Homotransplantation of Endocrine Tissues in a Diffusion Chamber *

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CONSIDERABLE evidence indicates that the rejection of homotransplanted tissue is an immunologic phenomenon.[5] Neither the exact mechanism by which the homograft functions antigenically nor the manner in which induced resistance destroys the graft is completely understood.

Several investigators have noted that homografts which do not become vascularized in the host will survive. Merwin and Hill [7] have clarified the nature of this protection. These investigators found that the site of implantation of a graft in the subcutaneous tissue of a mouse would determine whether the graft would become vascularized. Grafts placed close to a host vessel became vascularized, whereas those more distantly situated did not. Employing this laboratory model, these investigators noted that if a graft became vascularized, its rejection would occur. However, nonvascularized grafts survived indefinitely. To determine whether the survival of nonvascularized grafts was owing to their antigenic inertness, or to their resistance to elaborated antibody, mice were prepared so that each carried a vascularized and a nonvascularized graft. Both grafts were destroyed at the same time. In another study, grafts were placed so as to remain avascular in animals in which a vascularized graft had previously produced immunity. The subsequent avascular grafts were promptly rejected. These observations suggest that avascular grafts do not provoke an immune response, but that they are susceptible to immunity otherwise produced.

Algire, *et al.*[1, 2, 8] continued the study of the survival of the nonvascularized graft by devising a diffusion chamber which was permeable to essential metabolites, but which did not permit the passage of cells. He found that homografts protected in these chambers would survive. He postulated from these data that humoral antibody alone does not destroy grafts, and that if cells of host and donor are not brought into contact, homotransplants will survive. Algire's experiments utilized only small amounts of tissue in the laboratory mouse. It was believed that if this principle could be applied on a larger scale, it might be clinically useful. Specifically, it was decided to investigate the possibility of transplanting endocrine tissue in diffusion chambers. If successful and physiologically active, such grafts could be employed in hormonal deficiency states.

Method

Diffusion chambers were prepared in the following manner. A Millipore Filter † membrane, type HA, was first cemented to a methyl methacrylate ring to form a semipermeable disc. This membrane is composed of cellulose ester, is 150 micra in thickness, and has a pore size of approximately 0.45 micron. The cement used is

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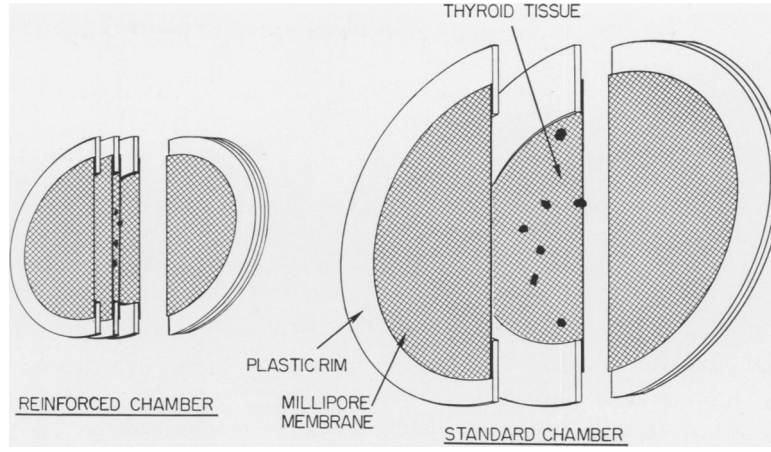

FIG. 1. Diagram of diffusion chamber. Insert —Diagram of reinforced chamber.

acryloid B-7.‡ The discs were then sterilized with ethylene oxide in a dessicator chamber with an attached air outlet for twelve hours. The gas was then removed by applying suction to the outlet, and was trapped in water. The discs were placed in sterile Petri dishes and were retained for the operative part of the experiment.

Paired, female, male, or male and female genetically unrelated mongrel dogs were anesthetized with intravenous pentobarbital sodium and were simultaneously subjected to laparotomy and thyroidectomy.

‡ Rohm and Haas Co., Philadelphia, Pa.

A total of 16 dogs was used. Half the dogs of the group had total thyroidectomy and the remainder had only one lobe removed. One lobe of thyroid gland from each dog was carefully sectioned with a sterile razor into sheets of tissue varying from 0.3 mm. to 1.0 mm. in thickness. The tissues were kept moistened with a tissue-culture medium composed of balanced saline solution, "Tris" buffer, horse serum, and penicillin and streptomycin. The sectioned tissue was placed on one of the previously prepared discs. To construct the diffusion chamber, the plastic ring of the tissue-containing

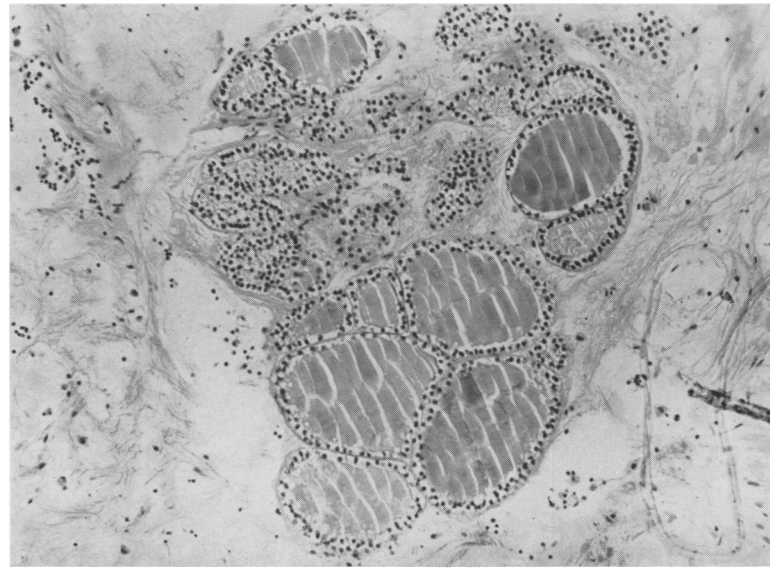

FIG. 2. Photomicrograph of thyroid acini after six weeks of homotransplantation. Note surrounding fibrosis. × 130.

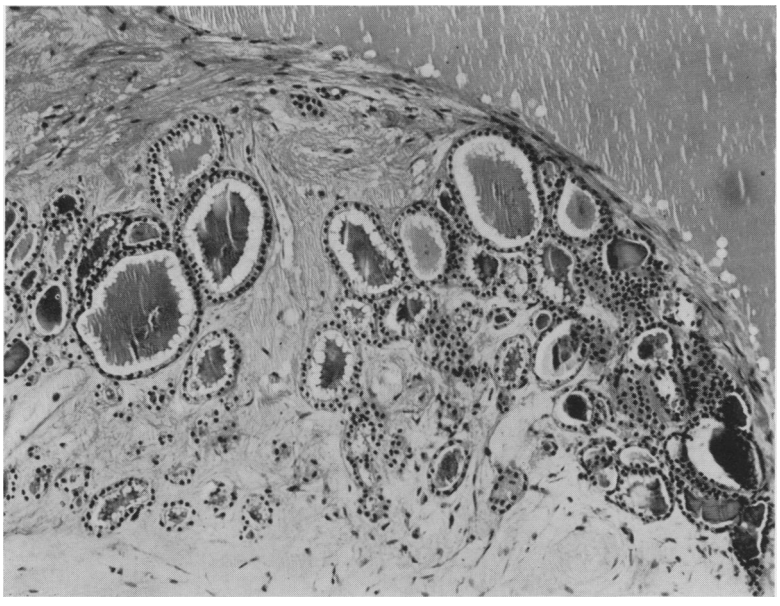

FIG. 3. Photomicrograph of thyroid acini after five months of homotransplantation. Note position adjacent to diffusion membrane. × 130.

disc was cemented to the ring of an identical disc. Chambers used during this study were of three types: (1) chambers with an internal diameter of 40 mm.; (2) chambers with an internal diameter of 15 mm.; and (3) chambers with an internal diameter of 40 mm., reinforced with another disc on either side of the chamber. In this type, two Millipore membranes instead of one separated the graft from the host on both sides (Fig. 1).

After drying of the cement, the chambers—three usually being necessary to accommodate one lobe of donor tissue—were placed intraperitoneally in the paired host animals. Postoperatively, the animals were

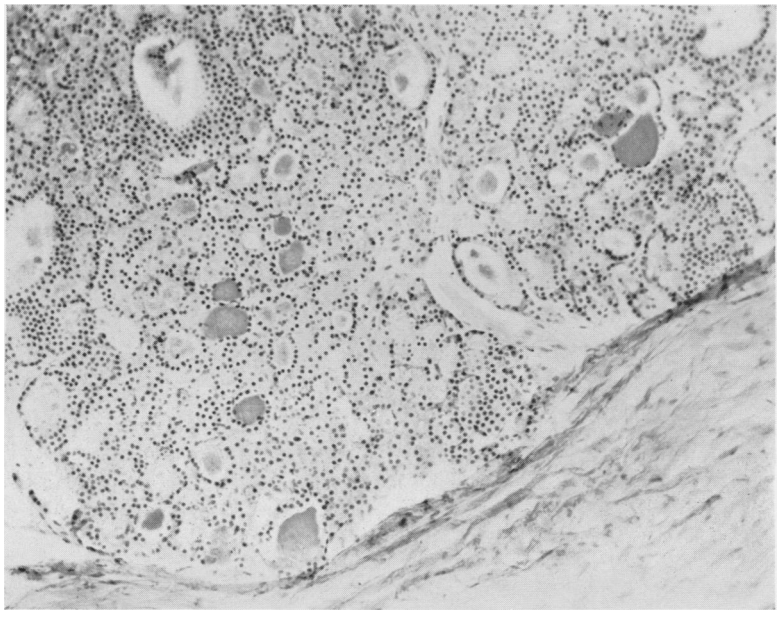

FIG. 4. Thyroid acini after 14 weeks following serial passage through two host dogs.

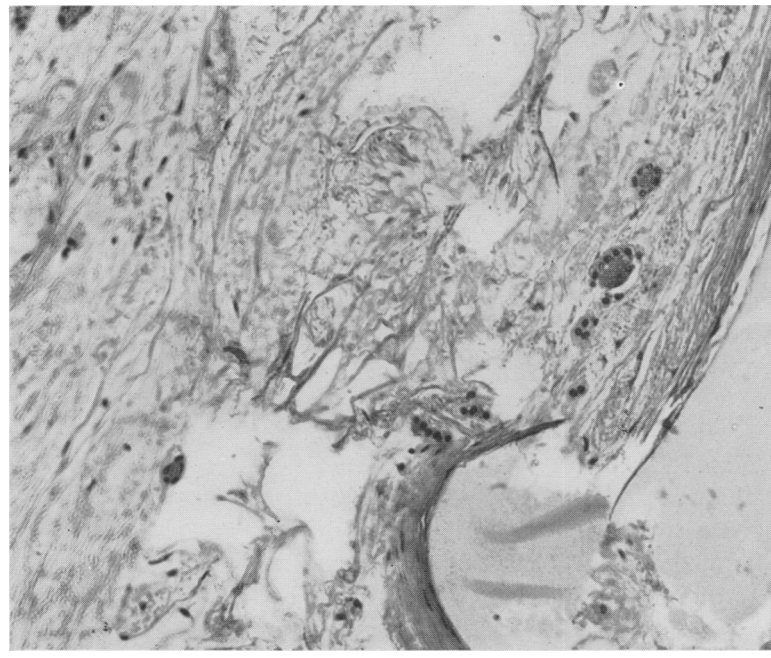

FIG. 5. Thyroid acini after 42 weeks. Fibrosis has replaced most of the graft.

maintained on standard diets, supplemented in the case of the totally-thyroidectomized animals with vitamin D and oral calcium. At varying time intervals, a diffusion chamber was removed from each animal and fixed in Bouin's solution. Microscopic sections were prepared and stained with hematoxylin and eosin.

Results

The diffusion chambers were, in general, well tolerated by the host animals. At laparotomy, the chambers were found encased in omentum, which had formed a mesothelial-lined membrane around the chamber. One of the 16 dogs died from intestinal obstruction presumably caused by the filters.

Homotransplantation of thyroid tissue was observed in this experimental system. Thyroid acini were demonstrated after 6, 8, 12, 14, 16, 20, and 42 weeks of transplantation (Figs. 2–5). The acini were composed of low columnar epithelium, with amounts of colloid carying from normal in some acini to scant or absent in others. The colloid appeared fragmented and contracted in certain sections. The most significant finding in this system was the fibroplasia which was noted in all specimens. Its extent was proportional to the duration of the homograft. In the six-week specimen, fibrosis was noted in about 50 per cent of the section. At 42 weeks, about 98 per cent of the tissue was fibrotic. After this time, fibrosis replaced the thyroid graft. In the specimens removed at the longest time intervals, the surviving thyroid acini were always found close to the membrane, and fibrosis was maximal in the center of the chamber. One thyroid homograft in a chamber was placed intraperitoneally in a host animal, was removed after one week, and was then placed in a second host. Removal three months later showed survival (Fig. 4).

Certain variables seemed to have no effect on homograft survival. No enhancement of homograft survival was noted in hosts with a total thyroidectomy as compared to the lobectomized group. As regards chamber design, an equally satisfactory result was noted with both the large

and small chambers. However, in the reinforced chambers with the doubled membranes, no acini survived.

One of the unsatisfactory technical features of this model was the occasional appearance of tears in the membrane in a peripheral position near the union of the membrane and the plastic ring. The stress on the filter is apparently greatest at this point. It was also observed that the tensile strength of the filter decreased with the duration of implantation.

Discussion

A study of the survival of nonvascularized grafts was undertaken to determine if such a system could be applied clinically. Following the experimental lead of Algire, a laboratory model was established, utilizing a semipermeable diffusion chamber in which acini of thyroid homografts have survived for as long as 10 months. Progressive fibroplasia has been noted in this system. Hallin [4] and Brooks [3] have had the same experience. This process is possibly the result of inadequate extracellular fluid diffusion because the fibrosis is noted maximally in the center of the chamber and the acini surviving the longest are located peripherally near the diffusion membrane. In addition, the reinforced chambers of doubled membranes were uniformly unsuccessful.

Summary

1. Successful homotransplantation of thyroid tissue has been achieved in dogs for periods extending up to 10 months.

2. The amount of thyroid tissue decreases and fibroplasia increases as a function of time.

3. The biologic principle underlying the survival of homografts in this system warrants further study, but it does not appear that clinical application of this laboratory model is feasible at present.

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