2. ———, PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 203.

3. Ornstein, L., Davis, B. J., *Disc electrophoresis,* Distillation Products Industries, Rochester, N. Y., 1962.

4. Scherr, G. H., *Trans. N. Y. Acad. Sci.,* 1961, Ser. II, v23, 519.

5. Robbins, J., Rall, J. E., *Physiol. Rev.,* 1960, v40, 415.

6. Robbins, J., Wolff, J., Rall, J. E., *Endocrinol.,* 1959, v64, 37.

7. ———, *ibid.,* 1959, v64, 12.

8. De Groot, L. J., Stanbury, J. B., *Am. J. Med.,* 1959, v27, 586.

9. Rilke, F., Colombo, F., Pecchiai, L., *Virch. Arch.,* 1956, v328, 552.

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The Persistence of Isotopically Labeled Cells in Corneal Grafts.* (27422)

FRANK M. POLACK† AND GEORGE K. SMELSER

Department of Ophthalmology, College of Physicians and Surgeons, New York City

It is essential to learn the fate of cellular constituents of homograft tissue if we are to decide whether donor cells can become incorporated in the host without an immunological reaction to them. It is possible that grafts of some tissues serve simply as an inert scaffold which is invaded by host cells which repopulate the structure after the death of the donor cells. Current interpretation of the rejection phenomena in skin and other organ grafts implies that donor cells remain. The frequent persistence of clear corneal homotransplants is an important variant from the usual experience with grafts of other tissues. Corneal grafts, because they consist largely of regularly arranged collagen with relatively few connective tissue cells, could well act as a scaffold which, following death of the donor cells, would be populated by host corneal corpuscles invading it. Certainly the covering epithelium of such grafts is regularly replaced by host epithelium. The endothelium covering the posterior surface is also known to regenerate rapidly and could behave as does the epithelium. However, ordinary histological study of such grafts have not been able to solve the problem of whether there is a replacement of the endothelial or the stromal cells because of the impossibility of distinguishing those of the host from those of the donor.

Basu, Miller and Ormsby(1) investigated the fate of corneal stroma cells by utilizing the sex chromatin as the identifying characteristic of the donor cells, and reported that cells in transplants to hosts of the opposite sex persisted by at least 3½ months. Using the same technic Espiritu *et al.*(2), reported that grafted endothelial cells survived for 4 months and then were replaced. Another possible means of investigating this problem depends on distinguishing host from donor cells by long lived isotopically labeled constituents incorporated in stable compounds in the cell structure. Tritiated thymidine is incorporated in DNA at the time of its synthesis and presumably undergoes no degradation or turnover. In cells with a low mitotic rate such a label would be extremely stable and not diluted by cell division. Mitosis is not found in the normal adult corneal endothelial or connective tissue cells so they form an ideal material for such a study. DNA synthesis occurs both during development and regeneration of these cells. Experiments were carried out in which tritiated thymidine was incorporated in the corneal endothelium and keratoblasts in adult rabbit corneas during regeneration of these cells after those in the central portion of the cornea had been killed by freezing. Labeled thymidine injected into the anterior chamber during regeneration, following mechanical injury in the endothelium

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† Special Fellow Nat. Inst. Health.

was found to be incorporated in the newly formed cells by Mills and Donn(3), and to persist in such corneas for at least 5 months by Bito and Harding(4). Hanna and O'Brien (5) have reported that rabbit corneal endothelial and stromal cells incorporate H^3 thymidine after penetrating injuries with a spatula. Such labeled corneas were transplanted and the labeled cells found to persist for 4 weeks. Incorporation of tritiated thymidine during regeneration of both keratoblasts and the endothelial cells after freezing and the persistence of the isotopic label for over 12 months has been established by Polack and Smelser. The persistence of such labeled cells after grafting is the subject of this report.

Methods. The central area of corneas of adult anesthetized albino rabbits was frozen by the application to its surface of a metal container filled with dry ice and alcohol(6). This procedure kills the corneal connective tissue and the underlying endothelial cells without affecting the general structure of the stromal collagen fibers. The keratocytes were replaced by mitosis of peripheral corneal cells especially during the first 7 days. Tritiated thymidine was injected repeatedly into the anterior chamber during this period. Approximately 1 month after this procedure, full thickness corneal disks 4.5 mm in diameter were cut with a trephine and transplanted to the host corneas which contained no isotopically labeled cells. In intervals up to 12 months such grafts (11 cases) were sacrificed, frozen sections of the corneal stroma cut tangential to its surface, and flat whole mounts made of Descemet's endothelium and membrane. Radioautographs were prepared of these sections and whole mounts by the stripping film technic. This report is based on the study of labeled transplants.

Results. Study of these autographs revealed that both the donor connective tissue and endothelial cells persisted in these grafts for at least one year (2 cases). Many cells were labeled at the end of this period and the proportion of labeled to unlabeled cells although difficult to determine was not appreciably less than that found in corneas which had not been grafted.

The surgical process involved in making the transplants no doubt destroys some cells at the edge of the trephine incision in both donor and host. Presumably most of the replacement comes from the host but in one case labeled endothelial cells migrated peripherally from the graft to cover the inner surface of the host cornea near the scar. Because of technical variation from animal to animal the number of corneal cells injured and amount of regeneration required in both host and donor tissue would necessarily vary.

Conclusion. These experiments demonstrate that both corneal stroma and endothelial cells persist for such long periods after transplantation that they may be regarded as essentially permanent residents in the host. The absence, in most cases, of an immunological reaction of the host to these cells is impressive and clinically fortunate. The fact that an immune response is not regularly observed may be due to the avascular character of the graft and the tissue surrounding it, or to the small amount of antigenic material (cells) introduced into the host.

Summary. Corneal homografts containing isotopically labeled cells (labeled with tritiated thymidine incorporated in the DNA of stromal connective tissue and Descemet's endothelial cells) were found to persist in apparently undiminished numbers for as long as 12 months. It is expected that studies of older grafts would reveal persistence of donor cells for still longer periods with no indication of an immunological reaction of the host.

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1. Basu, P. K., Miller, I., Ormsby, H. L., *Am. J. Ophth.*, 1960, v49, 513.

2. Espiritu, S., Kara, G. B., Tabowitz, D., *ibid.*, 1961, v52, 91.

3. Mills, N., Donn, A., in *The Structure of the Eye*, ed. by G. K. Smelser, Academic Press, New York, 1961, p435.

4. Bito, L. Z., Harding, C. V., *Arch. Ophth.*, 1961, v65, 553.

5. Hanna, C., O'Brien, J. E., *ibid.*, 1961, v66, 362.

6. Dunnington, J. H., Smelser, G. K., *ibid.*, 1958, v60, 116.

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