal communication.

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Activity of Vitamin B_{12} in the Growth of Chicks.*

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The requirement of the growing animal for certain dietary essentials can be increased by inducing a hyperthyroid condition. Ershoff,[1] and Betheil *et al.*[2] observed that liver effectively counteracted a thyrotoxicity in rats which were fed desiccated thyroid. Robblee *et al.*[3] found that the addition to the basal ration of either desiccated thyroid or iodinated casein improved the effective assay range for an unidentified chick growth factor. Work has been in progress[4] for some time on the properties of the growth stimulating components of condensed fish solubles. Fractionation procedures indicated similarities between the chick factor and the material in liver which is active in the treatment of pernicious anemia. Injectable liver extracts were found to be active in promoting the growth of chicks fed rations which were complete in the known growth essentials.[5]

Rickes *et al.*[6] reported the isolation from liver of a red crystalline compound termed vitamin B_{12} which was active in microgram quantities for the remission of pernicious anemia in relapse. More recently Ott *et al.*[7] showed that crystalline vitamin B_{12} had "animal protein factor" activity for the chick. Our data confirm this observation and indicate that vitamin B_{12} is highly active in stimulating the growth of the hyperthyroid chick.

Method. The chicks (New Hampshire ♂♂ x Single Comb White Leghorn ♀♀) were the progeny of hens fed diet B_1 described previously[8] in which protein was provided by

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by funds supplied by the Commercial Solvents Corporation, Terre Haute, Ind., and by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation.

[1] Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 500.

[2] Betheil, J. J., Wiebelhaus, V. D., and Lardy, H. A., *J. Nutrition*, 1947, **34**, 431.

[3] Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 400.

[4] Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *J. Biol. Chem.*, 1948, **173**, 117.

[5] Nichol, C. A., Robblee, A. R., Cravens, W. W., and Elvehjem, C. A., *J. Biol. Chem.*, 1947, **170**, 419.

[6] Rickes, E. L., Brink, N. G., Koniuszy, F. R., Wood, T. R., and Folkers, K., *Science*, 1948, **107**, 396.

[7] Ott, W. H., Rickes, E. L., and Wood, T. R., *J. Biol. Chem.*, 1948, **174**, 1047.

[8] Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *Poultry Sci.*, 1948, **27**, 442.