The Use of the Mouse Eye in Transplantation Experiments*

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The anterior chamber of the eye has proven an almost ideal site for tissue transplantation. The technic of transfer is simple, a high percentage of takes is obtained and the growing tissue can be followed by direct visual observation or even subjected to microscopic examination. A further advantage derives from the fact that the chamber supports the growth of heterologous tissues whereas, in other bodily sites, such tissues invariably fail to survive. Moreover, tissues grown in the chamber are usually readily separable from the tissues of the host; they contain little desmoplastic reaction, and in general are excellent material for chemical or immunological study.

In this laboratory, animals such as the guinea pig and rabbit have been used extensively in experiments on transplantation of organs and tissues to the anterior chamber of the eye. The expense of securing and maintaining such animals is considerable in relation to that entailed in obtaining and providing for mice and for this reason, a modification of the technic has been perfected to permit the easy and rapid utilization of mice for the same purpose.

METHOD

The technic used for anterior chamber transfer in larger animals is unsatisfactory when applied to mice. Securing the mouse to an animal board is difficult and time-consuming. A local anesthetic is inadvisable because of the proximity of the conscious animal's teeth to the operative field. The combination of a small eye, a resentful animal and a cumbersome restraining apparatus makes an ordeal of the procedure.

The technic adopted eliminates such difficulties. General anesthesia is effected by suspending the mouse by its tail in a drinking tumbler containing several gauze sponges soaked in ether. Sufficient anesthesia is obtained in 30 seconds, and as this step is carried out by an assistant, the time may be utilized by the operator in placing the tissue to be transferred in a trocar. The trocar is made by shortening the bevel at the tip of a 20-gauge hypodermic needle. A suitable, tight-fitting plunger can be manufactured or obtained simply by selecting a wire stylet of proper size from the stock supplied with the needles and applying a knob of sealing wax or other plastic material to one end.

A minute fragment of tissue is placed in the mouth of the trocar and manipulated into its barrel. This is usually readily accomplished by retracting the plunger to exert suction and prodding the fragment with a fine needle. Sometimes this operation may be irksome, particularly if the tissue is dry or sticky, but caution should be urged against the use of so-called physiological saline in an attempt to overcome the difficulty. Stock saline solutions in general use are not physiological and are often sufficiently toxic to cause death of the tissue.

The anesthesia rarely lasts longer than a minute and subsequent procedures must be executed rapidly. The mouse is held loosely in the left hand and the lids of its right eye forced apart with the thumb and index finger. Slight pressure with these fingers causes the eye to protrude sufficiently to allow adequate exposure for the operation. The anterior chamber is opened close to the upper border of the corneo-scleral junction by means of a short, quick jab with a double-edged corneal knife. The knife is of a size generally used in ophthalmological work and is readily secured in any instrument house. Single-edged knives or pointed Bard-Parker blades are not satisfactory for they result in a triangular incision through which the iris may herniate. The incision is made only of sufficient length to admit the trocar and must be accomplished entirely by the downward thrust of the knife for the instability of the protruding eye prohibits side cutting. The point of the knife should be directed slightly forward in making the incision in order to enter the chamber without cutting the iris.

The trocar, held between the thumb and middle finger of the right hand, is inserted into the chamber through the incision and the fragment expressed by pushing the plunger with the index finger. In order to prevent extrusion and escape of the fragment through the excision, all pressure exerted on

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the animal by the left hand should be released before withdrawal of the trocar. The fragment now free in the chamber, is forced into a wedged position at the inferior angle of the iris by applying light pressure along the corneal surface with a blunt instrument. The incision is not closed.

Considerable trouble may be encountered in preventing the escape of soft, slippery tissues such as embryonic brain when the trocar is withdrawn. This difficulty may be circumvented by incising the iris as well as the cornea at the limbus and directing the trocar behind the superior half of the iris, through the pupil and into the inferior portion of the anterior chamber. With withdrawal of the trocar, the fragment is almost invariably caught at the pupillary border and retained in the chamber.

The whole operation can be performed rapidly after short practice. The average speed in this laboratory is 2 mice a minute and further acceleration may be obtained if 2 anesthetists are employed. However, it should be emphasized that sterility is essential; instruments should be boiled and aseptic technic maintained throughout the procedure. The mouse eye is apparently much more susceptible to infection than is the eye of the guinea pig or rabbit.

**RESULTS**

The mouse eye has been used extensively in this laboratory for the past 3 or 4 years, particularly in the homologous and heterologous transplantation of cancer and embryonic tissue. The results have been most satisfactory in experiments in which only small growths of tissue were desired. The anterior chamber of the mouse eye is not large enough to contain growths of the size needed for

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**DESCRIPTION OF FIGURES 3 TO 8**

**Fig. 1.**—Mouse of strain A bearing anterior chamber transplant of embryonic mouse tongue treated with methylcholanthrene. This photograph was taken 83 days after transfer. Histologically, the tumor was an epidermoid carcinoma.

**Fig. 2.**—Mouse of C3H strain bearing anterior chamber transplant of Brown-Pearce rabbit tumor. This photograph was taken 140 days after transfer and represents a recurrence of growth after partial regression of transplant. Histological sections at death were identical with those of same tumor grown in rabbit.

**Fig. 3.**—Section of eye of C57 mouse bearing a transplant of a mouse ovarian embryoma. The animal was killed 2 1/2 weeks after transfer. Mag. X 35.

**Fig. 4.**—Higher power view of previous section. Mag. X 320.

**Fig. 5.**—Section of eye of Swiss mouse bearing transplant of mammary carcinoma originating in CBA mouse. Attempts to transfer tumor from CBA mouse to subcutaneous tissues of Swiss mice were unsuccessful but high percentage of takes was obtained when tumor tissue from Swiss eyes was transferred to subcutaneous spaces of other Swiss mice. Mag. X 50.

**Fig. 6.**—Higher power view of previous section. Mag. X 350.

**Fig. 7.**—Section of eye of Swiss mouse bearing a transplant of Cloudman melanoma. As in previous case, transfer from the eye to subcutaneous space resulted in takes whereas direct subcutaneous transfer from parent strain to Swiss was unsuccessful. Mag. X 50.

**Fig. 8.**—Higher power view of previous section. Mag. X 225.
Figs. 3-8
chemical or immunological studies and other species are more suitable for such purposes. Large growths do occur in the mouse eye but they are necessarily associated with rupture of the cornea and external protrusion. Infection is always present in such cases and the tissue is valueless for further passage or other experimentation.

Homologous transfers.—The transfer of mouse tumors to the anterior chambers of eyes of other mice results in a high percentage of takes and rapid growth. The age or sex of the recipient has played no observable part in the behavior of the graft in our series of transfers. Moreover, the so-called influence of strain appears to be largely negated when the anterior chamber is used.

Reports in the literature suggest that many mouse tumors are strain-specific. This has not been the case with the great majority studied in this laboratory. It is true that, in early stages of development, mouse tumors, like tumors in other animal species, are dependent in nature and are transplantable only autologously or to other animals of the same strain. But, with continued development in the original host, they attain autonomy and the ability to survive and to grow on homologous and heterologous transfer. Thus, only autologous takes or takes in animals of the same strain may result from the transfer of tissue obtained at biopsy whereas growth in foreign strains and even in alien species occurs with material derived from autopsy, 1 to 3 months later. It is suggested that the standard strain-specific mouse tumors, well known in all cancer research laboratories, were originally transferred from a spontaneous growth before autonomy had been attained.

In any case, it has been our experience that, if the primary host survives a sufficient period of time after the origin of the tumor, the growth loses its “strain specificity” and becomes transplantable in many strains. At the same time a variation persists in the case with which the autonomous tumor is transferred to various strains. Thus, one tumor originating in a C3H mouse could be transferred directly to dba mice but not to C57 mice. On the other hand, when the same C3H tumor grown in a dba mouse, was used, a high percentage of takes occurred in C57 mice. Such results suggested that the strain of the donor exerted an influence on the outcome of transfer and it was felt, for many reasons, that this might be referable to an antagonistic interaction between the connective tissues of the intended recipient and the tumor stroma carried along with the parenchyma at transfer (1). The point to be emphasized in the present connection bears on this suggestion, for takes occur on direct transfer from the primary host to the eye of the new strain in cases in which successful subcutaneous transplantation requires the intermediation of another host. After growth in the eye, the tumor can be readily transferred to the subcutaneous space of the new strain and carried by serial passage in that site. If, as suggested, incompatibility reactions between the connective tissues of the donor and recipient form the basis of the failure of primary subcutaneous transfer, then it must be assumed that such reactions do not occur in the anterior chamber. Other experiments substantiate this assumption and offer a plausible explanation. Histological study of anterior chamber grafts removed at short intervals after transfer shows that the transplanted stroma dies before vascularization by the new host begins while, in the interim, the parenchyma proliferates in the manner of a tissue culture. Thus, when vascularization eventually occurs and the connective tissues of the new host are brought in contact with the implant, the old stroma has largely disappeared and the basis for any serious interaction has been removed.

Growth is rapid in the anterior chamber so that after several weeks, the cornea ruptures and the tumor protrudes as a fungating mass. Large portions become necrotic, covered with dried crust and may eventually slough off. In such cases, the animal may live long enough for metastasis to occur, but usually the tumor becomes infected

DESCRIPTION OF FIGURES 9 TO 14

Fig. 9.—Section of eye of C57 mouse bearing transplant of the upper third of 2 mm. C3H embryo. Section was taken 80 days after transfer and shows growth of cartilage and squamous epithelium. Mag. X 50.

Fig. 10.—Section of eye of C57 mouse bearing transplant of intestine from C57 embryo. Section was taken 56 days after transfer. Mag. X 35.

Fig. 11.—Section of eye of Swiss mouse bearing transplant of lung from Swiss embryo. Section was taken at 94 days and shows an epithelialization of alveoli comparable to that seen in so-called lung adenomas of A strain. Mag. X 150.

Fig. 12.—Section of eye of Bagg albino mouse bearing transplant of brain from strain A embryo. Section was taken at 39 days and shows growth of ganglion cells as well as of glial elements. Mag. X 300.

Fig. 13.—Section of eye of strain A mouse bearing a transplant of spleen from strain A embryo. Section taken 390 days after transfer. Mag. X 70.

Fig. 14.—Higher power view of previous section. Note megakaryocytes. Mag. X 500.
and death follows without lymphatic extension or dissemination (Figs. 3 to 8). The transplantation of embryonic tissue is also readily performed in the mouse eye and at the present time, all of the various organs and tissues of the mouse embryo, with the sole exception of the liver, have been successfully transferred to this site. The anterior chamber possesses an advantage in the transfer of small organs as the gonads, spleen and esophagus in that they remain in view and are not lost during early growth phases, as is the case in the expanse of the subcutaneous space. In general, the subcutaneous space is a better nidus for larger organs such as the stomach or lung for it allows their full expansion, whereas in the chamber normal contours and relationships are soon lost and continued growth may lead even to corneal rupture.

A high incidence of takes is obtained and failure appears to depend entirely on infection or faults in technic. The transplanted organ usually reaches its maximum size in from 2 to 3 weeks and no further increase in size occurs. To date, mice bearing organ transplants have been held under observation for as long as 18 months without sign of regression and there is no reason to believe that the grafts will not persist throughout life.

Sections of the transplants obtained several weeks or months after transfer show well developed organs (Figs. 9 to 18). Modifications in differentiation sometimes occur, notably in the lung where squamous metaplasia of bronchial epithelium and epithelialization of alveoli (so-called lung adenomas) are most common.

In several experiments, fragments of adult organs have been used for transfer but potentialities in this direction have not been adequately explored. The fragments survive and actually show some increase in size. Histologically, the structure of the parent organ is duplicated and in view and are not lost during early growth phases, as is the case in the expanse of the subcutaneous space. In general, the subcutaneous space is a better nidus for larger organs such as the stomach or lung for it allows their full expansion, whereas in the chamber normal contours and relationships are soon lost and continued growth may lead even to corneal rupture.

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In several experiments, fragments of adult organs have been used for transfer but potentialities in this direction have not been adequately explored. The fragments survive and actually show some increase in size. Histologically, the structure of the parent organ is duplicated and organization appears normal (Fig. 19). Transplants of adult nerve are an exception in this respect and the aberration is of some interest. The fragments grow rapidly to fill the chamber and, on section, show a disorganized proliferation of Schwann cells bearing a distinct resemblance to tumors derived from these elements (Fig. 20).

The susceptibility of embryonic tissues to the action of carcinogenic chemicals has been reported (2, 3, 5, 6) and the anterior chamber of the mouse eye has been investigated as a nidus for carcinogenesis of this type (Figs. 1, 21 to 24). The technic is essentially identical with that employed in the transplantation of normal embryonic tissues, the only alteration being the addition of a minute crystal of methylcholanthrene to the fragment before transfer. As a rule, an interval of 60 or more days is required before the occurrence of indicative morphological changes, a period of approximately twice the duration required in the subcutaneous space. However, there are distinct advantages, apart from the opportunity for direct visual observation, associated with the use of the eye in such experiments. The eye affords a better medium for the growth of epithelium than does the subcutaneous space, takes occur with higher frequency and structures are reproduced with greater fidelity. Moreover, the connective tissues of the eye are more resistant to the action of carcinogenic chemicals, and sarcomatous growths of the host which might be interpreted as arising in the transplant are far less common.

Heterologous transfers.—Tumors of human and rabbit origin have been successfully transplanted to mouse eyes, although a considerable variation exists in the ease with which the two types of transfer are effected. Transfer from man directly to the mouse gives rise to relatively few takes and much better results are obtained if the tumor is first passed through a guinea pig generation. In contrast, the mouse is a better host for rabbit tumors than is the guinea pig.

The species studied may be classified with respect to the ease of heterotransplantability. A curious relationship was revealed: The mouse and the rabbit constitute one group and man and the guinea pig the other. Transfer within these groups is comparatively easy while transfer between the groups is difficult and attended with a much smaller percentage of takes. It is obvious that

**DESCRIPTION OF FIGURES 15 TO 20**

**Fig. 15.**—Section of eye of Strain A mouse bearing a transplant of kidney from a Strain A embryo. Section was taken 28 days after transfer. Mag. × 35.

**Fig. 16.**—Higher power view of previous section. Note persistence of embryonic type glomeruli. Mag. × 300.

**Fig. 17.**—Section of eye of ZBC mouse bearing a transplant of testicle from C3H embryo. Section was taken 38 days after transfer and shows growth of epididymis as well as of testicle. Mag. × 30.

**Fig. 18.**—Higher power view of previous section. Mag × 225.

**Fig. 19.**—Section of eye of C3H mouse bearing transplant of ovary from another adult C3H mouse. Mag. × 85.

**Fig. 20.**—Section of eye of Swiss mouse bearing a transplant of sciatic nerve from another adult Swiss mouse. Proliferation of cells resembles a neurofibroma of Antoni type. Mag. × 50.
this grouping of species also represents a division with reference to the ability to synthesize vitamin C. The relationship may be purely coincidental but metabolic differences between tumor and host tissues presumably occur and might well account for the observed variations. Pertinent investigations are in progress.

The heterologous tumors most fully studied in the mouse have been a human fibrosarcoma (4) (Figs. 25, 26) and the Brown-Pearce rabbit tumor (Fig. 2). Both give rise to a high percentage of takes, grow to fill the eye and are easily carried by serial transfer if the donor mice are killed before the expanding tumor ruptures the cornea.

The only noteworthy alteration in the growth of the fibrosarcoma in the mouse is a tendency of its cells to round off and assume an epithelioid character in sharp contrast to the obvious fibroblastic nature of the tumor in the guinea pig and in man. Curiously, the same alteration occurs on transfer of this growth to the rabbit. The histological appearance of the Brown-Pearce tumor does not change in the mouse (Figs. 27, 28). A peculiarity of its behavior in this species is the frequency of regression and recurrence. The tumor may grow to form a fungating mass as large as the mouse's head, then undergo regression so complete that no trace of tumor can be found in the atrophied eye. However, recurrence is the rule and in several instances renewed growth was not evident until after the lapse of 6 months.

The mouse eye also affords good growth for the embryonic tissues of other species (Figs. 29 to 32). The same species relationships observed in the heterologous transplantation of tumors holds here but is less pronounced and it is much easier to grow human embryonic tissue in the mouse than it is to grow human cancer. The tissues undergo differentiation and organization and, despite the distinct environmental difference, little variation from normal intrauterine development can be found.

DISCUSSION

The object of the present paper was to point out the potentialities of the mouse eye as a transplantation site. The experiments cited and the results obtained require further consideration in relation to the special fields to which they pertain but such discussion is not essential to the immediate purposes of this report and will be presented in later papers describing the experiments in more detail. Sufficient evidence has been offered to justify the conclusion that the mouse eye is a good transplantation site offering a better approach to special problems than other bodily regions and deserving more widespread use than is at present accorded.

SUMMARY

A simple technic of anterior chamber transfer in the mouse has been devised. The technic has been successfully applied to the homologous and heterologous transplantation of tumors and of embryonic tissue, the homologous transfer of adult tissues and the production of carcinomas in transplanted embryonic organs. Illustrative experiments are described.

REFERENCES


DESCRIPTION OF FIGURES 21 TO 26

Fig. 21.—Section of eye of strain A mouse bearing a transplant of esophagus from strain A embryo. A crystal of methylcholantherene was added to esophagus before transfer. Section was taken 69 days after transfer and shows epidermoid carcinoma developing from esophageal mucosa. The excessive keratinization appears to be characteristic of epidermoid carcinomas of the mouse produced in this manner. Mag. × 32.

Fig. 22.—Higher power view of previous section. Mag. × 225.

Fig. 23.—Section of eye of C3H mouse bearing a transplant of forestomach from a strain A embryo. A crystal of methylcholantherene was added to the fragment before transfer and the animal was killed 55 days afterwards. The stomach with its lumen filled with keratin occupies the expanded anterior chamber and small epidermoid carcinoma is arising at one pole. Mag. × 50.

Fig. 24.—Section of eye of C57 mouse bearing transplant of forestomach from a ZBC embryo. A crystal of methylcholantherene was added to the fragment before transfer and animal was killed 98 days afterwards. Section shows an invading epidermoid carcinoma with much less keratinization than is usually observed. Mag. × 85.

Fig. 25.—Section of eye of C57 mouse bearing a transplant of a human fibrosarcoma. Mag. × 50.

Fig. 26.—Higher power view of previous section. Mag. × 300.
Figs. 21-26
DESCRIPTION OF FIGURES 27 TO 32

Fig. 27.—Section of eye of strain A mouse bearing transplant of Brown-Pearce rabbit tumor. Animal was killed 10 days after transfer. Mag. × 60.

Fig. 28.—Higher power view of previous section. Mag. × 300.

Fig. 29.—Section of eye of Swiss mouse bearing transplant of embryonic human lung. The animal was killed 40 days after transfer. Mag. × 60.

Fig. 30.—Higher power view of previous section to show contiguous bronchial buds. Mag. × 500.

Fig. 31.—Section of eye of Bagg albino mouse bearing a transplant of kidney from guinea pig embryo. Animal was killed 43 days after transfer. Mag. × 50.

Fig. 32.—Higher power view of previous section. Mag. × 225.
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