same secretory cells or different ones with the same secretory proteins.

**Key words:** basal secretion, cholinergic agonists, lacrimal gland fluid proteins, nondenatured gradient gel electrophoresis, reflex secretion

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**References**


**A New Technique For Subchoroidal Implantation of Experimental Malignant Melanoma**

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A new technique for implanting Greene hamster amelanotic melanoma cells into the rabbit eye is described. The technique involves the deposition of a tumor fragment into the subchoroidal space via a transvitreal approach. Thirty rabbit eyes were implanted with 26 successful tumor growths producing solitary choroidal nodules. This technique offers the advantages of rapid implantation, the ability to precisely choose the site of implantation including posterior sites, and eliminates the need for a large scleral incision. Invest Ophthalmol Vis Sci 29:995-998, 1988

The Greene hamster amelanotic malignant melanoma is frequently transplanted into the subchoroidal space of the rabbit and used as a model for choroidal melanoma. The current technique of implanting the tumor involves making a posterior scleral incision, implanting the tumor and resuturing the sclera. Since spread of the tumor is often posteriorly through the sclera, any artificially created defect in the sclera may enhance extrascleral extension. The purpose of this study is to describe a new technique of transvitreal transplantation of Greene melanoma into the subchoroidal space of the rabbit that eliminates the need for posterior scleral disruption.

**Materials and Methods.** The Greene melanoma was propagated in the anterior chamber of adult, pigmented rabbits (2–3 kg). Rabbits were anesthetized with intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg). After topical administration of 0.5% proparacaine, 100,000 viable melanoma cells were injected intracameraly. Visible tumor nodules appeared on the iris surface within 1 week. By 2 weeks the nodules were large and confluent and the animal was anesthetized and sacrificed using intracardiac pentobarbital sodium. Under sterile conditions, the eye was enucleated, tumor removed and kept moist with saline while the recipient eye was prepared.
The recipient rabbit was anesthetized as above and a 3 mm inferotemporal conjunctival peritomy was performed. A sclerotomy was then made 1 mm posterior to the limbus using a 22-gauge needle. Under microscopic control (Weck, Durham, NC) the tip of the needle was then bent to a 45° angle and attached to a tuberculin syringe. This was then inserted through the sclerotomy and into the mid-vitreous cavity. The intraocular pressure was raised by the injection of physiologic saline. Using the tip of the needle, a retinotomy and choroidal rupture was produced in an area at the surgeon’s discretion and the needle removed.

A 0.25 mm fragment of tumor was then aspirated into the tip of a 25-gauge cannula and this was placed into the eye. Under microscopic control the tip of the cannula was placed in the subchoroidal space, tumor deposited, and needle removed (Fig. 1). The conjunctiva was then placed over the unclosed sclerotomy site. The entire implantation procedure could be completed in 2 minutes.

Animals were examined with indirect ophthalmoscopy twice weekly and sacrificed at intervals ranging

![Fig. 1. Illustration demonstrating the placement of a tumor fragment into the subchoroidal space using a transvitreal 25-gauge cannula.](image)

![Fig. 2. Solitary choroidal Greene melanoma nodule 5 days after implantation with small overlying retinal tumor nodule (H & E, original magnification ×25).](image)
from two to 12 weeks. The eyes were enucleated, fixed in 10% formalin and prepared for light microscopy. All studies were in compliance with the ARVO Resolution on the Use of Animals in Research.

**Results.** The tumor was implanted in 30 rabbit eyes with 26 successful takes. Initially eyes were pretreated with argon photocoagulation, producing a demarcation line around the anticipated implantation site. However, as no cases of retinal detachment were noted, subsequent eyes were not pretreated with laser. Occasionally, a small amount of vitreous hemorrhage occurred but in only one case did it interfere with implantation. After 1 week, a small tumor nodule could be visualized (Fig. 2). Large solitary tumors were present by 2 weeks (Fig. 3). By 3 weeks the tumor had spread more diffusely throughout the choroid. Rapid growth continued with retinal detachment, vitreous hemorrhage and complete intraocular filling by 6 to 8 weeks. Extrascleral extension occurred posteriorly through the optic nerve or by perforating at the limbus in eyes followed for greater than 8 weeks. No evidence of extension through the sclerotomy site was seen. Distant kidney metastases were documented at 12 weeks in one of two rabbits studied.

**Discussion.** The subchoroidal transplantation of Greene melanoma cells in the rabbit is a frequently used model for choroidal melanoma. The current technique of transplantation involves the production of a 2 to 3 mm scleral incision which may enhance extrascleral extension. If one is using the model to test new treatment modalities, any enhancement of tumor extension may prove deleterious to the evaluation of the treatment. This is particularly true in the evaluation of intraocular treatments that rely on focally treating visually apparent tumors. For this reason we developed a transvitreal tumor transplantation technique which, like the scleral incision technique, produces reliable and reproducible solitary tumor nodules. This technique offers three advantages. First, the lack of posterior scleral disruption eliminates the possibility of artifactitious extrascleral extension. None of our cases developed tumor extension at the sclerotomy site. Second, this technique is rapidly performed with complete implantation taking only a few minutes. Third, the tumor may easily be placed at any location. Thus, one can produce peripheral or posterior tumors.

There are two potential disadvantages of the technique. First, a surgical microscope is required and many animal laboratories may not be equipped with one. Second, one is trading a disruption of the sclera for a disruption in the retina and choroid. However, this may be acceptable in the evaluation of new treatment modalities that are aimed at treating small, solitary nodules. We have found that five out of eight small solitary nodules produced by the scleral incision technique developed evidence of extrascleral extension through the site of implantation (unpublished data). Using the transvitreal technique, however, no evidence of extension through the sclerotomy site could be documented in 26 eyes, though occasional small growths of tumor in the retina were noted.

We feel that transvitreal subchoroidal implantation
of tumor represents a useful model for the evaluation of focal surgical treatments of ocular tumors.

Key words: choroidal malignant melanoma, animal model, transplantation, Greene melanoma

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References