STUDIES ON X-RAY EFFECTS.

XIII. HISTOLOGICAL STUDY OF THE FATE OF CANCER GRAFTS INOCULATED INTO AN X-RAYED AREA.

BY WARO NAKAHARA, PH.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATES 22 AND 23.

(Received for publication, May 28, 1923.)

The familiar phases of cancer cell degeneration following x-ray treatment in man have generally been attributed to the direct injury produced by the irradiation, but there is little experimental proof to support this idea. On the contrary, recent studies have indicated that the treatment dose of x-rays has little, if any, direct effect on the cancer cells, and that the curative action of this agent depends largely, if not entirely, on the reaction induced in the surrounding normal tissue. The basis for this conclusion is to be found in the fact that a cancer graft will rarely grow when inoculated into a region which has previously been exposed to an erythema dose of x-rays, while grafts of the same tumor grow actively in unirradiated parts of the same animal.

Under the conditions of an experiment such as that described above, the cancer cells inoculated into an x-rayed area themselves received no x-rays, and therefore any changes taking place in them must be secondary to the altered condition induced by x-rays in the surrounding tissue. The object of the present investigation is to examine histologically the series of degenerative changes taking place in cancer cells implanted in a location made unfavorable for their growth by a previous exposure to x-rays.

Sixteen mice, after etherization, were shaved in the upper abdominal region down to and including both groins, and were then secured on a small board. An opening, measuring about 15 by 20 mm., was cut out of a piece of sheet lead, and this was placed over the animal so as to expose the left groin, to the midline, and completely protect the rest of the body. This area was then given a dose of x-rays governed by the following factors: 3 inch spark-gap, 10 milliampere, 6 inch distance from the target, and time of exposure 2½ minutes.

7 days later, when a mild erythema was first appearing, cancer grafts (Bashford Adenocarcinoma No. 63) were inoculated intracutaneously in the x-rayed area and like grafts in the corresponding location of the right, non-radiated groin.

Six of the sixteen mice were killed, a pair at a time, 48 hours, 4 days, and 7 days after the cancer inoculation. The cancer grafts with the surrounding tissue in the x-rayed and protected groins were removed and fixed in Carnoy's chloroform-alcohol-acetic fluid. The remaining ten mice were killed in pairs 24 hours, 48 hours, 3 days, 5 days, and 7 days after cancer inoculation, and the grafts were fixed in formaldehyde. Sections were stained with methylene blue or hematoxylin and eosin.

In order to determine what would have been the outcome of the tumor inoculations, six additional mice were subjected to the same x-ray treatment and inoculations, but were allowed to live. In the course of 3 weeks five out of six inoculations in the protected area resulted in healthy tumors, while only one tumor developed out of six inoculations made in the x-rayed area.

**Histological Description.**

The grafts removed 24 hours after inoculation were found to consist of a necrotic mass with islands of healthy cancer cells scattered here and there. In and around the grafts there was an acute polymorphonuclear and a mild lymphoid reaction. In the condition of the inoculated material and the reaction immediately surrounding it, no difference could be discovered between the grafts from the
two sides, but in the x-rayed area there was a marked lymphoid reaction in the skin layers, which, however, did not come in direct contact with the graft.

In the x-rayed area the necrotic debris of the original mass of graft tissue had been largely removed at the end of the 48 hour and 3 day periods, and the islands of healthy looking cancer cells showed frequent mitotic figures and seemed larger than at the previous period. These islands often formed a more or less continuous ring around the remains of the necrotic mass. The polymorphonuclear reaction had almost subsided and was replaced by a more extensive reaction of the cells of the lymphoid variety (Fig. 1). There was also pronounced activity of the fibroblastic tissue, and the cellular infiltration of the skin layers was still very prominent. At the same period cancer grafts in the protected areas were found to be in a more active stage of growth, islands of cancer cells tending to coalesce and to form irregularly shaped masses of healthy looking tissue. The polymorphonuclear reaction was much reduced, as in the x-rayed area, while the lymphoid cell infiltration was now present to only a limited extent around the cancer grafts.

At the 4 to 5 day periods the difference between the cancer grafts in x-rayed areas and those in protected areas was that degenerative changes had become even more prominent in the former, while the latter continued to grow actively.

The first event in this degenerative process, as seen under the microscope, was the swelling of both nucleus and cytoplasm. The cytoplasm gradually became more acidophilic, and the nucleus hyperchromatic (Fig. 2). The nucleus finally lost its structure, became more deeply stained, and later uniformly pycnotic. The general cell structure also became less and less distinct, and with the fragmentation of the pycnotic nuclei the cells were reduced to debris.

It was observed also that two or more cells often coalesced to form giant cells with highly vacuolated cytoplasm (Figs. 3 and 4), this type of change being found more frequently in small groups which had become imbedded in a fibrous matrix (Figs. 3, A and 4, B). It was not uncommon to find the nuclei pushed to the periphery of some cells by large masses of inclusion bodies (Fig. 3, B). In later stages of degeneration the nuclei lost their staining capacity and
appeared as the so called "ghost nuclei" (Fig. 4, B), and then finally became unrecognizable, leaving the cells as irregularly shaped masses of homogeneous appearance.

This degeneration process was generally complete by the 7th day, when the remains of the graft in the x-rayed area were represented by a small mass of necrotic debris, attended by some polymorphonuclear cells and macrophage reaction.

DISCUSSION.

It may be noted that the series of changes described above are in every respect similar to those that have been reported by Apolant, Contamin, Clunet, Marie and Clunet, Colwell and Russ, Knox, and others, as the typical changes taking place in cancer cells following radiation. To summarize the statements of these authors, all of whom describe essentially the same series of events, the first change occurring after the irradiation of cancer is a lymphocytic and to a less extent a polymorphonuclear exudation, and a connective tissue proliferation around the neoplasm. A few days later the cancer cells themselves begin to show signs of degeneration. These degenerative changes entail a marked swelling of both nucleus and cytoplasm, loss of structural details, hyperchromatism, pycnosis, and finally fragmentation of the nucleus, appearance of vacuoles, with increase in acidophilic affinity of cytoplasm, and giant cell formation by cell fusion. As the lymphocytes and connective tissue stroma increase, the cancer tissue is broken up into small islands, and at the final stage the pycnotic nuclei disintegrate and all traces of living cells are lost.

The general deduction has been that these characteristic changes resulted from the direct injury inflicted on the cancer cells by the x-rays. That this deduction is open to question is shown by the facts presented here; namely, that cancer cells implanted in an area of skin previously exposed to x-rays undergo exactly the same series of degenerative changes as those supposed to result from the direct injury.

In the case of radium treatment, especially when the emanation tube is imbedded in cancer tissue, there is little doubt that some of the cancer cells are directly killed by this agent. Recent observations by Ewing and Alter suggest, however, that the direct injury alone can hardly account for the entire process, and, furthermore, the effect of imbedded emanation tube on normal tissue as reported by Bagg indicates that the local accumulation of lymphocytes, rather than the necrosis of tissues immediately surrounding the tube, may be the more extensive of the induced changes. It would seem probable, therefore, that with radium as well as with x-rays, the induced reaction in the surrounding tissue plays the important rôle in bringing about the destruction of the cancer cells.

SUMMARY.

Cancer cells implanted in a skin region previously exposed to an erythema dose of x-rays show a series of degenerative changes in every way comparable to the frequently described stages of cancer cell degeneration following x-ray treatment. The findings contrast strongly with the survival and growth of grafts implanted in unexposed regions in the same animal. Since the changes are the same whether the cancer cells have been directly exposed in situ or merely implanted in the previously exposed skin, it follows that it is impossible to establish microscopically a direct injury from the x-raying as the principal factor in the therapeutic action of x-rays on cancer.

EXPLANATION OF PLATES.

PLATE 22.

Fig. 1. 48 hour cancer graft in the area of skin exposed to an erythema dose of x-rays 7 days previous to cancer inoculation. Small islands of cancer cells are shown in the midst of the skin layers thickly infiltrated, principally by cells of the lymphoid variety.

Fig. 2. Part of a 5 day graft in x-rayed skin. Hyperchromatism, pycnosis, and fragmentation of nuclei are represented. Some healthy living cancer cells are shown at the top.

PLATE 23.

Fig. 3. A small group of cancer cells imbedded in fibrous tissue, showing binucleated and highly vacuolated giant cell (A) and giant cells with nuclei pushed to the periphery (B).

Fig. 4. The same as Fig. 3, showing a binucleated giant cell (A) and so called "ghost cells" (B).
(Nakahara: Studies on x-ray effects. XIII.)
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