HISTOGENESIS OF THE THYROID GLAND DURING CULTURE IN DIFFUSION CHAMBERS

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Histogenesis regulation and sources of thyroid gland regeneration in white rats and steppe tortoises under conditions of auto- and allotransplantation in diffusion chembers are described. The dynamics of the reactive changes indicates the heterogenous character of morpho-functional differentiation of the thyroid structures. Differences in the adaptive plastic rearrangement of the thyroid gland of the vertebrates studied are related to the histogenesis rate and depend on evolutionarily fixed stability of the tissue to the hypoxic state.

Transplantation of organs and tissues in diffusion chambers allowing free diffusion of fluid but impermeable to cells is a convenient model with which to study many aspects of regeneration and histogenesis of organ structures [5-7, 10-12].

The object of this investigation was to study the potential value of the method and the character and sources of regeneration of thyroid epithelium from the comparative aspect in mammals (albino rats) and reptiles (Horsfield's tortoise) after auto- and allografting of the thyroid gland in diffusion chambers.

EXPERIMENTAL METHOD

Experiments were carried out on 140 adult noninbred male albino rats (group 1) and 80 tortoises (group 2). Under aseptic conditions and under ether or hexobarbital anesthesia, one-third of the thyroid gland was removed from the animals. Small slices of the gland (0.2-0.3 mm thick) were placed in diffusion chambers and transplanted intraperitoneally. The diffusion chambers, made from millipore filters (type HA, USA) with a pore size of 0.45 ± 0.01 μ and a thickness of 150 μ, were mounted on rings [9]; chambers of "packet" type also were used.

The chambers were removed 2, 6, 8, 12, and 18 h, 1, 3, 5, 7, and 15 days, and 1, 2, 4, 6, and 8 months after transplantation, fixed in Carnoy's mixture and neutral formalin solution, and embedded in paraffin wax. Sections were stained by general histological methods and histochemically for nucleic acids (by Feulgen's and Brachet's methods) and for mucopolysaccharides (the PAS reaction and Hale's method). To judge the functional activity of the thyroid gland, the isotope ^{131}I was injected subcutaneously into the animals in a dose of 0.05 μCi/g. Accumulation of the isotope in the chambers was determined 24 h later on the B-2 apparatus in the lead container of the MST-17 counter.

EXPERIMENTAL RESULTS

Desquamation of the thyrocytes and liquefaction and evacuation of the colloid were observed in the large and small follicles present in the chamber in rats 2 h, and in tortoises 8-12 h after implantation of the thyroid gland. The concentration of acid mucopolysaccharides was increased in the peripheral zone. The cytoplasm of the desquamated cells was strongly oxyphilic in character and the nuclei were pycnotic. The character of the pathological changes were the same after auto- and allografting and in both species of animals. Later (6 h in group 1, 18 h in group 2) evacuation of colloid was intensified. PAS-positive granules were found in the cytoplasm of the remaining cells (Fig. 1a). Gross degenerative changes developed in the thyrocytes of the central follicles, and some of the cells were dead and converted into cellular detritus. Desquamation of the cells and destruction in the central parts of the graft led to total disintegration of the follicles.

After 1-3 days more severe destructive and degenerative changes had developed in the grafts from the albino rats; these processes were particularly well marked in the central zone and they terminated in necrosis.

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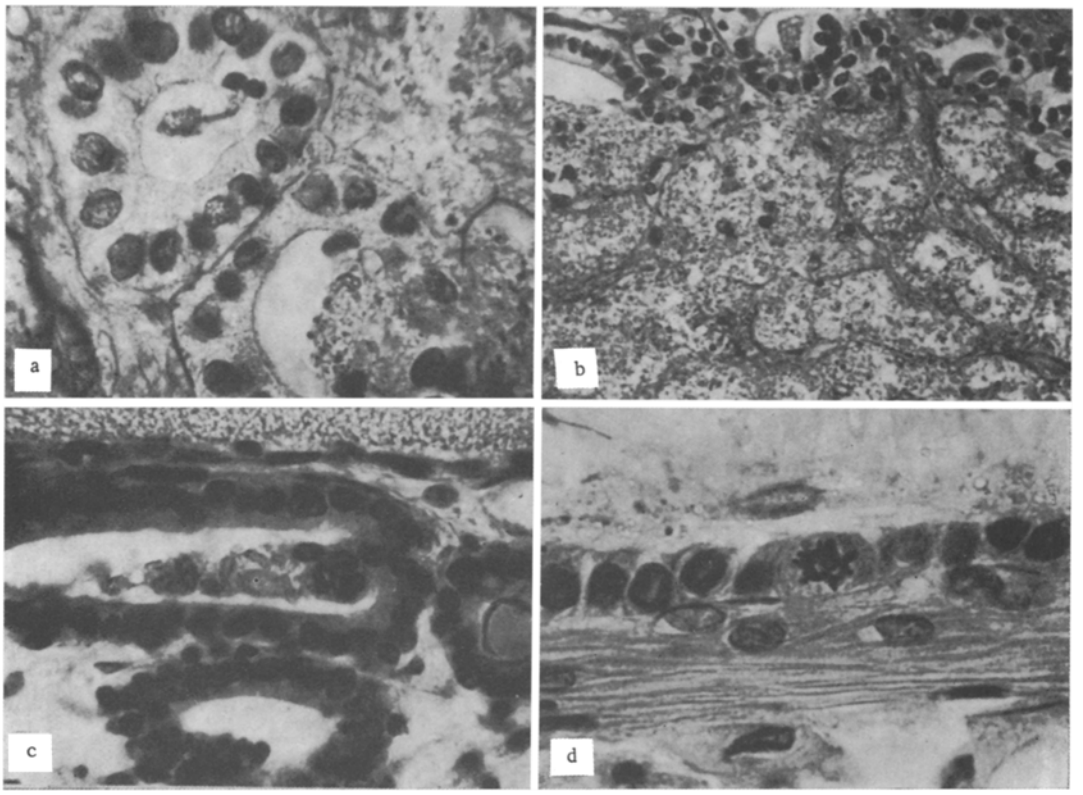

Fig. 1. Auto- and allografts of albino rat thyroid gland: a) evacuation of colloid accompanied by marked hypertrophy of thyrocytes and appearance of PAS-positive granules in their cytoplasm (6 h after transplantation), 900×, PAS reaction, counterstained with hematoxylin; b) autograft (24 h). Dead and viable cells of thyroid parenchyma clearly distinguishable, 200×, hematoxylin–eosin; c) regenerating region of allograft (7 days). "Stable" and "transient" follicles, 400×, hematoxylin–eosin; d) polymorphic growth of thyroid epithelium as a stratified sheet. Allograft (15 days), 600×, hematoxylin–eosin.

Viable thyroid tissue was preserved as separate epithelial clusters and follicles at the periphery of the graft, in the immediate vicinity of the chamber wall, because of the better nutritional conditions as the result of diffusion of fluid (Fib. 1b).

The thyroid tissue of reptiles is more resistant to harmful factors, as shown by the slower development of destructive processes. The reason for this may be the low level of metabolism and the greater resistance of cells of poikilothermic animals to hypoxia [13]. On the 7th day, for instance, separate follicles or parts of their walls were still preserved in the graft and desquamation of the epithelium and emptying of the follicles developed more intensively at the periphery of the grafts (Fig. 2a).

Morphological and histochemical analysis of the residual structures and observations on the course of their further development showed different levels of differentiation of thyroid organ complexes in mammals and reptiles. The heterogeneous character of morphological and functional differentiation of thyroid structures has been demonstrated during reparative regeneration [1-3, 8] and during tissue culture by Lazarenko's method [4]. This phenomenon is manifested very clearly in tissue grafted in diffusion chambers. The residual cells have greater future potential and in the later stages they serve as the source for regeneration of functional units of the gland.

On the 3rd day (group 1) and 10th day (group 2) the cellular detritus after complete proteolysis was replaced by exudate and by actively proliferating fibroblasts. The initial stages of regeneration of the gland cells were characterized by dedifferentiation. Integration and heteropolarity of the thyrocytes in the walls of the small and medium-sized follicles were lost after evacuation of the colloid. The RNA content was increased in the cytoplasm of the cells.

Proliferation of epithelial cells observed on the 4th (group 1) and 15th days (group 2) led to the formation of epithelial clusters of nonpolar gland cells. An important feature of this period is that it is possible to

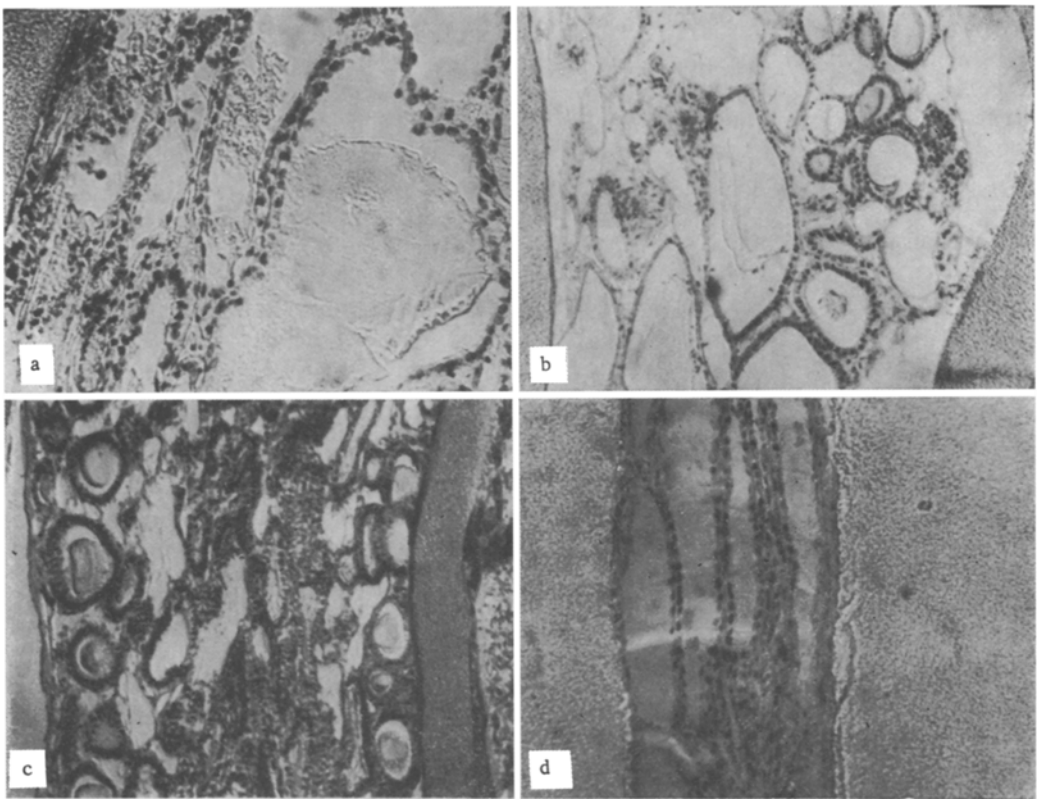

Fig. 2. Grafts of tortoise thyroid gland at different times after transplantation: a) allograft (7 days), desquamation of epithelium and destruction of follicles, 140×, hematoxylin–eosin; b) autograft (15 days), proliferating epithelial bands, newly formed small and medium-sized follicles, 140×, Brachet's method; c) allograft (30 days), epithelial islets and functioning follicles at periphery of graft, 140×, hematoxylin–eosin; d) large distended follicles with homogeneous colloid, allograft (4 months), 140×, hematoxylin–eosin.

determine the sources of regeneration. They include cells of the interfollicular islets and also thyrocytes of the small follicles, and individual cells of the medium-sized and large follicles (Fig. 2b). In the course of development of the repair process these cells formed epithelial bands and cell groups, and later their cells underwent secondary differentiation. Morphologically, this process was characterized by displacement of the nucleus and polar redistribution of RNA. Hale-positive granules appeared in the apical part of the cells, but later they were discharged into the narrow intercellular space. Sometimes the intercellular space appeared even before synthesis of precolloid material by the cells. These cells aggregated into complexes and formed microfollicular structures. The colloid droplets became oxyphilic in character and gave a clear PAS-positive reaction. The fate of the newly formed follicles differed. Some of them grew as a result of cell division, whereas others lost their colloid and their complex arrangement, their cells dedifferentiated once again and began to proliferate, replenishing the interfollicular aggregations. Follicles of the first type could be described as "stable," those of the second type as "transient" (Fig. 1c). In the tortoises extrafollicular proliferation of the cells of the residual small and medium-sized follicles was more intensive. The newly formed follicles were able to accumulate radioactive iodine. The highest level of accumulation of the isotope in rats was observed on the 7th-15th day (112-142 cpm) and in terrapins on the 15th-30th day (90-116 cpm).

Regeneration of the thyroid parenchyma depended on the state of the connective-tissue stroma. Fibroblasts in all cases were better preserved than the remaining cells, proliferated, and exhibited high fibrogenic activity. Proliferation of fibroblasts was particularly intensive along the inner wall of the chambers. Many lymphocytes and polymorphs, macrophages, and fibroblasts were concentrated near the chambers, outside them. The fibroblastic response led subsequently to the formation of connective-tissue capsules around the chambers. In the tortoises this first appeared on the 30th day after transplantation and led to a marked disturbance of nutrition of the graft.

Differences in the character of structural changes in the thyroid tissue of the homoiothermic and poikilothermic animals persisted at later stages. For instance, in the rats on the 15th day in some cases loss of organogenetic potential and distortion of the histotypical reaction were observed. The epithelium spread in the form of stratified polymorphic sheets (Fig. 1d). The genetic nature of the thyroid epithelium as a derivative of the prechordal plate is reflected in such polymorphism of the epithelial proliferates. Some cells evidently preserve their lability of determination and, if the conditions of diffusion worsen they exhibit an old type of response, by forming stratified epithelial sheets. Toward the end of one month only half the grafts were viable. The quantity of thyroid tissue in them varied from single follicles to well-formed functional lobules of glandular tissue. Later the repair processes weakened sharply. Proliferation of connective tissue inside the chamber and the formation of a connective tissue capsule outside it interfered greatly with diffusion and were accompanied regularly by death of the grafts. The longest period of survival of the graft, in the form of a small group of follicles between bundles of collagen fibrils, was 6 months in rats. Signs of destruction were well marked in the follicles and desquamated cells could be seen in the colloid.

In the tortoises by the 30th day of the experiment the grafts were more uniform in appearance and their zonal structure had disappeared (Fig. 2c). Thinly scattered deformed follicles with a slit-like lumen, containing liquid, intensively vacuolated colloid, predominated. The thyroid epithelium was prismatic and the cytoplasm of the cells weakly pyroninophilic. Later the follicles grew larger, and by the 4th month of the experiment large extensive follicles with thick colloid, formed of squamous epithelium (Fig. 2d), remained in the grafts. The interfollicular islands disappeared. A well-developed fibrous capsule formed around the chamber, interfering with diffusion and causing death of the glandular structures.

The investigations thus showed that the thyroid gland, transplanted in diffusion chambers preventing immunologic conflict, is capable of regenerating its follicular structures under conditions of autologous and allogeneic transplantation. Dystrophic and destructive changes, leading to death of the greater part of the graft, were observed in the initial stages of development of regeneration. Later the more intensive proliferation of the thyroid epithelium of the interfollicular islets and of the residual small and medium-sized follicles and differentiation of thyrocytes led to the formation of new follicles. Later the number of successful transplantations decreased and death of the grafts took place. Differences in the character of adaptive and plastic structural changes in the thyroid gland of these representatives of mammals and reptiles were reflected in the speed of histogenesis and depended on properties of their tissues established in the course of evolution.

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