DEVELOPMENT OF THE CORTEX DURING REPARATIVE REGENERATION OF THE ADRENALS

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The original cell form in reparative regeneration of the adrenal cortex after subtotal removal of its parenchyma is a mesothelial cambial cell (adrenocorticoblast), located in the inner layer of the capsule, which can differentiate in two directions. Some adrenocorticoblasts are converted into primary adrenocorticocytes and migrate into the subcapsular layer where, together with dedifferentiated adrenocorticocytes remaining after the operation they form the anlage for regeneration of the definitive cortex, from which the zona glomerulosa, zona fasciculata, and zona reticularis are subsequently formed by further proliferation and differentiation. Other adrenocorticoblasts differentiate into provisional adrenocorticocytes which form the provisional cortex of the regenerating gland, and after a certain period this undergoes involution.

There is still considerable discussion on the question of adrenocortical cytogenesis. Whereas some investigators [2, 3, 5, 8, 11, 12, 14, 15] have demonstrated cambial cells in the adrenal capsule, from which adrenocortical glandular cells differentiate during both physiological and reparative regeneration of the adrenal cortex, other workers [4, 6] stubbornly deny the existence of cambial cells in the capsule or their role as the source of development of adrenocorticocytes.

The object of the present investigation was to study the cytological and cytochemical changes in the adrenal capsule in the course of reparative regeneration of the adrenal cortex in connection with the cytogenesis of its parenchymatous structures.

EXPERIMENTAL METHOD

Experiments were carried out on 350 male albino rats weighing 120-160 g, divided into three groups. Group 1 consisted of intact animals (control). Bilateral adrenolectomy, followed by free autografting of the left adrenal in its normal situation at the upper pole of the left kidney, was performed on the animals of group 2. In the animals of group 3 the left adrenal was enucleated and the right adrenal simultaneously removed.

The animals were sacrificed and histological material taken 1, 2, 3, 4, 5, 7, 10, 13, 15, 20, and 30 days after the operation. Pieces of tissue were fixed in Bouin's, Carnoy's, and Becker's fluids. Paraffin sections from material fixed by Bouin's method were stained with hematoxylin and eosin, with azan and iron hematoxylin by Heidenhain's method, and with picrofuchsin by Van Gieson's method. After fixation by Carnoy's method histochemical methods were used (staining for RNA by Brachet's and for DNA by Feulgen's method, for polysaccharides by the PAS reaction). Frozen sections from fragments fixed by Becker's method were stained with Sudan IV by Goldman's method and with Sudan black B, and treated with Schiff's reagent to detect ketosteroids and with digitonin to detect cholesterol in the polarization microscope. During microscopic examination particular attention was paid to the study of tangential sections through the adrenals as the most suitable for studying the capsule, because of its relative thinness.

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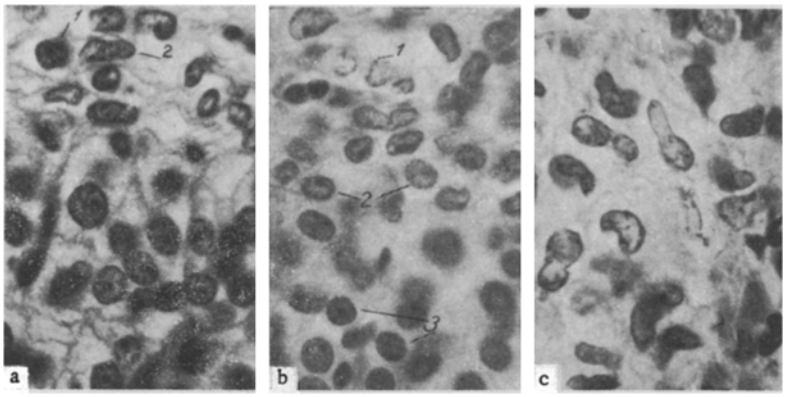

Fig. 1. Adrenocorticoblasts in the adrenal capsule: a) capsule in intact adrenal: 1) group of adrenocorticoblasts, 2) fibroblasts; b) differentiation of adrenocorticoblasts into primary adrenocorticocytes: 1) band of adrenocorticoblasts; 2) transitional forms; 3) primary adrenocorticocytes; c) amitotic division of adrenocorticoblasts of the capsule (tangential section). Here and in Figs. 2 and 3, hematoxylin-eosin, 630×.

EXPERIMENTAL RESULTS

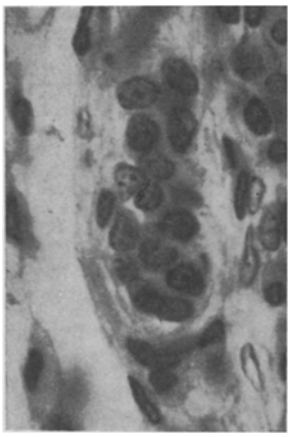

Fig. 2. Nodule of adrenocortical tissue in adrenal capsule five days after enucleation.

The structure of the adrenal capsule of the control rats was found to be complex and heterogeneous. The outer layer of the capsule consisted of dense connective tissue, formed from bundles of collagen fibers and fibrocytes. Numerous small cells with elliptical, oval, or irregular translucent nuclei containing a few diffusely scattered tiny granules of chromatin, and a narrow band of pale basophilic cytoplasm were found in the inner layer of the capsule (Fig. 1a). These cells (adrenocorticoblasts) were arranged in small groups of bands, between which fibroblasts and thin bundles of collagen fibers could be seen. In some parts the inner layer of the capsule was almost absent, and the fibrous layer was directly next to the zona glomerulosa, while in other parts the inner layer of the capsule was well-marked, and there was no sharp line of demarcation between it and the zona glomerulosa.

In the early periods after the operation (2-5 days) the inner layer of the capsule was considerably thickened and looser in structure. Intensive differentiation of the adrenocorticoblasts was observed, and they quickly acquired the features of adrenocortical glandular cells.

Usually in those parts of the capsule which bounded the parenchymatous structures remaining intact after the operation, adrenocorticoblasts differentiated into primary adrenocorticocytes. The nuclei of the cells became rounded, distinct nucleoli appeared in them, the chromatin content increased (Fig. 1b), and the Feulgen reaction became more strongly positive; the cells also increased in size, and the RNA content of their cytoplasm rose. Meanwhile intensive proliferation of adrenocorticoblasts by both mitotic and, evidently, amitotic division was observed (Fig. 1c). During their differentiation the primary adrenocorticocytes usually migrated into the subcapsular layer, but some of them remained in the capsule where nodules of adrenocortical tissue formed from them (Fig. 2).

Glandular cells remaining behind in the subcapsular layer after the operation in turn dedifferentiated approximately to the level of primary adrenocorticocytes: they lost the peripheral part of their cytoplasm, while the RNA content in the remaining cytoplasm increased and the lipid inclusions disappeared.

After 10-13 days the anlage of the regenerating definitive cortex was formed from primary adrenocorticocytes. The primary adrenocorticocytes occupying the central position in this anlage usually differentiated into provisional adrenocorticocytes, but differentiation of the main mass of the cells took place in three directions: toward the formation of cells of the zona glomerulosa, zona fasciculata, and zona reticularis, i.e., the three types of definitive adrenocorticocytes. An increase in the size of the cells was observed,

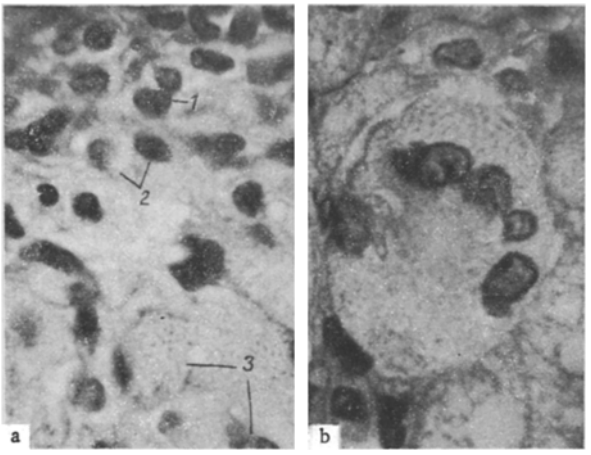

Fig. 3. Provisional adrenocorticoblasts in the adrenal: a) Differential of adrenocorticoblasts into provisional adrenocorticocytes: 1) Adrenocorticoblasts, 3) transitional forms; 3) provisional adrenocorticocytes; b) multinuclear provisional adrenocorticocyte.

especially in the zona fasciculata, the RNA content in their cytoplasm was reduced, while the number of lipid droplets was increased. Proliferation of the cells at this period took place by mitotic division. The main morphogenetic processes in the definitive cortex of the regenerating gland were complete one month after the operation.

In areas of the capsule under which no parenchyma remained after the operation adrenocorticoblasts differentiated into provisional adrenocorticocytes. The cells increased in size, their cytoplasm became highly vacuolated (Fig. 3a), and numerous lipid droplets, reacting strongly for keto groups and showing birefringence, appeared in it. As a result of amitoses of these cells, multinuclear provisional adrenocorticocytes with a denser centrally situated endoplasm, and a peripheral ectoplasm filled with tiny vacuoles appeared. The provisional adrenocorticocytes differed from the definitive adrenocortical cells, characterized by their merocrine secretion, in their marked ability to produce a holocrine secretion. In the course of formation of the definitive cortex of the regenerating gland, its provisional cortex gradually underwent involution.

Adrenocortical cytogenesis during reparative regeneration after subtotal loss of the parenchyma of the adrenal cortex thus begins with proliferation and differentiation of adrenocorticoblasts, cambial cells of mesothelial origin found in the adrenal capsule together with cells of mesenchymal nature – fibroblasts and fibrocytes. Proliferation of the adrenocorticoblasts is accompanied by desmolysis, i.e., by destruction of the collagen structures of the capsule, a characteristic feature in general of regeneration of the epithelium of parenchymatous organs [1, 9]. Amitosis [10] evidently plays an important role in the increase in number of adrenocortical glandular cells. The adrenocorticoblasts differentiate on the one hand into primary adrenocorticocytes, which later form components of the regeneration anlage of the definitive cortex, and on the other hand into provisional adrenocorticocytes which form the provisional cortex of the regenerating gland, subsequently undergoing involution. This type of cytogenesis is characteristic of reparative regeneration after subtotal loss of the parenchyma, whereas physiological regeneration of the zona glomerulosa, zona fasciculata, and zona reticularis takes place chiefly through proliferation of their own adrenocorticocytes [7, 13].

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