The Cultivation in the Living Organism of the Thymus Epithelium of the Guinea-Pig and Rat

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With one plate (fig. 1)

SUMMARY

In autografts of thymus enclosed in porous cellulose membranes, the epithelium grows out in the shape of nodules, compact sheets, cords, and scattered isolated islands. During the experimental period, regeneration of the thymocytes did not take place in the grafts, provided that exogenous lymphocytes from other parts of the body were prevented by the cellulose barrier from penetrating the remnants of the thymic epithelium.

INTRODUCTION

IN autografts and in syngenesiografts of the thymus, immigration of lymphocytes into the epithelial remnants of the transplant makes an important contribution to the regeneration of the organ (Jolly and Lieure, 1932; Grégoire, 1935, 1939; Law, 1952).

In previous investigations on the reciprocal reactions of the thymocytes and of the epithelial reticulum (Grégoire, 1935), I tried to prevent this immigration by isolating the grafts inside filters that allowed the passage of metabolites but were impermeable to infiltrating cells. Fragments of lobules from normal and irradiated, adult and embryonic thymuses were enclosed in collodion bags (Rezzesi's procedure, 1932) or in peritoneum impregnated with metallic silver (Irwin, Gairns, and Banting's procedure, 1934), or were wrapped in filter paper and in hydrophil cotton sheets. The loaded containers were implanted subcutaneously or intraperitoneally.

Proliferation of the thymic epithelium was recorded, especially in autologous and in embryonic grafts wrapped in cotton envelopes and temporarily protected by these envelopes from lymphovascular connexions and from infiltration of migrating cells and of lymphocytes (Grégoire, 1935, plate XIX, figs. 15, 16, and 39, and text-fig. 12). However, the cotton procedure was rather unsatisfactory, owing to the unknown variations in the weave of the cotton material, and owing to the difficulty of detecting the thinly spread epithelial structures growing inside the granulome with giant cells developed around the cotton fibres.

In further investigations, reported in the present paper, advantage has been taken of the improved methods of manufacturing cellulose membranes of standard grades of porosity.

[Quarterly Journal of Microscopical Science, Vol. 99, part 4, pp. 511–515, Dec. 1958.]

Material and Methods

Thirty guinea-pigs, 2½ to 3 months old, were used for the autografts, and 20 rats, 6 weeks old, for the homografts. Approximately 120 transplants were studied. In the guinea-pigs, total thymectomy was performed during the operation, and the grafts were the only thymic tissue present during the experimental period.

After excision of the parathyroid gland included in the thymus, the interlobular connective tissue was trimmed away and the thymic lobules were cut into small fragments. These were placed in the centre of the porous membranes, which were rolled or folded around the pieces of tissue. In order to insure a close contact between transplants and membranes, the folds were held tight by means of catgut knots or silver clamps. The bags were not as completely closed as if they had been sealed, but several folds separated the grafts from the surrounding fluids and penetration of free migrating cells into the bags was sufficiently delayed and was not noticeable during the experimental period. The loaded bags were placed in the subcutaneous connective tissue or in the peritoneal cavity. The 'diffusion chamber technique' recently developed (Prehn, Weaver, and Algire, 1954) has also been used in the present study.

Some of the porous cellulose membranes used were prepared according to the methods initially elaborated by Elford (Elford and Ferry, 1934). Others were 'dry filter membranes of Zsigmondy', of graded porosity, manufactured by the Membranfiltergesellschaft, Sartoriuswerke, Göttingen, Germany. The diameter of the pores of these membranes is 1–3 μ for the coarse grade, 0·5–1 μ for the medium grade, and 0·2–0·5 μ for the fine grade.

The membranes with their content were removed from the guinea-pigs between the 6th and 29th day, and from the rats on the 16th day after the operation. They were fixed in Bouin–Hollande–sublimate or in Susa, embedded in paraffin, cut in semi-serial sections (5 μ), and stained with Masson's trichrome and May–Grünwald's stains.

Results

During the days following transplantation, the debris of the massive destruction of the thymocytes remained in place, underwent slow dissolution, or was resorbed by epithelial and mesenchymatous phagocytes. The epithelial reticulum, initially loose, shrank into a denser stroma, in which the large characteristic mitoses of its cells appeared and increased in number with time (fig. 1, C). The epithelial remnants of the grafts grew out and formed nodules and compact sheets (fig. 1, A–E), cords (fig. 1, G), or small scattered islands consisting of a few cells. In some areas the epithelial cells were stretched and spindle-shaped. In these areas it was sometimes difficult to distinguish these elongated elements from the mesenchymatous cells mixed with them.

A luxuriant epithelial proliferation occurred in the places where the epithelial remnants were in close contact with the inner surface of the porous

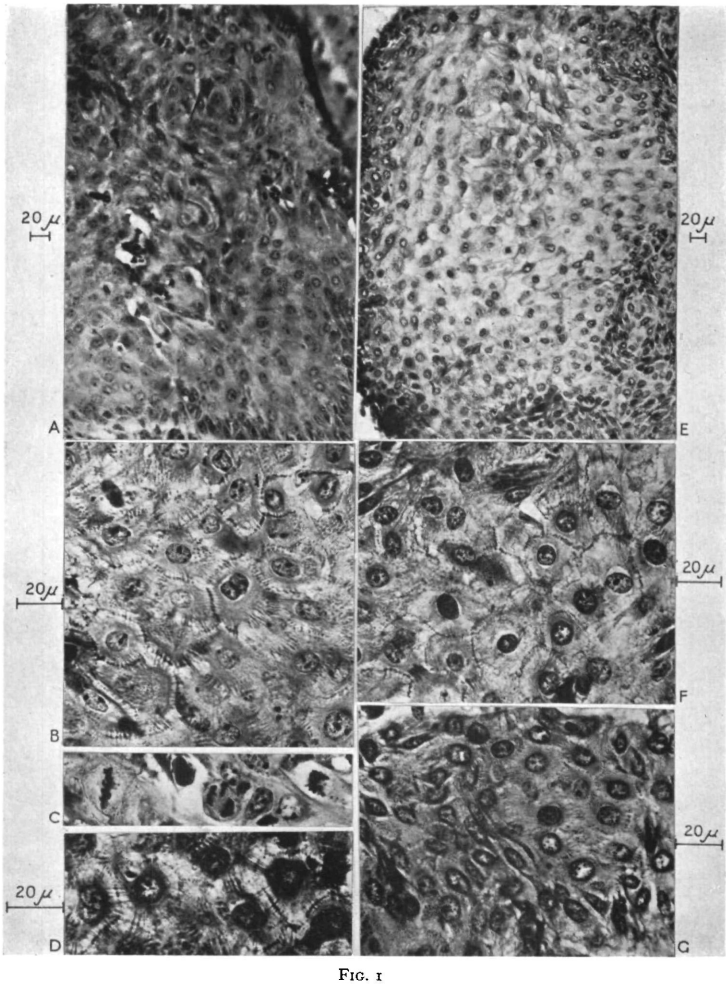

FIG. 1

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membranes (fig. 1, A–E). In the central parts of the bags, the outgrowth of the thymic epithelium was less active. Floating fragments, in too loose bags, were necrotic.

In the sections of the nodules and in various parts of the cords (fig. 1, B, D, F, G) the epithelial tissue looked like a flagstone pavement in which the individual cells assumed the shape of flat polygons, connected together by intercellular bridges, on which knots of Bizzozero-Ranvier were visible (fig. 1, D).

Thymocytes were absent in the sheets of the actively growing epithelium. In some portions of the epithelial remnants corresponding topographically to the former medullary zone of the lobules, a few thymocytes, with a darkly staining nucleus, had escaped the massive disintegration at the beginning of transplantation, and were scattered between the epithelial cords. These cells had obviously not increased in number during the experimental period, which extended, in several grafts, up to 29 days after transplantation.

The appearance of the grafts enclosed in porous membranes contrasted strikingly with that of the free grafts after the same period of time. In these free grafts, the three successive phases of regeneration of the thymocytes—(1) the immigration of exogenous small lymphocytes, with a darkly staining nucleus, into the epithelial remnants of the grafts; (2) development of a transient lymphoblast phase; and (3) the active mitotic proliferation and transformation of these blasts into small thymocytes with faintly staining nuclei (Grégoire, 1935, 1939)—resulted in reconstitution of the normal appearance of the lobules, in about 10 to 15 days. In the reorganized lobules, swarming thymocytes concealed the cells of the epithelium.

Sixteen days after transplantation, the portions of the homologous grafts in contact with the porous membranes had survived. The histological features of these grafts were similar to those described above for autografts.

FIG. 1 (plate). Guinea-pigs, 100 days old. Autografts of thymus, enclosed in collodion bags (diameter of the pores, 200–500 mμ) placed for 22 days in the subcutaneous connective tissue.

A–E, portions of epithelial nodules spread out along the membranes in close contact with them. In A, a part of the membrane is shown at the top right side of the picture. In E, the membrane has been detached from the graft during the sectioning process (top and bottom left). Notice, in both pictures, the mosaic-like architecture of the cells, an arrangement common to all epithelial cells in cultures *in vitro*. A, the epithelial cells are elongated and disposed in palisades along the boundaries of the nodule. E, in the central part of that nodule, the epithelial cells are spread out (pale cytoplasm). In the peripheral regions of the same nodule, the epithelial tissue is denser and the cells are grouped in strands and in cords, in continuity with other epithelial structures scattered in a loose mesenchymatous tissue in the more central portions of the bag. Hassall's corpuscles (not included in the pictures A–E) are located inside the epithelial outgrowth. B, D, F show portions of nodules more highly magnified. Individual epithelial cells appear in section like a pavement of polygonal flagstones, and are connected together by intercellular bridges (with knots of Bizzozero-Ranvier on their middle part), disposed all around their boundaries. Granules gathered in small fields (B–D) correspond to intercellular bridges in transverse section. In B, a binucleated cell appears in the centre of the picture. C shows three epithelial mitoses (2 metaphases, 1 anaphase) in a cord such as that illustrated in G. G shows a section of cords visible in the right part of E. In the central part of the cords the cells are polygonal. At the periphery they assume an elongated fusiform aspect. Mesenchymatous cells in small numbers are scattered between the cords.

Discussion

In the present material, survival and proliferation of the epithelium was recorded in membranes of the three porosities used. The grade of porosity seemed to play a less important part than the adherence of the fragments to the cellulose membranes.

Enclosed in porous membranes during the life of the animal, the thymic epithelium assumes the characteristic mosaic-like architecture recorded in all epithelial tissues *in vitro* (Fischer, 1946). Its outgrowth does not essentially differ from that described in cultures *in vitro*, especially by Pappenheimer (1913), Wassén (1915), Tschassownikow (1926), Popoff (1927), Schopper (1934), Emmart (1936), and Murray (1947).

Intercellular bridges, of controversial significance (Pease, 1951; Medawar, 1953), have been observed in autografts of thymus by Tschassownikow (1926) and by Jolly and Lieure (1932), and in thymic epithelial sheets growing inside cotton envelopes (Grégoire, 1935).

According to Algire (1957), membranes with pore diameters of $0{\cdot}8\mu$ allow the passage of migrating cells. Owing to the relatively short duration of the present experiments, infiltration by migrating cells through membranes corresponding to those used by Algire was not recorded.

In the transplants of thymus enclosed in porous membranes, the only sources available for the proliferation of thymus lymphocytes and for the repopulation of the epithelial stroma, are either the rare small cells mentioned in the description (homoplastic lymphocytopoiesis) that escaped the initial massive disintegration, or the mesenchymatous elements blended with the epithelial components of the grafts (heteroplastic lymphocytopoiesis).

An inadequate supply of metabolites essential for the multiplication or neoformation of these lymphocytes seems to be unlikely, especially in the vicinity of the porous membranes, where mitoses were observed in the connective cells as in the epithelial cells.

The present results rather support my former conclusions (Grégoire, 1935) that, as long as the lymphocytes cannot reach the epithelial cells of the thymus transplanted during life, the transplant remains purely epithelial. The results are also consistent with previous observations that immigration of exogenous lymphocytes, with duplication of the histogenetic process, plays an important part in the regeneration of the experimentally involuted thymus, especially in grafts (Jolly and Lieure, 1932: Grégoire, 1935, 1939; Law, 1952), and that heteroplastic lymphocytopoiesis is weakly developed in the thymus (see discussion in Grégoire, 1935, 1939, 1945, 1956; Downey, 1948; Frank, Kumagai, and Dougherty, 1953; Santisteban and Dougherty, 1954).

In former studies on the influence of endocrine imbalance on the regeneration of the irradiated thymus, alterations of the epithelial cells were recorded after adrenalectomy (Grégoire, 1942) and after injection of testosterone propionate (Grégoire, 1945). The procedure of culture during the life of the animal in porous membranes reported in this paper has been found to be an

adequate technique for reinvestigating these reactions in the absence of the lymphoid components of the organ. The results will be reported later.

The present work was aided by the Fonds National de la Recherche Scientifique.

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