Transplantation of a Thyroid Carcinoma within the Canine Species*

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Few reports of successful transplantation of canine tumors have been found in the literature, with the exception of the venereal sarcoma and the infectious papilloma. McWhorter and Prime (3) transplanted a chondro-osteosarcoma through three series of dogs, only to lose it in the third passage. In the first transfer of tumor tissue, growth was obtained in 25 per cent of the animals. The authors reported that they obtained the best results when using newborn puppies. Mann (2) transplanted a mammary carcinoma which grew in approximately 3 per cent of the animals in the first-generation transplant. In three-fourths of these successful transplants, the tumor was undergoing necrosis and absorption by the 7th-12th day after injection. In one animal carcinoma cells which appeared healthy were seen 41 days after injection. No attempt was made to pass the tumor through a second generation. Imamaki (1) reported the successful propagation of a canine endothelioma for nineteen generations within a 2-year period. The author stated that the original tumor had developed spontaneously in the abdominal wall and tumor nodules were present on the penis. Owing to the designation “endothelioma,” as applied to the venereal sarcoma (Beebe and Ewing), the ease in transplanting the tumor, and the location in the original host, there is good reason to believe that it might be the venereal sarcoma.

Tumor transplantation has been undertaken to find a spontaneous canine tumor that can be transplanted through a series of dogs with a high percentage of growth in each passage group. The ultimate goal is to standardize the procedure of transplantation in order that a larger tumor-bearing animal might be made available for oncologic research.

Transplantations have been attempted with thirteen spontaneous canine tumors. Inoculations of the tumor material have been performed by the subcutaneous, intramuscular, subarachnoid, intracerebral, intraocular, intratesticular, and intraperitoneal routes, with both purebred Beagle and mixed-breed puppies. After numerous attempts by the above methods proved unsuccessful, it was decided to treat the experimental animals with irradiation and cortisone. Results with cortisone alone did not improve the outcome. After the instigation of total-body irradiation followed by the administration of cortisone, similar to the method used by Toolan (4), a spontaneous thyroid carcinoma has been transplanted successfully through seven generations to date. In one group of second, fourth, and seventh serial transplant puppies, nitrogen mustard (Mustargen, Merck) was substituted for irradiation. The procedure is described below.

MATERIALS AND METHODS

Mixed-breed puppies of both sexes, ranging in age from 3 weeks to 3 months, received either 800 or 500 r total-body irradiation in one dose. The 500-r dose was discontinued in favor of the 300-r for the third and succeeding serial transplants, since the lesser dose resulted in as high a percentage of growth without appreciably shortening the life of the animal. X-radiation was administered at 220 kv. and 15 ma. at a TSD of 50 cm. (48 r/min). Added filtration was 0.5 mm. Cu and 1.0 mm. Al to give a HVL (half value layer) of 1.05 mm. Cu. Animals were given implants of the tumor tissue 4–5 days following irradiation. When nitrogen mustard was substituted for irradiation, the dose was 0.4 mg/kg of body weight, administered intravenously 1 hour to 2 days prior to implantation.

The first series of transplants was composed of four puppies, the only animals available at the time the tumor presented itself. The second series was composed of sixteen puppies; the third, elev-

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en; the fourth, fourteen; the fifth, twelve; the sixth, 22; and the seventh, 22.

The original tumor was obtained from a 5½-year-old Boxer female with a history of a cough for 2 weeks prior to biopsy, listlessness, and enlargement in the ventral cervical region. Biopsy revealed the tumor to be an undifferentiated medullary carcinoma. Necropsy disclosed a diffuse, multi-lobulated, soft tumor mass, which extended approximately 11 cm. from the posterior part of the larynx (Fig. 1). The entire thyroid gland was replaced by the tumor tissue. The regional lymph glands and lungs were involved with metastatic tumor tissue (Fig. 2). Histologically, the tumor was markedly cellular and contained numerous mitoses and giant cells (Fig. 5). The microscopic picture of the tumor nodules within the lymph glands and lungs was similar to that seen in the primary.

The tumor tissue for transplantation was obtained aseptically from the thyroid gland of the donor while the animal was still living. Twelve- or 13-gauge needles were filled with finely minced tumor tissue and were kept at approximately 4° C. Transplantations were made both subcutaneously in the left flank region and intraperitoneally to the right of the umbilicus in the first through the fifth transfers. In one group of the fifth, in two of the sixth, and in all of the seventh series, only the subcutaneous route was used. Passage of tumor material from one series to another was made after 2 or more weeks of active tumor growth in one host.

The initial dose of cortisone (Cortone, Merck) or hydrocortisone (Hydrocortone, Merck) was given orally on the day of tumor transplantation and subsequent administration on alternate days that followed. Doses given to the first two serial transfers varied from 2–5 mg/pound of body weight administered on alternate days. For the third and succeeding transfers the dose was kept at 5 mg of cortisone or hydrocortisone/pound of body weight given on alternate days. The administration of cortisone was continued either for a 2 weeks’ period or for the duration of the animal’s life.

RESULTS

First generation.—In the first transplant, in which one-half of the recipients received 500 r total-body irradiation and the remaining 300 r, both receiving cortisone for 2 weeks’ period, the transplants continued to grow up to the time of death in all animals. The animals getting 500 r (LD 50/30) died or were destroyed within 27 days. The puppies receiving 300 r either died or were destroyed within 46 days. Deaths were frequently due to infections; to counteract this, streptomycin was given daily following irradiation. All puppies received distemper and hepatitis antiserum at weekly intervals.

One of the animals in the first series was destroyed on the 46th day after subcutaneous and intraperitoneal transplantations because of the markedly debilitated and cachectic state of the animal. At necropsy a large subcutaneous tumor mass with ulceration measured 4.2 x 3.3 x 3.0 cm. The tumor was surrounded by an area of hemorrhage. There was diffuse tumor involvement of the omentum, mesentery, and serosal surface of the intestines and invasion of the abdominal musculature at the site of subcutaneous inoculation. The intraperitoneal growths varied from 0.1 cm. in diameter to 6.0 x 5.5 x 3.2 cm. Degeneration had occurred within both the subcutaneous nodule and the largest intraperitoneal ones. However, each contained a rim of healthy-appearing tumor tissue measuring 2.5–3.0 cm. in thickness. The microscopic pattern of the tumor resembled that of the original tumor (Figs. 6 and 7).

The remaining three animals in the first series demonstrated growth in varying degrees of the intraperitoneal tumor inoculum in the mesentery and omentum as well as extension of the tumor tissue in the abdominal musculature at the site of the subcutaneous inoculation. The average measurement for the subcutaneous nodules in the first series taken 21 days after transplantation was 4.3 x 3.0 x 2.5 cm.

No alterations were noted in the thyroid glands of these animals.

Second generation.—In the second series of transplants, one group of animals was given total-body irradiation, half receiving 500 r, and half 300 r; a second group received nitrogen mustard in place of irradiation; and a third group received only cortisone. The first group received cortisone for the duration of life; the second, for a period of 2 weeks; and the third, for the duration of the experiment. All animals in the hydrocortisone-treated, irradiated group developed tumors; the average measurement for the subcutaneous nodule taken 21 days after transplantation was 5.3 x 1.8 x 0.9 cm. In the group receiving nitrogen mustard followed by cortisone tumor growth occurred in 15 per cent of the animals. The nonirradiated group receiving 5 mg of cortisone/pound of body weight on alternate days presented evidence of tumor growth at the site of subcutaneous inocula-
tion from the 8th to the 12th day; however, by the 16th day, the nodules had completely regressed. Biopsy taken 8 days following transplantation disclosed that the tumor was undergoing degeneration and necrosis, although there were areas of apparently healthy tumor cells. No mitoses were observed.

Third generation.—In the third series of transplants, in which all puppies received 800 r total body irradiation and hydrocortisone for the duration of life, the transplants grew in every animal. Animals dying on the 14th to the 25th day following transplantation demonstrated widespread growth of the intraperitoneal tumor inoculum (Fig. 8). Tumor nodules varying in size from 0.1 cm. in diameter to 2.2 X 1.7 X 1.0 cm. were found on the parietal peritoneum, omentum, mesentery, diaphragm, ovarian ligament, and on the serosal surfaces of the kidneys, urinary bladder, and intestines. Hemoperitoneum was observed in five animals. The individual tumor masses contained areas of hemorrhage, and blood clots were attached to the tumor nodules. The tumor invaded the abdominal musculature, and mesenteric and sternal lymph node metastases were found. The average measurement for the subcutaneous nodules in the third series at 21 days was 3.2 X 2.2 X 1.5 cm. The microscopic pattern in the third series resembled that of the original tumor and that of the preceding transplants. However, in scattered areas tumor cells adhered closely to one another in groups or islands (Fig. 8). Examination of the thyroid glands in this series revealed no apparent alterations.

Fourth generation.—Of the fourth series of transplants, one group received nitrogen mustard together with hydrocortisone for a 2-weeks’ period, while the two remaining groups received total-body irradiation followed by hydrocortisone for a 2 weeks’ period. In the first group the transplant of only one animal grew, whereas in the latter groups all transplants grew; necropsy findings for animals dying from the 12th to the 15th day were similar to those observed in the third series. Hemoperitoneum was noted in four animals. The average measurement for the subcutaneous nodules in the fourth series on the 14th day following transplantation was 2.4 X 2.1 X 1.2 cm. The histologic picture in the fourth series was similar to that of the preceding series.

Fifth generation.—Of two groups of fifth serial transplant puppies that were given irradiation followed by hydrocortisone for the duration of life, one received only a subcutaneous transplant, whereas the other was given inoculations of tumor material by both subcutaneous and intraperitoneal routes. The transplants grew in all the above animals. The average measurement for the subcutaneous tumor nodules in the two groups was 3.2 X 2.6 X 1.8 cm. at 21 days. Two of the irradiated puppies were still living by the 32d day, and the tumor nodules continued to increase in size. Growth occurred in one of three animals of a third group of fifth-series puppies receiving neither irradiation nor cortisone, and given tumor material by the subcutaneous route. The measurement in the one taken 21 days following transplantation was 2.5 X 1.7 X 1.4 cm. By the 32d day the tumor had completely regressed.

Sixth generation.—Animals in the sixth transplant series were divided into four groups: the first was composed of irradiated, hydrocortisone-treated puppies which received transplants by the subcutaneous route; the second, untreated animals receiving subcutaneous transplants; the third, irradiated, hydrocortisone-treated animals given intrasplenic vein inoculations; and the fourth, nonirradiated, hydrocortisone-treated ani-
FIG. 5.—Original canine tumor: thyroid carcinoma from the necropsy specimen. Hematoxylin & eosin. X300.

FIG. 6.—Subcutaneous tumor nodule, first-generation transplant, 24 days following transplantation. Hematoxylin & eosin. X300.

FIG. 7.—Subcutaneous tumor nodule, first-generation transplant, 24 days after transplantation. High power of Figure 6. Hematoxylin & eosin. X700.

FIG. 8.—Subcutaneous tumor nodule, third-generation transplant, 14 days after transplantation. Hematoxylin and eosin. X300.
mals inoculated through the intrasplenic vein. Groups one, three, and four received hydrocortisone for the duration of life, whereas group two received none. In the first group all transplants grew. At 21 days the average measurement for the tumor was $4.5 \times 3.5 \times 2.3$ cm.; one of the puppies which was alive and in good health by the 31st day had an actively growing tumor which measured $7.5 \times 6.4 \times 5.8$ cm. Growth of the transplants was not observed in the second and fourth groups, whereas tumor growth in the third occurred in all animals with tumor nodules in the liver, hepatic lymph glands, lungs, and spleen. Histologically the tumor cells in the various organs either were surrounded by areas of hemorrhage or grew along the circumference of an area of hemorrhage.

Seventh generation.—The seventh passage of the tumor was made into two groups of animals, one receiving x-radiation and the other, nitrogen mustard, both to receive hydrocortisone for the duration of the experiment. At present, the percentage of success cannot be fully ascertained, although it is 100 per cent in both groups in animals where 2 or more weeks have passed since transplantation.

One eighth-series group has been initiated in x-irradiated, hydrocortisone-treated puppies.

DISCUSSION

With the use of total-body irradiation and cortisone or hydrocortisone, it has been possible to transplant a spontaneous canine thyroid carcinoma through seven generations by using mixed-breed puppies with a transplant growth that approaches 100 per cent. The substitution of nitrogen mustard for total-body irradiation has failed to give comparable results. The use of cortisone alone, as seen in the third group of the second generation, resulted in growth of the transplants up to the 8th–12th day, but with complete regression by the 16th day. Transplantation of the tumor to untreated puppies, as seen in the third group of the fifth generation and in the second and fourth groups of the sixth, has resulted in the lowest percentage of growth.

Marked differences were not observed between animals receiving cortisone or hydrocortisone for 2 weeks and those receiving it for the duration of life. Since the tumor now appears to be well established, it is planned to reduce the amount of irradiation and cortisone and to attempt to obtain tumor growth in untreated animals. By reducing the dose of irradiation and cortisone, it is reasonably certain that the life expectancy of the experimental animals can be lengthened. The high incidence of deaths in our puppies is due mainly to bacterial and viral infections, to which the animals are more susceptible following treatment with irradiation and cortisone. Some of the deaths in the intraperitoneally inoculated animals could be attributed to the tumor, because of its widespread growth in the peritoneal cavity, the associated hemoperitoneum, the anemic state of the animal, or to the cachectic state of the animal associated with marked tumor degeneration within the peritoneum.

SUMMARY

By the use of total-body irradiation followed by cortisone or hydrocortisone, a thyroid carcinoma spontaneously occurring in a Boxer dog has been transplanted with a high degree of success through seven generations of mixed-breed puppies and is now in its eighth passage. Lymph node metastases have been observed.

REFERENCES

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*Cancer Res* 1954;14:734-737.

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