THE ROENTGEN RADIATION OF PAPILLOMA VIRUS (SHOPE)

I. The Effect of X-Rays upon Papillomas on Domestic Rabbits*

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Induced by a specific virus, the rabbit papilloma described by Shope (1) is an epithelial growth with the characteristics by which tumors are usually recognized (2). Benign at the onset, the papillomas undergo progressive alterations in both domestic and cottontail rabbits until they become malignant epidermoid carcinomas (3–6). The relation of the virus responsible for the initial growth to the final cancer is not known. As a means for throwing light on this papilloma-to-carcinoma sequence, we have studied the effect of Roentgen rays *in vivo* and *in vitro* on papilloma virus. Correlated observations on other viruses, on bacteria, and on a yeast have also been made.

An important point at issue in the present studies was whether the curative effect of Roentgen radiation on virus-induced tumors results from effects upon the cells of the host or upon the virus. That the curative effect of x-rays on rabbit papillomas (Shope) is due to their direct action on the rabbit's cells, and that the virus appears to be unaffected by doses of a magnitude that are curative, has been briefly recorded (7). The work on which this preliminary note was based has been amplified by further experimentation and will be presented in detail in a series of papers.

The studies described in the present report deal with the effects of Roentgen radiation *in vivo* on papillomas that have been induced on domestic rabbits by papilloma virus (Shope). The experiments were designed to determine the minimal dosage necessary to bring about the complete disappearance of all, and of 50 per cent, of the tumors irradiated, respectively. Further, they were planned to compare the efficacy of a single large dose of x-rays with that of divided doses. Finally, the work included a

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study of the response of the rabbit's tissues following complete or partial eradication of the tumors. (The surviving rabbits have been under observation for over 3 years.)

Materials and Methods

Virus.—The papilloma virus was obtained from papillomas that were present on 8 cottontail rabbits (genus, *Sylvilagus*) when they arrived from Kansas, where they had become infected under natural conditions.

Papillomas.—Tumors to be irradiated were induced on 62 domestic rabbits (genus, *Oryctolagus*) by inoculating them with papilloma virus.

Inoculation of Rabbits.—The viral suspension to serve as inoculum was prepared from papillomatous tissue immediately after its removal from a cottontail rabbit. The tissue was thoroughly triturated with Locke's solution and alundum[1] to yield a 10 per cent suspension. This suspension was centrifuged horizontally at 1500 R.P.M. for 30 minutes, and the supernatant fluid was used to inoculate domestic rabbits. Inoculation was effected by rubbing 0.1 ml. of this material on a lightly scarified site, approximately 2 cm. in diameter, that had been prepared on the inner aspect of the distal half of each ear. The resultant tumors, which appeared between 8 and 20 days later, were subjected to Roentgen radiation from 29 to 192 days after inoculation. The ears were used as the sites for the papillomas since they could be conveniently irradiated while the rest of the rabbit was protected from the radiation by a shield.

Method of Radiation.—In order to bring the inner surfaces of their ears into a convenient position for irradiating, the rabbits were placed on a board in a supine position, their ears extending out flat. The irradiated area was limited to the lesion and to a zone around it that was approximately 1 cm. in width. The remainder of the animal's body was protected by a lead shield. The radiation was administered in fractional, or in massive, doses. The Roentgen rays were generated by a current of 25 milliamperes, having a 200 kilovolt peak. They were filtered through 0.5 mm. of copper and 1.0 mm. of aluminum. Radiation was administered at a target-skin distance (measured from the base of the tumor) of 30 cm. The output, as determined by a Victoreen r-meter, was 160 r units per minute (in air). The rabbits that received fractional radiation were given 600 r units on successive days until the total dosage was achieved, whereas the animals that received massive treatment were given the whole dose at one time.

EXPERIMENTS

For purposes of orientation, the first experiment was designed to indicate the minimal amount of radiation that would cause the complete local disappearance of papillomatous tissue. Since the papilloma described by Shope has certain resemblances to infectious human warts, it seemed possible that its sensitivity to radiation might fall within the range of Roentgen dosage that leads to the complete and permanent disappearance

[1] Alundum, an electrically fused crystalline alumina prepared by the Norton Company, Worcester, Massachusetts, was used because of its excellent "cutting" qualities.

of such warts. This dosage rarely exceeds 1800 r. Accordingly, 2000 r was the largest dose used in this experiment. Graded massive doses were employed.

Experiment 1.—21 aural papillomas on 11 rabbits were selected for irradiation. All of the papillomas were actively growing and were between 21 and 59 days old. Each tumor was irradiated with a single massive dose, the doses ranging from 250 to 2000 r.

TABLE I

The Results of Single Massive Doses of X-Rays Ranging from 250 to 2000 r on 21 Papillomas on 11 Domestic Rabbits

Dosage in r units	No. of lesions irradiated	No. of lesions that disappeared
250	1	0
300	1	0
500	2	0
600	1	0
800	1	0
1000	4	0
1200	1	0
1300	1	0
1500	3	0
2000	6	1

TABLE II

The Results of Single Massive Doses of X-Rays Ranging from 2500 to 5000 r on 47 Papillomas on 25 Domestic Rabbits

Dosage in r units	No. of lesions irradiated	No. of lesions that disappeared	Percentage of "cures"
2500	18	6	33
3000	15	9	60
3500	10	10	100
4000	2	2	100
5000	2	2	100

The results of the first experiment are summarized in Table I. It may be seen that single irradiations of less than 2000 r failed to eradicate a single tumor, while a dose of 2000 r caused only 1 of 6 papillomas to disappear. The higher doses within this range resulted in noticeable regression, but the surviving papillomatous tissue grew actively, showing the subsequent changes that are characteristic of the papilloma-to-carcinoma sequence.

It was shown in the first experiment that a single dose of x-rays of 2000 r or less was insufficient to "cure" actively growing papillomas. In the second experiment, larger single doses were used.

Experiment 2.—49 aural papillomas on 25 rabbits were selected for irradiation. All of the papillomas were actively growing and were between 59 and 172 days old. Each tumor was irradiated with a single massive dose of x-ray, the doses ranging from 2500 to 5000 r.

The results of the second experiment are set forth in Table II. (Since 2 of the papillomas irradiated with 5000 r were present on a rabbit that died of an intercurrent infection 8 days after irradiation, these tumors have not been included.) It may be seen that radiation in a dosage of 3000 r sufficed to effect the disappearance of 60 per cent of the papillomas irradiated, while a single dose of 3500 r "cured" all of them.

Having determined that 3500 r was the critical amount of radiation necessary to "cure" when the x-rays were administered in a single massive dose, a third experiment was carried out to investigate the effects of dividing the dose (fractional dosage).

Experiment 3.—52 aural papillomas on 26 rabbits were selected for irradiation. All of the papillomas were actively growing and were between 49 and 120 days old. Each tumor was irradiated with divided doses of 600 r each, which were administered on successive days until the desired total dosage had been achieved. The total dosages ranged from 2400 to 6000 r.

The results of the third experiment are summarized in Table III. It may be seen that the effects of fractional dosage closely approximate those of single massive dosage. Thus, a total dosage of 3000 r, applied fractionally, "cured" approximately 50 per cent of the tumors irradiated, while a dosage of 3600 r was uniformly effective. So, too, were larger doses.

DISCUSSION

The experiments described in the present report show that the virus-induced rabbit papilloma described by Shope, when present on domestic rabbits, disappears after adequate amounts of Roentgen irradiation. Similar findings have been recorded in a brief note by Lacassagne (8). He found that a total dosage of 5000 r, or more, administered on 2 or 4 days, "cured" the tumor, whereas a dosage of 2200 r was ineffective. He does not give the number of tumors irradiated, nor does he state whether a graded series of doses was used. The present study in which 120 tumors were irradiated establishes the limits within which "curative" irradiation may be achieved—3600 r is uniformly effective; 2000 r ineffective. This amount of irradiation is within the range of Roentgen dosage that normal tissues are able to withstand and is no greater than that used for the roentgenotherapy of certain human neoplasms. Because the rabbit papilloma

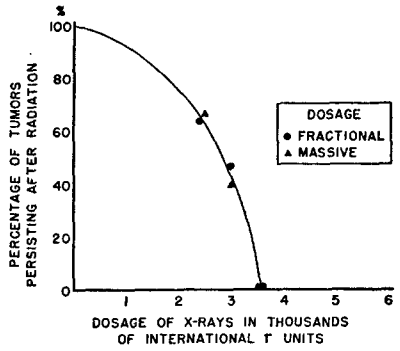
has been compared to human warts, it may be significant that much more irradiation is required for its eradication than for that of the human warts.

The relative efficacy of fractional and massive dosage in the roentgenotherapy of tumors is an important question. In the case of the virus-induced papilloma of Shope, within the limitations under which the present

TABLE III

The Results of Divided Doses of X-Rays, the Total Radiation Ranging from 2400 to 6000 r on 52 Papillomas on 26 Domestic Rabbits

Dosage in r units	No. of lesions irradiated	No. of lesions that disappeared	Percentage of "cures"
2400	12	4	33
3000	17	9	53
3600	11	11	100
4200	4	4	100
4800	2	2	100
5400	3	3	100
6000	3	3	100



TEXT-FIG. 1. The curative effect of x-rays on virus-induced papillomas (Shope) on domestic rabbits. The percentage of tumors persisting after irradiation is plotted against the dosage, expressed in international r units.

experiments were conducted, the two methods gave similar results. These results, which are summarized in Tables II and III, are depicted graphically in Text-fig. 1.

As has been indicated in a preliminary report (7), the enormous dosage of 14,000,000 r is required to render non-infectious a cell-free suspension of papilloma virus. It is clear, therefore, that the effective elimination of papillomas from domestic rabbits by roentgenotherapy must be due to detrimental effects on the rabbit's cells and not to detrimental effects on the

virus. This was also Lacassagne's opinion (8). Subsequent papers in the series of which the present report is the first will give detailed accounts of the effects of Roentgen radiation *in vitro* on papilloma virus and of the response to radiation of papillomas on cottontail rabbits. A consideration of the possible effects of Roentgen radiation in modifying the papilloma-to-carcinoma sequence in the papillomas that survive irradiation will be presented and the reaction of the host's tissues to the injurious effects of Roentgen radiation will be described.

CONCLUSIONS

Virus-induced papillomas (Shope) on domestic rabbits are susceptible to Roentgen rays. The dosage which uniformly brings about their complete and permanent disappearance has been found to be 3600 r (200 kilovolts), whether this dose be administered at one time or fractionally. 60 per cent of the tumors are cured when irradiated with 3000 r.

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