THE ACTION OF BURIED TUBES OF RADIUM EMANATION ON NEOPLASIAS IN PLANTS

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Received for publication September 20, 1922

The insertion into an animal tumor of buried capillary glass tubes containing radium emanation presents a comparatively new departure in radium therapy and is undoubtedly destined to play a prominent part in the future development of the whole field of radiotherapeutics. The technique employed on animals and human beings is as follows: Radium emanation, the first active product of decomposition of radium, is an elementary body in a state of a heavy gas, and by means of appropriate apparatus may be collected in capillary glass tubes 3 to 5 mm. long, and 0.25 mm. in diameter. These tubes may be made to contain anywhere between 0.1 to several millicuries of radium emanation each. They are inserted into the tumor tissue by aid of a trocar.

The tubes exert a comparatively weak but continuous action on the tissues which lasts for several weeks. The cumulative action of 1 mc. when buried permanently in the tissue is calculated to equal 132 mc. hours. The tissue immediately surrounding the capillary is influenced by the soft beta rays and may become necrotic. Figure 1, *A*, shows such an area of necrosis in the spleen of a rabbit into which a radium emanation capillary was inserted. A priori it is feasible to anticipate that this necrotic area acts as a filter on the soft rays and the next zone of tissue is then influenced only by the hard gamma rays of radium.

In the course of the last two and a half years, the senior writer has used this method extensively in his clinical work. As a general rule the insertion of capillary glass tubes in carcinoma

and sarcoma in the human patient is followed by the replacement of the tumor tissue, in the vicinity of each capillary tube, by a connective tissue capsule which wholly encloses the tube. The whole tumor shrinks in size, a process which takes place gradually in the course of six to eight weeks after the insertion of the capillary tubes.

The clinical results to date appeared to be of such importance that it seemed imperative to investigate biologically the mechanism of the action of this method of radium therapy.

In a previous study on the influence of x-rays on the development of the crown gall, the writers (1) have shown that neoplasia of plants presents an ideal tissue for the study of the subject since the results of the action of the rays on the plant tumor cells are not obscured in plants by blood, lymph, and the connective tissue, as is the case in animal tumors. This study of the action of the x-rays on the crown gall indicated that the main immediate action of the rays consists, not in a direct destruction of the cells, but in the arrest of their proliferating power. The death of the cells follows as a consequence of the aging of the individual tumor cell.

The present investigation consists in the application of the method of insertion of buried capillary glass tubes filled with radium emanation in plant tissue, and in a gross and a microscopical study of the resulting changes.

Adult normal plant tissue, and particularly young growing tips of plants, were used for purposes of comparison, while crown gall and club root tissues were the main materials.

MATERIALS AND METHODS

The normal tissues used in our experiments consisted of young and adult roots of the purple-top turnip and the growing tips of the tobacco plant. Club roots on cabbage and kohlrabi, artificially produced, and crown galls on the geranium were the main material. Capillary tubes 3 mm. long and 0.25 mm. in diameter containing radium emanation were introduced into the plant. This was done by making a pin-hole opening in the desired part of the plant by means of a sterile needle and then introducing the sealed tube containing the emanation.

The tube of radium emanation was left buried in the tissue for from one to fifteen days and the plants were observed carefully at regular intervals. Empty tubes equal in size to those containing the emanation were inserted in identical tissues as controls. The irradiated and non-irradiated tissues were killed in a variety of fixing agents, of which Flemming's strongest solution and Cornoy's fixative gave the best results.

The material was then cut in sections 5 to 7.5 microns thick. They were stained with iron hematoxylin and Flemming's triple stain.

OBSERVATIONS

The effect of the insertion of a tube of radium emanation into young root tissue is shown in figure 1. This figure represents a longitudinal section of the root through the region where the tube was inserted. The tube contained 2.2 mc. of radium emanation and the photograph was made fourteen days after the needle was buried in the tissue.

The dark area represents the area of necrosis surrounding the tube for a distance of 2 to 3 mm.

A control, empty tube was inserted into the root of a young turnip plant fourteen days previously, growing in the same plot, is shown in figure 2. The tube may be seen in place and the necrotic area here is represented by a small narrow line where the sterile needle made an opening to permit the insertion of the tube. In mature roots, similar results were obtained.

Figure 4, *A*, and 4, *B*, represents cross sections of two purple-top turnips growing side by side. The plant shown in 4, A, received a glass tube containing 3.4 mc. of radium emanation while 4, *B*, received only a sterile glass tube. Figure 4, *A*, shows a necrotized area surrounding the radium tube while 4, B, shows a narrow line representing the region where the empty glass tube was inserted, fourteen days previously, similar to our observations made in young roots.

The effect of a tube of radium emanation 1.8 mc. after being inserted into the growing tip of a mature tobacco plant, is shown in figure 5, *A*. The tube was inserted at the time this branch

was beginning to form flower buds, just below the tip of the branch but still in its growing region.

Another branch of the same plant shown in figure 5, B, in the same stage of development, received an empty capillary tube in the same region of the branch. No interference with the growth of the flower stalk or buds is visible here.

In the case of the first branch which received the radium emanation, a change was noted twenty-four hours after insertion of the tube. A small necrotic area appeared forty-eight hours afterward, and this blackened area around the tube grew larger and larger until it cut off the tip of the plant, or the part above the necrotic area. The part below the necrotic area showed no visible effect. Similar changes were noted in the growing tips in young plants before flowering stalks had developed. Similar results with quantities of radium emanation varying from 0.3 to 3 mc. were also noted. With the larger quantities of radium emanation, however, the necrotic areas, it appears, were more readily noticeable after a relatively short period.

THE EFFECT OF RADIUM EMANATION ON CROWN GALL TISSUE

The study of the effect of radium emanation on crown-gall tissue was done on the geranium. Inoculation of the apical part of the geranium stem with Bacterium tumefaciens was followed by the insertion of a tube of radium emanation one to thirty days after the inoculation was made.

A large number of geranium plants grown during the same period were matched for size and put in pairs. One of a pair received a tube of radium emanation in the region of inoculation, while the other served as a control and received an empty tube.

Figure 6 represents two plants growing in 6-inch pots in a green house under similar conditions. Both were inoculated at the same time with a culture of Bacterium tumefaciens. *A* received an empty tube in the region of inoculation,—two days after the inoculation was made. *B* received a tube containing 0.4 mc. radium emanation. Very early in the study of these plants, of which these were two of a series of thirty such experiments, *A* showed a slight swelling, a week after the inocu-

lation with the Bacterium was made, while *B* showed very early a blackened area around the tube and no swelling.

When the photograph (shown in fig. 6) was taken, two weeks after inoculation and twelve days after the application of the radium emanation, *A* showed a recognizable crown gall, while *B* showed a black necrotized area surrounding the tube and no evidence of crown gall. In all these experiments similar results were obtained.

Microscopic studies of small crown galls approximately one month after infection, which were irradiated with tubes of radium emanation, were studied from one to fifteen days after the radium had exerted its influence on the neoplasm. In the early stages after the insertion of the tube is made into the crown gall, the effect becomes noticeable two to three days later. Examination of sections under the microscope shows that the tissue surrounding the tube is necrotized and drawn away from the glass tube; the radial cell walls have collapsed so as to form a more or less compact layer of cell walls or cellulose around the glass tube; the cells immediately surrounding the cellulose capsule have disintegrated; and one occasionally finds the nuclei and cytoplasm in the process of disintegration, while the layer of cells beyond this zone is apparently unaffected. The first zone or cellulose capsule is characteristic of the effects of the radium. While the disintegrating zone is present, it blends with the outer zone, which is not visibly affected.

Figure 7 represents a microscopic section of a young crown gall into which a tube of radium emanation was inserted for five days. The cellulose capsule is markedly visible in *B*. The plasmolized and disintegrating cells are visible in the enlargement shown in figure 8, *C*. Normal nucleated cells are visible in the area beyond *C*.

THE CLUB ROOT

Club roots artificially produced by infusion inoculations in cabbage and kohlrabi roots were also tested with tubes of radium emanation. Figure 9 represents the thickened finger-like roots of a young kohlrabi plant into which a tube containing 0.4 mc.

of radium emanation was inserted. As in the case of the irradiated crown-gall and normal tissues, a small necrotic area appeared around the edge of the tube several days after its insertion.

Microscopic sections of the root are shown in figure 10. *A* marks the region where the tube was inserted. The cellulose cushion is here visible at *B*. The xylem tubes shown to the left have not collapsed. The hypertrophied parasitized cells are not markedly affected but the hyperplastic tissues are devoid of cytoplasm and nuclei. In general, the same appearance is presented here that was observed in the case of the irradiated crown galls.

DISCUSSION

In the normal adult tissue the only perceptible result of an insertion of a radium emanation tube is complete destruction of tissue in the immediate vicinity of the capillary. The tissue beyond this area does not seem to be influenced in any way which corresponds with results obtained in animal tissue. Adult tissue is not affected by moderate amounts of gamma radiation.

FIG. 1. Microscopic section of the spleen of rabbit showing the effect of buried radium emanation tube.

FIG. 2. Young root of turnip showing effect of buried radium emanation tube.

FIG. 3. Young root of turnip showing effect of buried empty tube.

FIG. 4. Cross section of mature root of turnip. *A*, received tube of radium emanation; *B*, control, received empty tube.

FIG. 5. Shows the effect of buried radium emanation tube in the growing point of a tobacco plant, *A*, and of control empty tube buried in *B*.

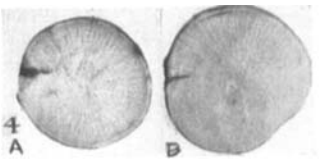
FIG. 6. Shows the effect of buried radium emanation twenty-four hours after the geranium was inoculated with bacterium tumefaciens. *A*, control; *B*, received tube of radium emanation.

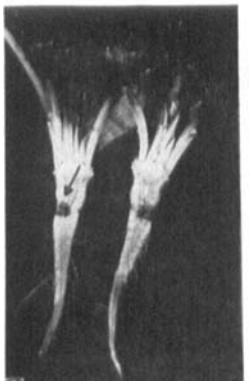
FIG. 7. Microscopic section of a crown gall in which a tube of radium emanation was buried. *A*, region tube occupied; *B*, cellulose capsule; *C*, disintegrating cells.

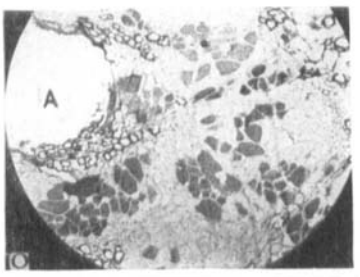
FIG. 8. Microscopic section of same part, position of crown gall, higher magnification.

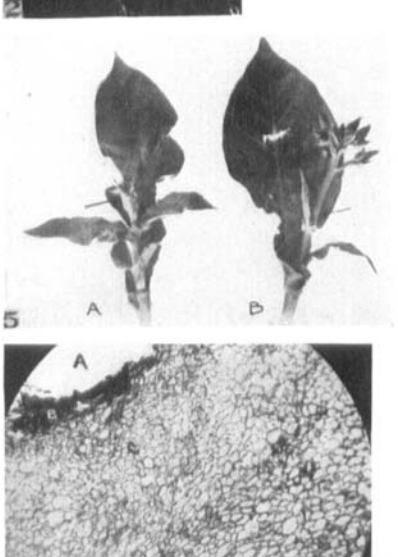
FIG. 9. View of root of young kohlrabi plant infected with club root organism showing buried radium emanation tube.

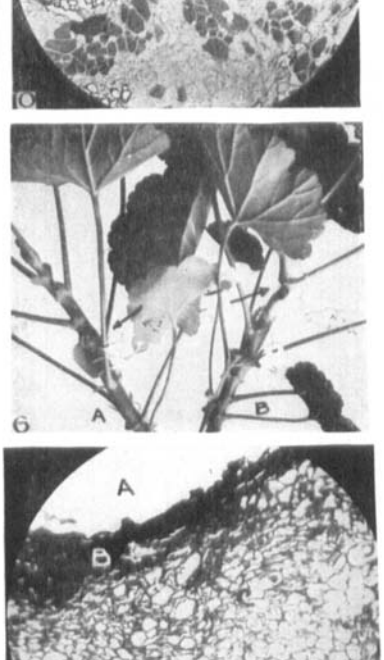
FIG. 10. Microscopic section of club root; *A*, region of tube.











The insertion of radium emanation tubes into the crown gall tissue is followed by an inhibition of the development of the neoplasia which is evidenced by the reduction of the size of the tumor as compared with the controls. This is an indication of the inhibition of the nuclear proliferating activity of the gamma rays of radium on the tumor cells.

The tumor tissue in the immediate vicinity of the buried radium emanation tubes is affected mainly by the soft beta rays. Here, therefore, deeper changes take place in the tumor tissue. Sections of this region show the collapse of cell walls radially to the capillary tube, forming a cushion of cellulose. The cells immediately behind this cushion are devoid of both nucleus and cytoplasm. Occasionally one finds a nucleus in process of disintegration. This disintegrated tissue and the cellulose cushion filters off the soft gamma rays. Cells further back of this area are consequently acted on only by the gamma rays.

As was shown in previous studies of I. Levin and M. Levine (2), and I. Levin and B. Joseph (3), such action of gamma rays may not be followed by evident morphological changes in the tumor cells. None the less, this proliferating power will be inhibited, and the tumor will not increase in size.

It is significant that the cellulose cushion seems to play a rôle in plants, in walling off the necrotic area about the radium emanation tubes and filtering off the soft beta rays, similar to that played by the connective tissue stroma in animal tumors.

In club root tissue the degenerated cells immediately adjoining the so-called cellulose cushion do not seem to contain the Plasmodiophora brassicae, while the parasite is present in the cells at a distance further from the capillary tube.

This apparent action of radium on the parasite as well as the more minute study of the intracellular changes caused by the irradiation is a subject of further study by the writers and will be reported later.

REFERENCES

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