EFFECTS OF X-RAY IRRADIATION ON THE TRANSMISSIBLE VENEREAL TUMOR OF THE DOG
(Plates XXII—XXV)
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INTRODUCTION

The transmissible venereal tumor of the dog is a neoplasm developing in the external genitals of both male and female dogs. It has been encountered in approximately three percent of dogs seen in the University of Tokyo Veterinary Hospital. In Japan it is usually called "polyp" because of its gross appearance whereas in the United States and European countries a variety of names has been applied including Sticker's sarcoma, venereal granuloma or contagious lympho-sarcoma. Although the etiology has not been fully clarified, it is generally accepted that the tumor is transmitted between sexes by copulation (Smith 1898, White 1902, Watanabe 1956).

Pathologically the tumor has usually been classified as a round cell sarcoma (Smith 1898, Sticker 1906, Yamagiwa 1908, Matsui 1909, Imamaki 1932, Watanabe 1956). Other pathologists have considered it to be an endothelioma (Beebe 1906), a neuroblastoma (Jackson 1944), a histiocytoma (Mulligan 1948), or a reticular monocytoma (Nanta 1949). The tumor appears to be malignant on the basis of its histological characteristics since abundant mitoses are ordinarily observed. Furthermore, metastasis may occur to the inguinal lymph nodes or, less commonly, to the spleen, liver, lung, or subcutaneous tissues. Rarely, death ensues as the result of widespread metastases, uremia due to urethral obstruction, or septicemia following necrosis and secondary infection.

The tumor is carried by the great majority of dogs for long periods, up to several years, without serious deleterious effects on the general condition of the animals. In some instances, the tumor appears to shrink and disappear spontaneously. Consequently, the malignancy of this tumor is regarded by most authors to be of relatively low degree.

Using viable cell suspensions experimental transplantation from dog to dog, either by scarification of the genital mucosa or by subcutaneous implantation, has been accomplished frequently (Wehr 1889, Smith 1898, Sticker 1904 and 1906, Wade 1908, Matsui 1909, Matsuba 1927, Imamaki 1932, Stubbs 1934, Tsuchie 1941,
Thiery 1950). Further, experimental passages of the tumor through many
generations have been reported (Karlson 1952). The tumor has never been trans-
planted by cell-free materials. However, attempts to demonstrate filtrable
causative agents have been unsuccessful (Sticker 1906, Stubbs 1934, De Monbreun
1934). Nevertheless, the possibility of a viral origin must be considered (Nanta
1951).

In therapy, surgical excision or cauterization has been generally employed but
incomplete removal frequently results in recurrence. Recently, treatment by x-ray
irradiation has been reported. Banks (1954) described a single case treated
successfully with a total dose of 2244r given in seven exposures over a twenty-two-
day period. Marquès et al (1952) reported a high recovery rate in cases treated
by irradiation but no details were given.

This paper describes the results of x-ray irradiation therapy in eight dogs.
Histologic changes produced by treatment were followed by serial biopsies in all
animals. In five dogs, permitted to survive, follow-up observations have been
carried out for two years or more.

**MATERIAL AND METHODS**

Eight female dogs with spontaneous single or multiple transmissible venereal
tumors of the vagina, of one to twelve month duration, were used in these studies.
In each case, a part of the tumor protruded between the labia so that observation
and biopsy were convenient.

X-ray treatments were administered twice a week over a period of three weeks.
Total dosage ranged from 1200r to 2400r with six exposures of 200r in two dogs
(No. 1 and 2), or 300r in four dogs (No. 3, 4, 5 and 6) and 400r in two dogs (No. 7
and 8). The quality of radiation employed was as follows: 150 KVP, 3 mA., filter
0.5 mm. Cu. plus 1.0 mm. Al, anode-skin distance 30 cm., field 4x6, 6x8, or 8x10 cm.
corresponding to the size of tumor, dose rate 10.56 to 16r per minute. The dogs
were irradiated under general anesthesia with thiopental sodium.

Biopsies were made a few days before, immediately before, immediately after,
and one, three, six, twelve, twenty-four and forty-eight hours after the first
irradiation treatment. Thereafter, biopsies were made at variable intervals
following therapeutic exposures until the tumor disappeared. Tissues taken for
histological study were fixed in Bouin's solution and ten percent formalin, and
sections were stained with hematoxylin-eosin, Heidenhain's iron hexatoxylin and
Heidenhain's azan. For fat staining, frozen sections were stained with Sudan III.

Hematological studies were also performed during and following treatment.
Hemoglobin contents were determined by the oxy-hemoglobin method. Total white
blood count using four counting chambers was carried out in the usual manner.
Blood smears were stained with Wright's stain and 400 leukocytes were counted.

**Results**

A. **Gross Changes in the Tumor**

Observations before irradiation (Table 1, Fig. 1, Fig. 5):

In some cases (No. 1, 2 and 3), one or more tumors involved only the vaginal wall adjacent to the labia whereas in others (No. 4, 5, 7 and 8) massive tumors spread widely over the entire vaginal wall almost occluding the vulvo-vaginal lumen and elevating the skin over the perineum. Some tumors were pedunculated, others were attached by a broad base to the vaginal wall. The pink or red surface was finely granular. Consistency varied from soft and fragile to firm. They bled easily when handled and had a characteristic disagreeable odor.

Observations after irradiation (Fig. 2, Fig. 3, Fig. 6):

The surface of the tumors was covered by a seromucous discharge six to forty-eight hours after the first irradiation, and the granular appearance gradually became smooth during the period of the treatment. Reduction in size and decolorization became evident following the third or fourth treatment. Thereafter, the tumors regressed rapidly. In one dog (No. 2) the tumor disappeared completely three days after the fourth irradiation treatment, whereas in a less sensitive case (No. 8) complete regression of the tumor did not occur until one month after the sixth treatment. After six exposures (1200 to 2400 r), complete disappearance of the tumors was observed (Fig. 3, Fig. 6) in all cases except one (No. 5). In the latter animal, the superficial masses adjacent to the vulva disappeared following the final x-ray exposure, but the autopsy revealed surviving tumor tissue located deep in the vaginal lumen adjacent to the cervix (Fig. 4).

Follow-up observations on the results of irradiation have been made for more than two years. No relapse of the tumor has occurred in five surviving dogs. In addition, two dogs have undergone successful pregnancies with normal parturition (Table 1).

B. **Histologic Changes in the Tumor**

Observation before irradiation (Fig. 7, Fig. 8 and Fig. 9):

In the tumor tissues from eight dogs examined before irradiation, parenchymal tumor cells were round, oval or polyhedral and displayed a striking uniformity in size and shape. Cell boundaries were clearly revealed with either the azan or iron hematoxylin stains; with the hematoxylin-eosin stain cell borders were less distinct. The nuclei were large, round or oval in shape, and included clumps of fine chromatin together with a single prominent nucleolus. Mitoses in various stages were frequently observed.

Micrometer measurements revealed diameters of cells, nuclei and nucleoli ranging
from 8 to 12 µ, 6 to 7 µ and 1 to 2 µ, respectively. The cells also contained considerable cytoplasm which stained light pink with eosin. Small lipid droplets, varying widely in size and number, were demonstrated in the cytoplasm with Sudan III stain. A small number of karyolytic or pyknotic cells was also observed. The fibrous stroma was usually scant but was increased in some cases. The azan stain clearly showed a small number of collagenous and reticulum fibers among the parenchymal cells (Fig. 8). Small numbers of lymphocytes and granulocytes were scattered throughout the stroma.

Observations after irradiation:
Within three to six hours after the first irradiation, mitoses of the tumor cells almost entirely disappeared in all cases. A few abnormal mitotic figures with chromosome bridge formation, acentric chromosomes, or scattered chromosomes

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Breed</th>
<th>Sex</th>
<th>Age</th>
<th>Duration after detection of growth</th>
<th>Location and size of the tumor</th>
<th>Observations after recovery and other remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1</td>
<td>Mongrel</td>
<td>f.</td>
<td>2</td>
<td>1 year</td>
<td>Vaginal vestibule 5x7x1.5 cm.</td>
<td>No sign of recurrence in more than two years.</td>
</tr>
<tr>
<td>Na. 2</td>
<td>Japanese Akita</td>
<td>f.</td>
<td>3</td>
<td>7 months</td>
<td>All over the vaginal wall near the vulva 7.5x6x1.5 cm.</td>
<td>No sign of recurrence in more than two years.</td>
</tr>
<tr>
<td>No. 3</td>
<td>Japanese Shiba</td>
<td>f.</td>
<td>4</td>
<td>1 month</td>
<td>Left vaginal wall near the vulva 3x4x2 cm.</td>
<td>No sign of recurrence in more than two years.</td>
</tr>
<tr>
<td>No. 4</td>
<td>Mongrel</td>
<td>f.</td>
<td>6</td>
<td>1 year</td>
<td>All over the vaginal wall. 5x7x4 cm, measured over the skin</td>
<td>Destroyed five months after recovery. No vestige of the tumor.</td>
</tr>
<tr>
<td>No. 5</td>
<td>Mongrel</td>
<td>f.</td>
<td>Uncertain</td>
<td>All over the vaginal wall, 1 to 2 cm. thick.</td>
<td>A part of the tumor remained at autopsy 32 days after the end of the treatment.</td>
<td></td>
</tr>
<tr>
<td>No. 6</td>
<td>Mongrel</td>
<td>f.</td>
<td>Uncertain</td>
<td>A pigeon egg size tumor at the ventral vaginal wall.</td>
<td>Parturition two times, died two years after recovery. No vestige of the tumor.</td>
<td></td>
</tr>
<tr>
<td>No. 7</td>
<td>Mongrel</td>
<td>f.</td>
<td>4</td>
<td>6 months</td>
<td>All over the vaginal wall, and the vagina was full of the growth. 13x7x6 cm. measured over the skin.</td>
<td>Destroyed one month after the end of the treatment. No tumor tissue.</td>
</tr>
<tr>
<td>No. 8</td>
<td>Mongrel</td>
<td>f.</td>
<td>6</td>
<td>1 year</td>
<td>All over the vaginal wall and the vagina was full of the growth. 10x5x5 cm. measured over the skin.</td>
<td>Parturition one time. Destroyed two years after the end of the treatment. No. vestige of the tumor.</td>
</tr>
</tbody>
</table>

Table 1. The dogs with transmissible venereal tumors treated by x-ray irradiation.
occasionally observed during this period (Fig. 19). After this temporary suppression, mitosis reappeared in the tissues biopsied at twelve hours after the first irradiation, but some of these mitotic figures showed abnormality. The number of abnormal mitotic figures was gradually increased in course of time after the first irradiation. The abnormal mitoses observed in the tissues biopsied twelve to forty-eight hours after the first irradiation represented, in the main, deviations from the stage of prophase or metaphase; less commonly, abnormalities of the anaphase or telophase were seen. Sticky chromosomes, chromosome bridges, asymmetrical division, scattered chromosome fragments or acentric chromosomes were the aberrations observed in this period (Fig. 10, Fig. 20, Fig. 21).

In one case (No. 8), perivascular cuffs of tumor cells were observed in the material biopsied forty-eight hours after the first irradiation. Tumor cells lying remote from the capillary vessels showed karyolysis or pyknosis, whereas the tumor cells in the layer near the capillary vessels were relatively unaffected (Fig. 16).

Enlargement of both the tumor cells and their nuclei was generally observed in the tissues biopsied twenty-four hours after the first irradiation; this change was less noticeable in earlier stages (twelve hours), but more distinct in later biopsies (forty-eight hours). However, after the second exposure, cellular and nuclear enlargement became more marked; the diameters of the cells and the nuclei increased to 13 to 15μ and about 10μ, respectively (Fig. 11, Fig. 12). In this stage of degeneration, cellular and nuclear disintegrations including deformity of the cell membrane, vacuolization, loosened chromatin network, marginal hyperchromatosis, karyolysis, and pyknosis were observed in almost all cells (Fig. 11, Fig. 12, Fig. 13). In addition, most of the mitotic figures still observed in the disorganized tissues were abnormal, with scattering of chromosomal materials in the enormously enlarged nuclei being predominant (Fig. 23).

There were no discernible changes in the stroma observed for a few days following the first irradiation. However, the stromal response became more conspicuous after the second irradiation (six to nine days after the first irradiation). In this stage of tumor degeneration, the nuclei of fibrocytes and endothelial cells of capillary vessels became round and the nucleoli of these cells were clearly visible with azan stain. In parallel with these changes, fibroblastic proliferation was observed (Fig. 12, Fig. 14).

Degeneration of tumor cells became remarkably intense after the third or fourth irradiation (nine to thirteen days after the first irradiation) and many granulocytes, histiocytes, lymphoid cells and plasma cells appeared (Fig. 12, Fig. 24).

In the final stage of tumor regression, after the fifth to sixth irradiation (fourteen to twenty days after the first irradiation), conspicuous proliferation of fibrous tissue (stained bluish with azan stain) was observed (Fig. 15, Fig. 18). In this
fibrous tissue a few degenerating tumor cells were found scattered with infiltration cells.

C. Hematological Observations

Before irradiation all dogs showed a leukocytosis, predominantly neutrophilic, up to 20,000 cells per cubic millimeter. By the time treatment was completed the white-cell count returned to normal. However, prior to the decrease in white-cell count, a transient increase developed in four cases a few days after the first irradiation. A slight fall in eosinophils was observed in five cases within ten days following the first irradiation. Lymphocytes and monocytes showed no definite or consistent changes in number. Both the hemoglobin content and hematocrit decreased slightly in some cases during treatment. Typical blood changes, observed in two cases, are shown in Charts 1 and 2.

DISCUSSION

A temporary inhibition of cell division seems to be a characteristic effect of radiation, having been demonstrated in a great variety of cells (Glücksmann 1941, Hamazaki 1954, Lea 1955). In this study, a comparable change in cells of the transmissible venereal tumor of dogs followed x-ray irradiation. An almost complete disappearance and a subsequent reappearance of mitoses were demonstrated three to six hours and twelve hours, respectively, after the first irradiation at
dosage levels of 200, 300 or 400 r.

Since the majority of mitotic aberrations observed twelve to forty-eight hours after the first irradiation occurred during the prophase or metaphase rather than during the anaphase or telophase, it would appear that most of the tumor cells exposed to irradiation died during the stages of prophase or metaphase without completing the subsequent mitotic division. These findings correspond with the theory that when a cell is killed as a result of irradiation, death does not occur immediately but during or following the next division that the cell undergoes (Glücksmann 1941, Lea 1955).

An increase in the size of cells, or of their nuclei, following irradiation has been observed in a variety of tissues including human tumors (Glücksmann 1941, Wood 1949, Lea 1955). In the present investigation cellular and nuclear enlargement of the tumor was seen twelve to forty-eight hours after the first irradiation, and it became more pronounced after the second exposure. The average diameters of these cells and nuclei were one and a half times as large as those measured before irradiation. The degenerative changes observed in almost all cells following cellular and nuclear enlargement would suggest that the latter alterations represent an initial manifestation of cellular degeneration induced by irradiation. Furthermore, it would appear from these studies that when cellular and nuclear enlargement with nuclear disintegration have become pronounced (e.g.: following four to six exposures to x-ray), cellular degeneration may occur directly rather than during a subsequent mitosis.

A peculiar histological change reminiscent of a perithelioma was observed early in the treatment of one case. The lesions consisted of agglomerations of intact tumor cells around small vessels. It seems possible that the nutrient supply of tumor cells lying remote from the capillaries was insufficient to sustain cellular function against radiation-induced damage whereas cells located adjacent to the capillary vessels survived.

The significance of stromal responses during radiation treatment of the tumor has been recognized by many investigators (Murphy 1923, Koller 1946, Windholz 1947, Jolles 1950). According to their views, tumor regression induced by irradiation is initiated by intra-cellular responses (or direct radiation effects) including suppression of mitosis, cellular and nuclear enlargement, chromosome fragmentation and nuclear disintegration. Following these changes, regression is manifested by inter-cellular responses (or indirect radiation effects) represented by reactions closely resembling inflammation, with hypertrophy of connective tissue elements resulting in the formation of fibrous tissue. The connective tissue responses observed in the tumor of dogs after irradiation treatment were essentially the same as those described above. In these tissues fibroblastic proliferation was observed
initially, followed by infiltration of white blood cells during the period when intra-cellular disintegration was most conspicuous; thereafter, degenerated tumor cells disappeared and fibrosis increased.

The present investigation did not demonstrate significant differences in the cellular degeneration or connective tissue proliferation attributable to differences in the dose levels of x-ray irradiation. The complete regression of the tumor following exposure to a total dose of 1200 to 2400 r would indicate that the venereal tumor of the dog is highly sensitive to irradiation. In view of this, the suggestion by De Monbreun (1934) that the venereal tumor of the dog may be of lymphocytic origin is of interest.

The tumor cell degeneration and stromal response became conspicuous after the third or fourth exposure. Thus, the remaining two exposures may have been excessive and unnecessary. Other experiments are now in progress in order to determine whether complete regression of the tumor can be induced with less therapeutic exposures or with a smaller total dose.

The survival of the tumor mass located deep in the vaginal lumen of dog No. 5 (Fig. 4, Fig. 17) was probably due to inadequate exposure to x-ray. Recent experiences in this department indicate that contact x-ray therapy or surface therapy with radium or radioactive cobalt needles via the vaginal cavity may be effective in such a case.

The elevated total white-cell counts of these dogs, probably due to the inflammatory reaction on the surface of the tumor, decreased to normal following the regression of the tumor. The usual hematologic changes produced by ordinary therapeutic doses of irradiation have been described (Minot 1924, Lasser 1954, Lavedan 1954) as follows: a transient neutrophilia followed by leukopenia and lymphopenia, and a corresponding eosinopenia followed by eosinophilia. In the present studies an initial temporary neutrophilia was observed in four cases, and an initial slight eosinopenia was observed in five cases. No subsequent leukopenia or eosinophilia could be disclosed. A modest decrease in hemoglobin content and hematocrit occurred during treatment; thereafter, these values soon returned to or above pre-treatment levels. The cause of this anemia has not been clarified. However, from the hematological and clinical findings, it seems probable that the systemic effects of irradiation in the doses employed were relatively minor.

**SUMMARY**

The response of the transmissible venereal tumor of dogs to x-ray irradiation has been studied. Tumors of eight female dogs, in the vaginal wall adjacent to the labia, disappeared completely following radiation therapy given in six exposures of 200, 300 or 400r over a period of three weeks (1200 to 2400 r total dose). From
the results obtained it is concluded that the transmissible venereal tumor of the
dog is sensitive to irradiation.

Histological studies revealed transient suppression of mitosis, cellular and nuclear
enlargement and mitotic aberrations in the earlier stages of treatment. In the
advanced stages of tumor regression, nuclear disintegration, infiltration with
granulocytes, lymphoid cells, histiocytes and plasma cells followed by proliferation
of fibrous tissue were observed.

Both clinical and hematological observations indicate that the irradiation therapy
was well tolerated.

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EXPLANATION OF FIGURES (Plates XXII—XXV)

Fig. 1. Appearance of the tumor of dog No. 1 before irradiation treatment. The tumor developed over the vaginal wall near the vulva protruding between the labia.

Fig. 2. Degenerating tumor of dog No. 1 after four exposures of 200 r of X-ray.

Fig. 3. Complete regression of the tumor of dog No. 1 after six exposures of 200 r (1200 r total dose).

Fig. 4. The unaffected tumor mass adjacent to the cervix of dog No. 5 following the irradiation treatment. Note the regressed tumor near the vulva. t: unaffected tumor, u: uterus, r: regressed tumor, b: bladder.

Fig. 5. External appearance of the tumor of dog No. 7 before treatment. The tumor which developed all over the vaginal wall protruded from the vulva, elevating the perineum.

Fig. 6. Complete regression of the tumor of dog No. 7 after six exposures of 400 r (2400 r total dose).

Fig. 7–Fig. 9. Photomicrographs of the tumor of dog No. 7 before irradiation. Parenchymal cells with round or oval nuclei containing a distinct nucleolus are intermingled with a fine network of stromal fibers. Several mitotic figures are observed. Fig. 7 H-E, ×200, Fig. 8 Azan, ×200, Fig. 9 H-E, ×400.

Fig. 10. The tumor tissue of dog No. 7 biopsied forty-eight hours after the first irradiation. Abnormal mitotic figures including fragmented and sticky chromosomes are observed. H-E, ×400.

Fig. 11. Pronounced degeneration of the tumor cells of dog No. 7, four days after the second irradiation. Note cellular and nuclear enlargement and many pyknotic and karyolytic figures of the tumor cells. Scattered and fragmented chromosomes are observed in dividing cells. H-E, ×400.

Fig. 12. Nuclear disintegrations including vacuolization, karyolysis or pyknosis were noted in dog No. 7 three days after the fourth irradiation. Cell infiltration and fibroblastic proliferation became marked. H-E, ×400.

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Fig. 13. Fat droplets in degenerated tumor cells of dog No. 7 three days after the fourth irradiation. Sudan III, ×400.

Fig. 14. Conspicuous fibroblastic proliferation associated with the advanced degeneration of tumor cells of dog No. 2, three days after the fourth irradiation. H-E, ×400.

Fig. 15. Prevailing fibrosis observed in dog No. 7 after the completion of treatment. H-E, ×200.

Fig. 16. Perivascular cuffs of tumor cells observed in dog No. 8 forty-eight hours after the first irradiation. H-E, ×100.

Fig. 17. An unaffected tumor tissue in the remained tumor mass located adjacent to the cervix of dog No. 5. H-E, ×200.

Fig. 18. Fibrous tissue near the vulva of dog No. 5. H-E, ×200.

Fig. 19. A chromosomal bridge observed in dog No. 7 immediately after the first irradiation. Heidenhain's iron hematoxylin, ×1500.

Fig. 20. Sticky chromosomes observed in dog No. 2 forty-eight hours after the first irradiation. H-E, ×1000.

Fig. 21. A tripple cleavage observed in dog No. 1, forty-eight hours after the first irradiation. H-E, ×1000.

Fig. 22. Chromosomal bridges observed in dog No. 7, four days after the second irradiation. H-E, ×1000.

Fig. 23. Scattered chromosomes in two mitotic cells observed in dog No. 7, four days after the second irradiation. H-E, ×1000.

Fig. 24. A ring-shaped histiocyte phagocyting cell debris observed in dog No. 2, two days after the third irradiation. H-E, ×1000.

要 旨

犬の可移植性性器腫瘍におよぼすX線照射の効果

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犬の可移植性性器腫瘍におよぼすX線照射の効果を、自然発生の本腫瘍を媒介に有する8頭の犬について検索した。

陰門部附近に発生した腫瘍はすべて1回線量200, 300, 400rの6回照射（間隔2〜3日）総量1200〜2400rの照射によって縮小治癒した。この結果本腫瘍が放射線に対する感受性の高い腫瘍であることが分った。

また組織学的検索の結果、治療初期に一時的な細胞分裂の抑制、種々の異常分裂像、細胞および核の大きさの増大が認められた。それ以後の時期では腫瘍細胞の著しい変性、遊走細胞の浸潤が認められ、線維芽球の増生によって腫瘍基底部に癒痕組織が形成された。

臨床所見および血液所見から考えるとX線照射の犬の全身状態におよぼす影響は少なかった。